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CHARACTERISATION OF CYTOKINE GENE POLYMORPHISMS IN PATIENTS WITH ACUTE PANCREATITIS

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CHARACTERISATION OF CYTOKINE GENE POLYMORPHISMS IN PATIENTS WITH ACUTE PANCREATITIS

by

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ABSTRACT

Background and Aims

Acute Pancreatitis is an inflammatory disorder of varied aetiology and outcome. Tumour necrosis factor (TNF) and interleukin-10 are important mediators of disease pathogenesis. To investigate if the TNF and IL-10 gene loci influence susceptibility to and severity of acute pancreatitis, 135 patients with acute pancreatitis, ethnically matched normal controls, and alcoholics without pancreatic disease were studied.

Methods

Aetiology was classified as being secondary to alcohol, gallstones, or idiopathic. Patients were stratified into groups according to disease severity by assigning an organ failure score. Three TNF microsatellite loci (TNFa, TNFb, and TNFc), the -308 polymorphism within the TNF gene, the IL-10.G microsatellite locus, and 3 bi-allelic polymorphisms in the 5' flanking region of the IL-10 gene were typed using the polymerase chain reaction.

Results

There was no difference in allelic frequency of any of the cytokine gene loci between groups stratified according to disease severity. When patients were stratified according to aetiology of disease there was a decrease in the frequency of the TNFa2 allele in those patients with alcoholic acute pancreatitis compared to controls (14.3 vs. 35.5%, χ^2 =7.24, p=0.007). There was also a reduction in the frequency of the IL-10.G13 allele in patients with alcoholic pancreatitis compared to controls (4.8 vs. 21.3%, χ^2 =6.46, p=0.011).
Data is also presented showing that a number of haplotypes exist as well as linkage disequilibrium across all 4 loci of the IL-10 gene, which contrasts with findings from previous work. The 3 locus haplotypes GCC and ATA are in strongest linkage disequilibrium, as is the microsatellite allele G9 and –1117.A and G9 with the 3-locus haplotype ATA.

Conclusions

This work has identified an allele within the TNF gene locus, and an allele within the IL-10.G locus which have different frequencies in patients with alcohol induced acute pancreatitis compared to other aetiologies. This finding may in part explain individuals' differing susceptibility to the development of acute pancreatitis after excessive alcohol consumption.

Haplotypes not previously described exist across the IL-10 locus.

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AUTHOR'S DECLARATION

At no time during the registration for the degree of Doctor of Medicine has the author been registered for any other University award.

Relevant scientific and clinical meetings and conferences were attended at which work was presented; external institutions were visited for consultation, and papers were prepared for publication.

Conferences attended:

American Gastroenterological Association (AGA) 1997 and 1998
British Society of Gastroenterology (BSG) 1998
British society of Histocompatibility and Immunogenetics (BSHI) 1997 and 1998
British Society of Immunology (BSI) 1997
European Pancreatic Club (EPC) 1997
United European Gastroenterology Week (UEGW) 1997
Pancreatic Society of Great Britain and Ireland 1997

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Polymorphisms of the tumour necrosis factor-α (TNF) gene in acute pancreatitis. 6th United European Gastroenterology Week (UEGW), Birmingham, October 1997.
Polymorphism of the TNF-α Promoter region in acute pancreatitis . Pancreatic Society, Lancaster, November 1997
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Cytokine Gene Polymorphisms In Acute Pancreatitis
Kevin Sargen, Andrew G Demaine, Andrew N Kingsnorth

Investigation Of Inerleukin-10 Gene Promoter Polymorphism In Normal Controls
K Sargen, A G Demaine

Signed.....

Date.....

1. Introduction to the pancreas and acute pancreatitis

Human acute pancreatitis is an inflammatory disorder of the pancreas which causes significant morbidity and mortality. Although it was previously recognised, it was not until 1889 that the disease was fully characterised by Reginald Fitz, the Professor of Pathological Anatomy at Harvard University, who published a full clinical description of acute pancreatitis in it's various forms of severity. He also catalogued pathological changes, both gross and histological, of the severe cases which died and came to post mortem (Fitz, 1889).

Acute pancreatitis has a varied aetiology, gallstone disease and alcohol predominating amongst identifiable causes, with a significant proportion being of undetermined aetiology. The presence of gallstones or a history of heavy alcohol consumption does not mean that the development of acute pancreatitis is inevitable, which indicates that there must be other factors involved relating to the individual or aetiological agent.

The clinical course of the disease is often mild with only minimal associated organ dysfunction, but a significant proportion of patients develop severe pancreatitis which is associated with organ failure, systemic complications, local inflammatory and infective manifestations and, in up to 10% of cases, death.

1.1 The Normal Pancreas

1.1.1 Gross anatomy

The pancreas is a retroperitoneal organ situated at the level of the second lumbar vertebra. The gland is described as having a head, neck, body and tail (Bannister, 1995). The head, lying to the right of the midline, is closely related to the C loop of the duodenum formed by it's 1st, 2nd, and 3rd parts. The neck, body, and tail extend upwards to the level of the 12th thoracic vertebra, where it is anterior to the superior pole of the left kidney and is closely related to the spleen. The aorta and inferior vena cava lie posterior to the gland, as do their branches the splenic artery and vein, which extend along the line of the body and tail. The

transverse mesocolon arises from the anterior-inferior surface. Commonly the distal bile duct passes through the head of the pancreas before entering the 2nd part of the duodenum. The pancreas is formed in the embryo from two endodermal buds arising from the second part of the duodenum. The smaller ventral bud forms the inferior part of the head of the pancreas and the uncinate process, whilst the larger dorsal bud forms the rest of the head, the neck, body and tail of the gland.

This developmental process means that the pancreas has two ductal systems into which lobular ductules ultimately drain. The main pancreatic duct (of Wirsung) drains the ventral pancreas, and opens into the 2nd part of the duodenum at the ampulla of Vater, usually in conjunction with the common bile duct (the 'common channel') (Lankisch and Banks, 1998b). The duct draining the dorsal pancreas is named the accessory pancreatic duct (of Santorini). It communicates with the main pancreatic duct, but also opens into a separate minor duodenal papilla. In up to 10% of individuals the accessory duct does not fuse with the main pancreatic duct and may form the principal drainage of the gland; this is termed *pancreas divisum* (Rosai, 1998).

The blood supply of the pancreas is from multiple anastomoses formed from branches of the coeliac axis and superior mesenteric artery, whilst venous drainage is into the portal vein. Lymphatic drainage is extensive, draining to retroperitoneal and mesenteric nodes. Both divisions of the autonomic nervous system, parasympathetic and sympathetic, have branches supplying the gland.

1.1.2 Histology

Both endocrine and exocrine systems are situated in the pancreas.

The exocrine component consists of lobular units of cells called acini , formed from the cells which synthesise digestive enzymes, and cells that form the ductal system. Each acinus is formed from a single layer of acinar cells, arranged in a spherical fashion, the apex of which secrete enzymes into the pancreatic ductal system which originates from the centroacinar cells.

The acinar cell is a relatively large, pyramidal shaped cell with prominent microvillae on the luminal surface (Fig 1.1). It is a highly polarised cell, the apical portion containing many eosinophilic zymogen granules, the basal cytoplasm being highly basophilic due to the presence of abundant rough endoplasmic reticulum (RER) (Stevens and Lowe, 1992). RER and other organelles are present in high numbers as the pancreatic acinar cell synthesises, stores, and secretes enzymes at a higher rate than any other cell in the body (Lankisch and Banks, 1998b).

The endocrine component of the pancreas is represented by the islets of Langerhans, and a smaller number of endocrine cells associated with the ducts and acini.

The islets form 1-2% of the adult pancreas and consist of four main cell types:

i. β cells which secrete insulin and islet cell amyloid polypeptide (IAPP), a molecule co-secreted with insulin. β cells are the commonest cell type in the islet (60-70%)

ii. α cells which secrete glucagon.

(both glucagon and insulin are involved in intermediary metabolism)

iii. δ cells which secrete somatostatin, a peptide with a broad spectrum of gastrointestinal activity.

iv. PP cells are very few in number and secrete pancreatic polypeptide (PP) which reduces pancreatic secretion and biliary tract motility.

The endocrine cells not contained within islets are serotonin and PP producing.

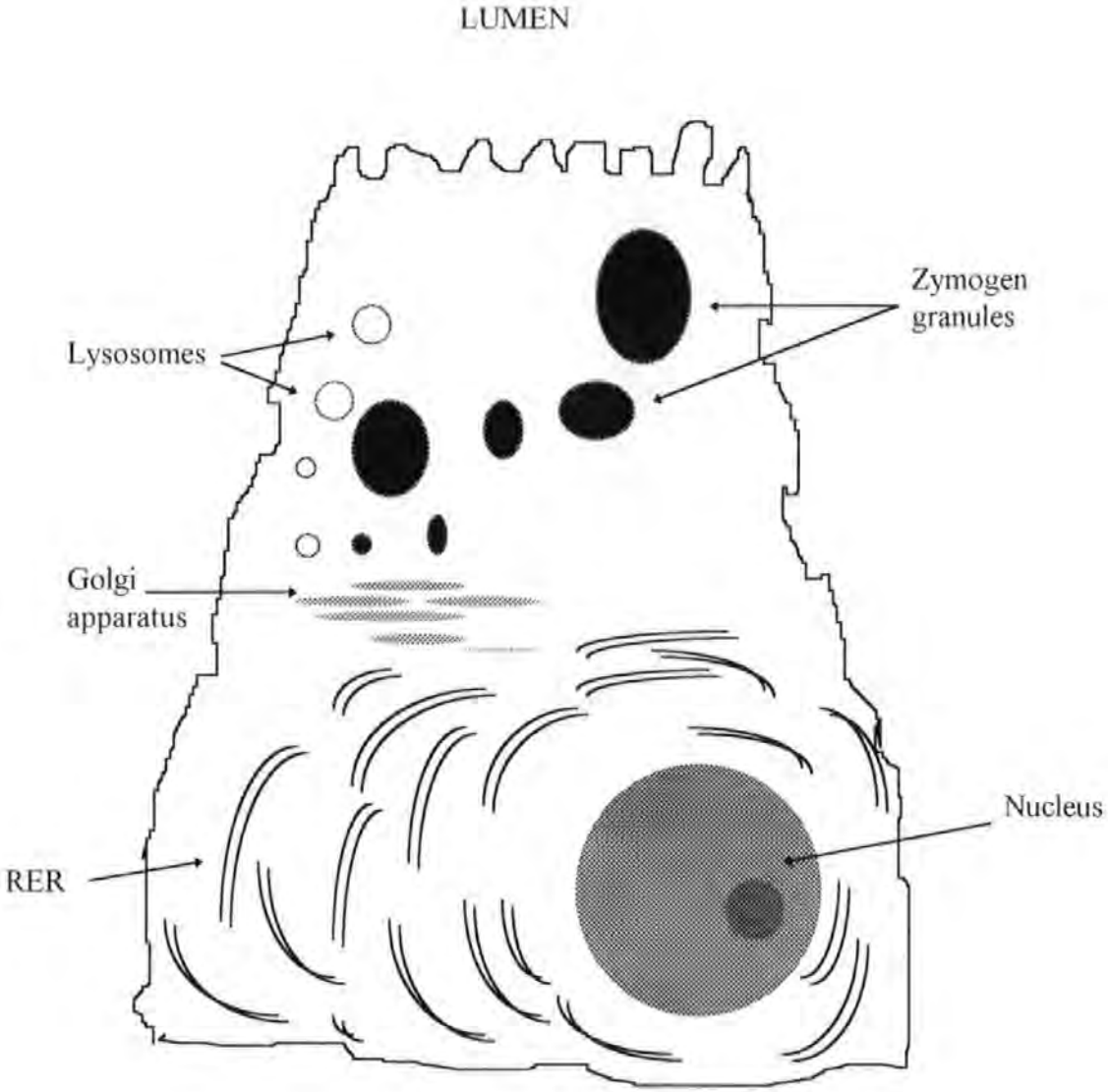

Figure 1.1 Representation of pancreatic acinar cell

The acinar cell is a relatively large, pyramidal shaped cell with prominent microvillae on the luminal surface (Fig 2). It is a highly polarised cell, the apical portion containing many eosinophilic zymogen granules, the basal cytoplasm being highly basophilic due to the presence of abundant rough endoplasmic reticulum (RER). Mitochondria (not shown) are also present in large numbers due to the high activity of the cell.
Proteins, synthesised in the RER, pass to the Golgi, where enzymes are processed and membrane bound into zymogen granules (pancreatic pro-enzymes) or lysosomes.
Lysosomal enzymes are mostly segregated from the digestive pro-enzymes into their separate 'compartments' when processing occurs at the Golgi apparatus, as they can activate trypsinogen, and hence activate the pancreatic 'enzyme cascade'.
In a process termed exocytosis, zymogen granules fuse with the apical cell membrane, and enzymes are released into the lumen.

1.1.3 Physiology

As discussed the pancreas consists of exocrine and endocrine components. The endocrine part has little relevance to the understanding of the pathophysiology of acute pancreatitis and will not be discussed further.

The exocrine portion of the gland produces a secretion which has two components: digestive enzymes secreted by acinar cells, and water, electrolytes and bicarbonate secreted by duct cells. The bicarbonate produces an alkaline pH (pH 8.0), optimum conditions for the function of digestive enzyme in the duodenum.

Secretion of water, electrolytes and bicarbonate anions is stimulated by both neural and humoral control. Neural control is via the vagal parasympathetic nerve and humoral control is principally via secretin, a peptide hormone. Secretin is produced by a sub-type of mucosal cell, the S cell, in the crypts of Lieberkühn of the proximal small intestine. It acts on the pancreatic ductal cells, via cAMP, to increase secretion. The stimulus for secretin production is the entry of acid into the duodenum.

Exocrine secretion from pancreatic acinar cells is under different control.

1.1.3.1 Acinar cell secretion

Enzymes produced by the acinar cell include proteases, lipases, nucleases, amylase, and trypsin inhibitors. Some enzymes are secreted as inactive proenzymes because they are capable of attacking cell membranes, and require activation by trypsin, which takes place in the duodenal lumen. This has been termed the pancreatic enzyme cascade (Rinderknecht, 1993) and is summarised in Figure 1.2. Trypsin is itself an enzyme produced from a pancreatic proenzyme, trypsinogen, by the action of enterokinase in the duodenum which prevents intrapancreatic activation. As well as the two endogenous protection mechanisms mentioned there exist a number of others to protect the pancreas from autodigestion. Some of these will also be mentioned in following paragraphs, but they are collectively listed here:

1. All digestive enzymes (not lipase/amylase) are synthesised in inactive, pro-enzyme forms.

2. The activating enzyme (enterokinase) is physically separate from the pancreas.
3. Digestive enzymes are compartmentalised within the acinar cell (see below).
4. Intracellular calcium concentrations are low, inhibiting trypsinogen activation.
5. The acinar cells secrete pancreatic secretory trypsin inhibitor (PSTI). This fits into a 'pocket' on the trypsin molecule and inactivates it.
6. Trypsin can autodigest itself if excessive activation occurs within the acinar cell (see section 3.3)

Amylase and lipase are not harmful to the healthy pancreas, so are secreted in active form.

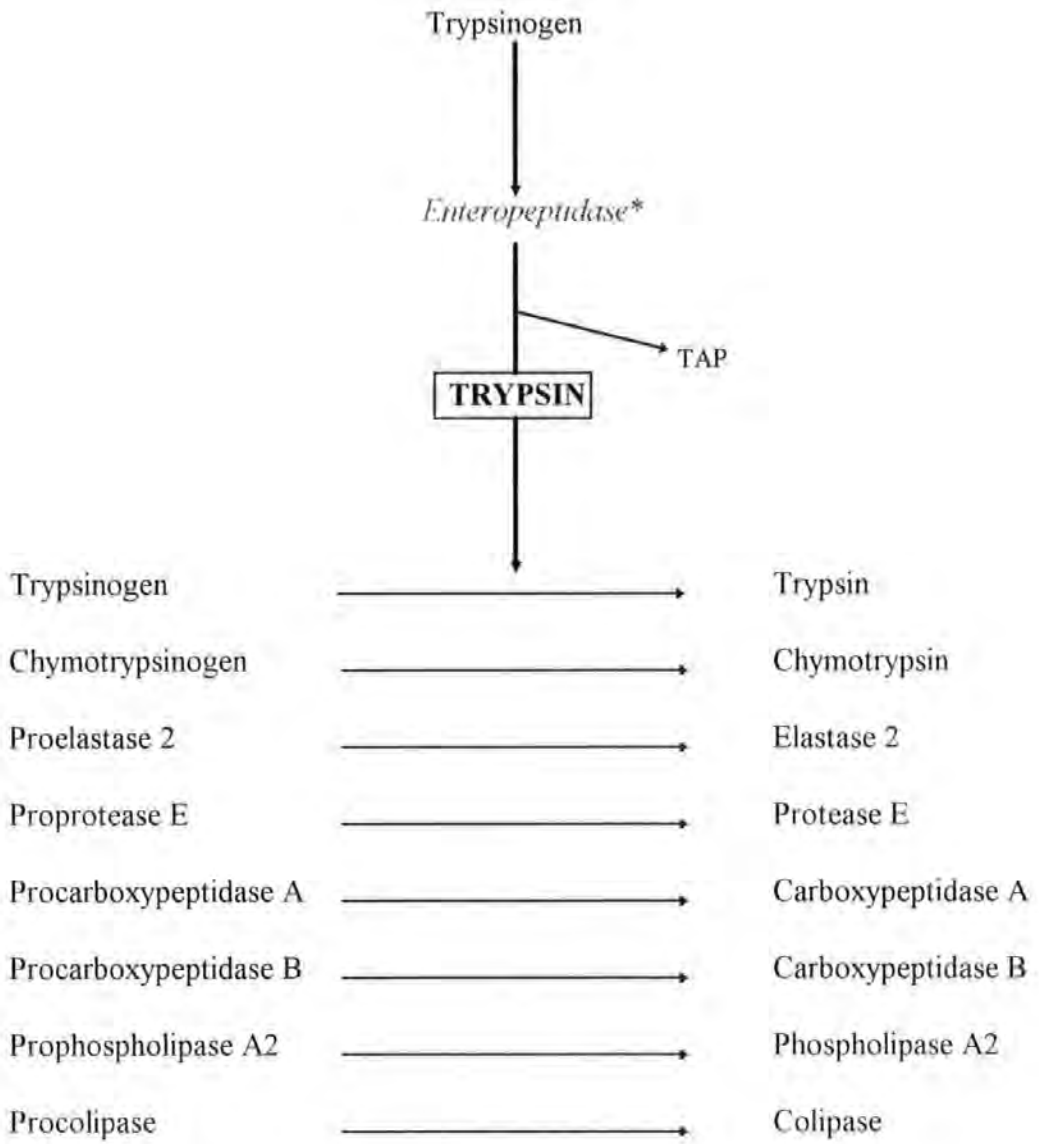

Figure 1.2 Pancreatic enzyme cascade

Most enzymes are secreted in pro-enzyme form., but amylase and lipase are not harmful to the healthy pancreas, so are secreted in active form. Trypsin activates all other pancreatic pro-enzymes to their active form.
* *Enterokinase* or trypsin. The process is normally initiated in the brush border by *enterokinase*. Trypsin can also activate trypsinogen by the cleavage of TAP.
TAP - Trypsinogen activation peptide

1.1.3.2 Enzyme synthesis and secretion

Pancreatic enzymes are synthesised by the pathway common to all eukaryotic cells. RNA polymerase catalyses the synthesis of a complementary strand of mRNA from the startpoint in a gene promoter to the terminator sequence. Regulatory proteins, which can be the gene product acting in an autogenous fashion, interact with the RNA polymerase to control this step of gene expression. Ribosomes on the RER then catalyse the translation of mRNA into proteins.

Newly synthesised proteins pass through membrane enclosed compartments to the Golgi apparatus (Palade, 1975). Proteins that are destined for export from the cell, such as digestive enzymes, are packaged within a glycoprotein membrane in the form of condensing vacuoles. These mature into zymogen granules as they migrate towards the apex of the cell for subsequent release. Lysosomal enzymes such as Cathepsin B, which is capable of activating trypsinogen produced by the acinar cell (Greenbaum and Hirschkowitz, 1961), are packaged in separate lysosomes during passage through the Golgi apparatus (Kornfield, 1986; Steer and Meldolosi, 1987) which is another mechanism to prevent intracellular activation of damaging digestive enzymes.

The migrated zymogen granules fuse with the acinar cell luminal membrane and contents are secreted into the lumen by exocytosis. This is a complex process mediated by protein kinase C and Ca^{2+} dependent protein kinase (Steer and Meldolosi, 1987).

1.1.3.3 Control of acinar cell secretion

Like exocrine ductal secretion, the secretion from pancreatic acinar cells is under several different influences. Neural control is from the parasympathetic vagal nerve, terminating in acetylcholine (ACh) receptors. Hormonal control is via a number of different pathways and the acinar cell has receptors for gastrin, cholecystokinin (CCK), secretin, and vasoactive intestinal polypeptide (VIP). CCK is the most potent stimulant of pancreatic enzymes. CCK is released by the cells of the duodenal mucosa in response to the entry of free fatty acids, amino acids, polypeptides, glucose, glyceride, and calcium into it's lumen (Adler et al.,

1995). CCK acts to stimulate secretion via binding to it's own receptor (Adler et al., 1991), and by an interaction with the cholinergic nervous system (Nishizuka, 1984; Soudah et al., 1992; Li and Owyang, 1994). Gastrin, a product of G cells in gastric mucosa is a weak stimulant of pancreatic exocrine secretion, as are secretin and VIP.

Both CCK and ACh stimulate the breakdown of a membrane phospholipid, phosphatidylinositol, into diacylglycerol which activates protein kinase C , and inositol triphosphate which mobilises calcium to activate Ca^{2+} dependent protein kinase (Streb et al., 1983). Both these kinases mediate exocytosis of digestive enzymes.

1.2 Pancreatitis

Inflammatory disease of the pancreas can occur in acute and chronic forms. This thesis is concerned with acute pancreatitis, but it will also be useful to discuss here the definition of chronic pancreatitis.

1.2.1 Clinical definition of acute pancreatitis

At a recent international symposium in Atlanta, Georgia, USA in 1992, an attempt was made by a group of 40 internationally recognised experts in acute pancreatic inflammatory disease to define and classify acute pancreatitis on a clinical basis (Bradley, 1993). The classification of the complications of acute pancreatitis is discussed in section 1.5.3.

Acute pancreatitis was defined by the Atlanta symposium as *an acute inflammatory disease of the pancreas, with variable involvement of other regional tissues or remote organ systems.* Two previous symposia in Marseille, France in 1984, (Singer et al., 1985) and Cambridge, UK in 1983 (Sarner and Cotton, 1984) both provided a similar clinical definition, and recognised that the disease can occur as a single episode or be recurrent.

1.2.2 Morphological definition of acute pancreatitis

The Marseille consensus group also provided a morphological definition, importantly recognising that there was a gradation of lesions seen in acute pancreatitis. Mild pancreatitis is associated with interstitial oedema within the gland, and a varying degree of

peripancreatic fat necrosis. More severe forms of the disease are associated with extensive peripancreatic and intrapancreatic fat necrosis, gland necrosis and haemorrhage. Lesions can be localised or diffuse (Singer et al., 1985).

Following acute pancreatitis, provided the aetiological agent and complications such as pseudocysts have resolved the pancreas recovers clinically, morphologically and functionally.

1.2.3 Clinical definition of chronic pancreatitis

The Marseille symposium of 1984 defined chronic pancreatitis as follows: *Clinically characterised by recurrent or persisting abdominal pain, though chronic pancreatitis may present without pain. Evidence of pancreatic insufficiency e.g.: diabetes or steatorrhoea may be present.* As with their definition of acute pancreatitis, a morphological description was given (Singer et al., 1985). The symposium in Cambridge, UK the previous year gives a more clinicopathological definition: *continuing inflammatory disease of the pancreas, characterised by irreversible morphological change and typically causing pain and/or permanent loss of function* (Sarner and Cotton, 1984).

1.2.4 Morphological definition of chronic pancreatitis

The gland becomes fibrotic, areas of which may subsequently calcify. The exocrine parenchyma is destroyed in a focal, segmental, or diffuse manner. Duct dilatation may be seen, associated with ductal strictures and/or calculi. Inflammatory cells of all different types are present within the substance of the gland, and there may be oedema and focal necrosis. Cysts and pseudocysts are often present (Singer et al., 1985).

1.3 Epidemiology

As the natural history of acute pancreatitis is short, incidence and prevalence rates do not differ. Wide variations in incidence rates are reported even within the UK, which may in part at least be due to methodological differences between studies. Investigators use differing diagnostic criteria and different methodology to identify cases. Also, figures

sometimes omit cases diagnosed at autopsy, which can account for 30-40% of fatal cases (Corfield et al., 1985)

1.3.1 Incidence

A study reporting cases occurring over a decade in the Bristol, UK area reported in 1985 that the annual case rate per million was 73.0, revealing a rise in incidence over the previous decade from 53.8 cases per million (Corfield et al., 1985). In comparison studies in Scotland, UK have reported mean annual incidence rates of 242 per million of the population (Thompson et al., 1987). Incidence rates in Scotland have also risen over the last twenty years (Wilson and Imrie, 1990).

Based on these figures, there are probably up to 10,000 cases annually in the UK.

1.3.2 Mortality rates

Mortality rates are also subject to wide inter-study variation. The highest case mortality rate of 20% has been reported in a study that included cases first diagnosed at autopsy. These accounted for 35% of total deaths from acute pancreatitis (Corfield et al., 1985). However a study also including cases diagnosed at autopsy gives an overall mortality rate of 8% (Mann et al., 1994). Studies from Scotland indicate that mortality rates have been falling over the past thirty years (Wilson and Imrie, 1990), which may reflect advances in critical care support and management of complications.

It is very difficult to draw comparison between these studies due to reasons outlined above. Consensus opinion regards overall mortality rates to be in the region of 8-12% (Kingsnorth, 1997).

1.4 Aetiology of acute pancreatitis

Although the list of aetiological factors of acute pancreatitis is long (Table 1.1), many are rare, and there remains a significant proportion of patients in whom aetiology is unable to be determined and whose disease is classified as idiopathic. Recent reviews reflect general consensus opinion that biliary and alcoholic acute pancreatitis account for the majority

(approximately 80%) of cases (Kingsnorth and Sargen, 1998; Mergener and Baillie, 1998).

1.4.1 Cholelithiasis

In the UK the reported incidence of gallstone pancreatitis has been variable. Studies report that biliary stone disease accounts for 30 to 50% of cases of acute pancreatitis (Thompson et al., 1987; Corfield et al., 1985; Mann et al., 1994; De Beaux et al., 1995). Within a region the incidence of disease attributable to gallstones appears to be stable as evidenced by two studies reporting on the same population over 30 years (Trapnell and Duncan, 1975; Corfield et al., 1985).

In the early part of this decade two groups of investigators have suggested that biliary stone disease accounts for a greater proportion of cases than earlier recognised. They both showed that the majority of cases previously labelled as idiopathic were in fact due to microlithiasis and 'biliary sludge' (Lee et al., 1992; Ros et al., 1991).

Although the number of patients with gallstones developing acute pancreatitis is small (Moreau et al., 1988), it is clear that even stones less than 5mm in diameter increase the risk of presenting with acute pancreatitis four fold (Diehl et al., 1997). Furthermore it is recognised that the risk of acute pancreatitis in patients with biliary stone disease is reduced to that of the normal population following removal of the gallbladder and it's stones (Moreau et al., 1988).

1.4.2 Alcohol

The reported incidence of alcohol induced disease is also highly variable, different studies estimate it accounts for between 11% and 38% of cases in the UK (Thompson et al., 1987; Corfield et al., 1985; De Beaux et al., 1995; Mann et al., 1994). This can be explained by two main factors. The first is that patterns of drinking within a local population vary, and these will influence the amount of cases of alcoholic pancreatitis. This is evidenced by studies from Finland, a country with a high per capita consumption of alcohol in which 70% of cases of acute pancreatitis are reported to be caused by alcohol (Puolakkainen et al., 1987). In contrast with other European countries ethanol consumption in Finland per

inhabitant has been increasing to between 6-7 litres per annum since the 1970's, correlating with the rise in incidence of acute pancreatitis (Jaakkola and Nordback, 1993).

Different epidemiological studies have no common defining criteria for when alcohol is considered the main aetiology . This may also explain variability in the reported incidence of alcoholic acute pancreatitis between studies. Only one published study has set an alcohol consumption level (consumption of $\geq$80 g day^{-1}) at which subjects were defined as having alcoholic acute pancreatitis (Wilson et al., 1985).

In 80% of alcoholics, neither pancreatic or liver disease develops, and in only 5% does pancreatitis (chronic or acute) develop (Meier, 1995). The reason for this is unclear, and various hypotheses have been suggested and tested. However, none of them provides an explanation, as alcoholics with pancreatic disease do not appear to have different drinking patterns or tobacco consumption compared with alcoholic controls, and conflicting data exists with regard to associated dietary factors (Haber et al., 1995). Genetic factors have also been studied, and these are discussed in section 4.3.

1.4.3 Drug induced pancreatitis

A recent retrospective study estimates that the incidence of drug induced pancreatitis is only 1.4%. It seems that this form of the disease usually runs a benign course (Lankisch et al., 1995). Agents known to cause acute pancreatitis include Thiazides, Azathioprine, Valproate, Oestrogens, Corticosteroids, Sulphonamides, and Tetracyclines. There are many other agents which are considered to possibly be a cause (McArthur, 1996).

1.4.3 Idiopathic pancreatitis

Idiopathic pancreatitis has been reported to account for between 14% and 23% of cases of acute pancreatitis (Mann et al., 1994; Thompson et al., 1987; De Beaux et al., 1995; Corfield et al., 1985). As discussed above, this figure may represent an overestimate as it can include unrecognised cases of both alcoholic and gallstone pancreatitis (Lee et al., 1992; Ros et al., 1991). It can be postulated that some cases of idiopathic pancreatitis are secondary to undiagnosed viral infections.

1.4.4 Infectious pancreatitis

Mumps virus, Coxsackie, Hepatitis B, Cytomegalovirus, and the Herpes simplex I & II viruses are some of the viruses believed to initiate pancreatitis (Parenti et al., 1996). Non-viral agents include Mycoplasma, Leptospira, Legionella, and the Ascaris worm. Some of these are probably very rare as only a few case reports exist in the literature. The actual incidence of viral and other forms of infectious acute pancreatitis is unknown. However, in the UK the Mumps virus, Coxsackie, Hepatitis B, Cytomegalovirus, and the Herpes simplex I & II viruses are very prevalent which increases the chance of cases of viral induced acute pancreatitis developing.

Aetiological factors in acute pancreatitis
Cholelithiasis (including microlithiasis)
Alcohol
Trauma (Blunt and penetrating)
ERCP
Obstruction (Ampullary stenosis, Duodenal diverticulum, neoplasm, parasites)
Hypercalcaemia
Hypertriglyceridaemia (Types I and V)
Infection (Cocksackie, Mumps, Mycoplasma)
Hypothermia
Ischaemia (Vasculitis, Hypotension)
Cardiopulmonary bypass
Drugs[1]
Miscellaneous (Scorpion bite, hereditary, pregnancy)

Table 1.1 Aetiological factors in acute pancreatitis

1 Thiazides, Azathioprine, Valproate, Oestrogens, Corticosteroids, Sulphonamides, Tetracyclines. Also many other possibles.
ERCP - Endoscopic retrograde cholangio-pancreatography

1.5 Clinical acute pancreatitis

The presenting features and natural history of human acute pancreatitis are well known and will be described in the following sections.

1.5.1 Presenting features

Patients usually present with an acute onset of upper abdominal pain (epigastric) which can be referred to the back in up to 50% of patients. Due to it's site and nature the pain can often be confused with that originating from a myocardial infarction. Other symptoms include anorexia, nausea, and vomiting. Some patients, especially the very ill and elderly, present in a confused state due to systemic hypoxia.

Clinical signs associated with acute pancreatitis include epigastric tenderness, abdominal distension, small bowel ileus, dehydration, and evidence of hypovolaemia . Jaundice can be apparent in a case secondary to gallstone disease, and in a severe case subcutaneous extraperitoneal tracking of inflammatory peripancreatic exudate is manifested as abdominal wall staining (Cullen's sign and Grey Turner's sign).

1.5.2 Natural history

It is a consistent finding in clinical series that the majority of patients with acute pancreatitis have mild disease, associated with minimal systemic upset or absence of local complications (Bradley, 1993; De Beaux et al., 1995). These patients can be managed by supportive care and careful monitoring for the development of complications (Banks, 1997), and require a relatively short hospital stay.

However, this still leaves a significant proportion of patients who develop complicated disease, evident by the development of distant organ failure or local pancreatic pathology. Exact figures for the proportion of patients developing complications are difficult to derive from many studies, as widely different classification systems are used and figures quoted are often anecdotal. Furthermore some studies rely upon predicted severity scores which do not always correlate with outcome. The wide variability of the figures quoted illustrates these problems.

1.5.2.1 Organ failure

The development of organ dysfunction usually occurs in the first two weeks of disease (Karimgani et al., 1992; Kingsnorth, 1997). Prospective series with comparable but not the same defining criteria of organ failure report that organ failure occurs in between 7 % and 27% of patients (De Beaux et al., 1996; Kingsnorth et al., 1995; Larvin and McMahon, 1989).

The respiratory system is most commonly affected (Kingsnorth et al., 1995) and minor respiratory insufficiency is often seen. In one selective series as many as 22% of cases had respiratory insufficiency of some degree (Talvik et al., 1977). Respiratory failure is a significant cause of mortality, 60% of patients who die within the first week of disease die from respiratory failure (Guice et al., 1988).

The development of respiratory failure can present as tachypnoea and confusion, and reduced arterial oxygen saturation is evident upon monitoring. In it's severe form respiratory failure develops into a clinical scenario labelled the Adult Respiratory Distress Syndrome (ARDS), in which refractory hypoxia occurs.

Renal insufficiency can also develop, evidenced by the development of oliguria and rising serum creatinine. The incidence of renal dysfunction in one series, as defined by a serum creatinine of >200μg/dl, was reported to be 8.5%, and 80% of these required dialysis (Lankisch and Banks, 1998e).

Cardiovascular insufficiency is another serious complication of acute pancreatitis, and manifests itself as refractory hypotension. This can be due to failure of the vasculature or myocardium, or both. Data on it's frequency suggests it occurs in up to 6.4% of patients (Satiani and Stone, 1979).

Metabolic complications requiring intervention are a rare but serious event in acute pancreatitis. Hypocalcaemia is said to be common in acute pancreatitis (McFadden, 1991), and may result from the hypoalbuminaemia of disease or from fat saponification in the peritoneum. The requirement of treatment for hypocalcaemia is uncommon.

Hyperglycaemia is a poor prognostic factor in acute pancreatitis (Ranson et al., 1974), and is reported to be associated with high levels of glucagon and cortisol, and a corresponding deficiency of insulin (McFadden, 1991).

Respiratory, renal, and cardiovascular failure can occur on their own or together, resulting in multisytem organ failure (MSOF). The development of MSOF also puts the patient at risk of hepatic failure and coagulopathy. MSOF or any of it's components can result from acute pancreatitis without sepsis or necrosis, but is also common when sterile necrosis or septic complications are present (Banks et al., 1996).

1.5.2.2 Local complications

Local complications are those that involve the pancreas or peripancreatic area. They include acutefluid collections, pancreatic necrosis, acute pseudocyst, and pancreatic abscess. Pancreaticocutaneous fistulae can occur also, usually after surgical intervention. Definitions are provided in the following section (Section 1.5.3).

Local pancreatic complications have been reported to occur in 19% of patients in a consecutive series (De Beaux et al., 1995).

Acute fluid collections and pseudocysts are often asymptomatic, but are often found on imaging investigations (Bradley, 1993). A pseudocyst, by definition, is only classified as such if present four weeks from the onset of disease, and has a reported incidence of 7% (De Beaux et al., 1995).

Pancreatic necrosis, occurring in 7% of cases (De Beaux et al., 1995), is often suspected in a patient with severe disease, or in one whose disease runs a protracted course. It can be relatively asymptomatic or fulminant. It is detected using contrast imaging techniques. There is disagreement as to whether pancreatic necrosis, whether infected or sterile, is predictive of mortality (Banks et al., 1996; De Beaux et al., 1995).

Pancreatic abscess has a reported incidence of 5% (De Beaux et al., 1995). It takes at least 4 weeks to form, and is associated with pain and systemic upset.

1.5.3 Clinical classification of acute pancreatitis

As previously mentioned, differing criteria employed by investigators has led to difficulty in comparing studies performed in clinical acute pancreatitis. Realisation of this led to the development of a clinically based classification system for acute pancreatitis, which was agreed by a consensus conference in Atlanta, Georgia, USA in 1992 (Bradley, 1993).

1.5.3.1 Mild acute pancreatitis

Defined as being associated with minimal organ dysfunction and an uneventful recovery, lacking the described features of severe acute pancreatitis.

1.5.3.2 Severe acute pancreatitis

Severe acute pancreatitis is defined as being associated with organ failure and/or local complications. The definitions are listed in Table 1.2.

CATEGORY	DEFINING CRITERIA
Mild acute pancreatitis	**Minimal organ dysfunction**
Severe acute pancreatitis:	**Organ failure and / or local complications**
Predicted severe disease	Apache II Score ≥ 8
Organ failure:	
i. Cardiovascular insufficiency	Systolic blood pressure < 90 mmHg
ii. Respiratory insufficiency	PaO_2 < 60 mmHg (8 KPa)
iii. Renal failure	Serum Creatinine > 177μmol l^{-1} (after rehydration)
iv. Gastrointestinal bleeding	> 500ml of blood loss in 24 hours
v. DIC	Platelets ≤ 100,000 per mm^3, Fibrinogen < 1g l^{-1}
vi. Metabolic failure	Serum calcium ≤ 1.87 mmol l^{-1}
Local complications	
i. Acute fluid collection	Located in or near the pancreas, lack a wall of granulation or fibrous tissue
ii. Pseudocyst	Collection of pancreatic secretion bounded by fibrous or granulation tissue wall, presenting 4 weeks after onset of disease
iii. Abscess	Circumscribed collection of pus
iv. Necrosis	Diffuse or focal nonviable pancreatic parenchyma

Table 1.2 Atlanta Convention Classification of acute pancreatitis[1]

Apache II	Acute Physiology and Chronic Health Evaluation II (Larvin and McMahon, 1989)
DIC	Disseminated Intravascular Coagulation (Coagulopathy)
mmHg	millimetres of mercury
Kpa	Kilopascals

[1] (Bradley, 1993)

1.6 Management of clinical acute pancreatitis

Management of acute pancreatitis is mostly supportive in nature, with initial effort directed towards restoring intravascular volume, ensuring adequate oxygenation, and relieving pain. Patients should be observed closely, to look for signs of developing organ failure and sepsis.

Investigations should monitor pulmonary, hepatic and renal function, and aim to identify the aetiological agent.

In severe cases intensive therapy may be required, including artificial ventilation, renal replacement therapy, and ionotropic support. The development of infected pancreatic necrosis warrants surgical debridement and drainage, whilst relatively asymptomatic cysts and sterile necrosis can be treated expectantly. Pseudocysts that do ultimately need drainage can often be dealt with by percutaneous techniques, not requiring open surgery.

2. Overview of the immune system

Acute pancreatitis is a disorder that is characterised by inflammation and activation of various components of the immune system. The structure and mechanisms of the immune system, and the inflammatory response that it generates in conditions including acute pancreatitis, merit consideration to enable understanding of the pathophysiology of the disease.

The immune system has two (arbitrary) functional components to deal with the presence of antigens (antigen; any substance that binds specifically to an antibody or T cell receptor) within the body, specific (acquired) immunity and non-specific (innate) immunity. Antigens can be exogenous, such as pathogenic organisms, or endogenous. Endogenous antigens include damaged cells (e.g. acinar cell in acute pancreatitis) and components of the body no longer recognised by the immune system as 'self'.

2.1 Non-specific (Innate) immunity

This is the initial non-specific response to tissue injury produced by the body in response to mechanical, chemical or biological stimuli. Of necessity it is an early response and is capable of rapid amplification.

Innate immunity has humoral and cellular components. The recruitment and activation of the cellular component occurs in tandem with the amplification of complement, kinin, coagulation and fibrinolytic cascades, a process known as the acute phase response.

The principal cellular effectors are the endothelial cell, neutrophil, and monocyte/macrophage. Regulation of this inflammatory response involves many physiological messengers. Cytokines are key mediators of the process, but others include adhesion molecules, prostaglandins, kinins, nitric oxide, oxygen free radicals, and proteases. Various mediators are produced by the effector cells of inflammation or as a direct consequence of cellular injury.

2.2 Specific (Acquired) Immunity

Specific immunity provides a mechanism for the body to selectively detect and eliminate foreign antigens. It exhibits specificity, recognising antigens that may differ by only one amino acid. This specificity means it has to be very diverse, recognising and responding to a multitude of different antigens. It also has a specific immunological memory, ensuring subsequent encounters with antigen are responded to quickly. As the immune system is so efficient at dealing with antigens it needs to be able to distinguish self from non-self, and so avoid the development of autoimmunity.

The acquired immune system has humoral and cellular components. Responses described as humoral involve the secretion of antibody by B-lymphocytes and the involvement of various soluble mediators in immune response and regulation.

The cellular component involves various types of T lymphocytes which recognise expressed antigen in association with MHC molecules, and carry out cytotoxic killing of such cells to destroy associated antigen. T cells also secrete cytokines which mediate inflammatory cell activation, differentiation, and therefore function.

Although they have been described separately, the innate and acquired immune systems do not function independently. Many of their cellular and soluble components have functions in both systems. Effector cells of the immune system, their cell membrane molecules, and the mediators which affect them are now described.

2.3 Cellular effectors of the immune system

These include the granulocytes (neutrophil, eosinophil, and basophil), the mononuclear cells (monocyte/macrophage), and the lymphocytes (B cells, T cells, and NK cells). Dendritic cells process and present antigens to subsets of T cells. Although not from the same cell lineage, the endothelial cells of the vasculature can be included in the category of immune system cellular effectors.

2.3.1 Neutrophils

The neutrophil is the characteristic cell of acute inflammation, inwardly migrating from blood to tissue in response to localised stimuli. It is a nucleated cell with phagocytic activity, and has numerous cytoplasmic granules containing substances including peroxidases, lysozyme, other hydrolytic enzymes, and lysozyme. Release of lysozyme and other reactive intermediates, such as oxygen free radicals (OFR's), nitric oxide, proteases, elastases, and collagenases, contribute to tissue damage and aid in the destruction of pathogens.

Neutrophils exhibit chemotaxis, and express high levels of receptors for chemokines, a large family of proteins which act as chemoattractants (Premack and Schall, 1996). Chemokines are expressed by a variety of leucocytes, and cells affected by inflammatory processes (Gerard et al., 1997; Grady et al., 1997). Some cytokines, components of the complement and blood clotting systems also act as chemoattractants. To get to areas of inflammatory activity neutrophils have to extravasate through the vascular endothelium.

2.3.2 Endothelial cells and adhesion molecules

The endothelial cell's immunological function is to facilitate the migration of inflammatory cells and proteins to sites of inflammation. This implies increased permeability to plasma molecules, and a mechanism for leucocyte extravasation. Leucocytes interact with the endothelial cell by sequential activation of families of adhesion molecules expressed on the surface of both types of cell (Panes and Granger, 1998). These include the selectins (L-, P-, and E-selectins), integrins (CD11, CD18, etc.), and the Ig supergene family (ICAM-1, VCAM-1, etc.). Expression of adhesion molecules is induced by cytokines such as TNFα and IL-1, transcription factors (NFκB and AP-1) (Ledebur and Parks, 1995), and lipopolysacharride (Fries et al., 1993). Initially weak adhesive interaction involving selectins and integrins causes leucocyte rolling, margination, and pavementing along the endothelial cell's luminal surface. Adhesion molecules from the Ig family, and others such as the

integrins mediate transmigration of cellular components through the endothelial barrier, most probably by them squeezing between intercellular junctions (Bianchi et al., 1997). Adhesion molecules and their ligands not only mediate neutrophil migration, but also monocytes, lymphocytes, eosinophils and platelets.

2.3.3 Mast cells

Mast cells are located near blood vessels in all connective tissue and are cells with dense collections of cytoplasmic granules which release substances such as histamine, prostaglandins, thromboxanes, and leukotrienes. These substances have been characterised as being vasoactive, chemotactic for other inflammatory cells, or having the ability to promote platelet aggregation and increased vessel permeability. IgE mediated release occurs during atopic responses, but degranulation of such cells is also caused by components of the complement system.

Mast cells also secrete a variety of pro-inflammatory cytokines, which serves to promote the inflammatory response to injury or infection.

2.3.4 Monocyte/Macrophage

Macrophages mature from circulating monocytes. Maturation involves an increase in size, number of organelles, secretory capability and phagocytic activity. They can be resident in tissues or migratory. They are able to secrete lysozyme and other inflammatory intermediates like superoxide radical and nitric oxide. Macrophages have the ability to secrete a wide variety of pro-inflammatory cytokines, and as such is the pivotal cell in eliciting and amplifying the early stages of inflammation, also called the acute phase response (Baumann and Gauldie, 1994). Cytokines produced by the macrophage include IL-1, IL-6, IL-8, IL-10, IL-12, and TNFα, all of which have effects upon components of the innate and adaptive immune systems (see Table 2.1).

Macrophages are also able to present antigens to naïve T cells and exhibit class I and II MHC expression . To avoid the development of autoimmunity this only occurs in the presence of inflammation or infection, and not when the macrophage is fulfilling it's normal

function of the phagocytosis of dead body cells.

2.3.5 Lymphocytes

Lymphocyte sub-populations consist of B cells, T cells, and null cells. They all have different cell surface/membrane components which reflect their different functions. Development of these different lineages from the common lymphoid progenitor cell require the presence of constitutively expressed transcription factors (Georgopoulos, 1997).

2.3.5.1 B cells

B cells mature in the bone marrow and have membrane bound immunoglobulin on their cell surface. B cells also express MHC class II molecules, so can act as an antigen presenting cell (APC).

Immunoglobulins act as receptors for specific antigens, and appropriate interaction between the two induces clonal proliferation of the antigen specific B cell. This produces plasma cells and memory B cells. Plasma cells have an innate capability to produce free (soluble) immunoglobulin, termed antibody.

It is the formation of ligand bound immunoglobulin and related plasma antibody that forms the foundation of the so called humoral immune response.

2.3.5.2 Immunoglobulins

Immunoglobulins are specific to antigens and as such are a very diverse family of molecules. The number of different antibody specificities able to be generated is estimated to be at least 10^8, far outnumbering the estimated number of potential antigens, so enabling the generation of a primary response to antigens not previously encountered.

Immunoglobulins have a structure which consists of heavy and light polypeptide chains, each type having variable and constant regions (Fig 2.1). The multi-gene families encoding for the polypeptide chains are located on different chromosomes and consist of different gene segments. These are termed V (variable), D (diversity), J (joining), and C (constant). The light chain families have V, D, and C gene segments, the VJ segments encode the variable region of the light chains. The heavy chain family contains V, D, J, and C segments,

the VDJ segments encode the variable region of the heavy chains. Random mutational and recombinational events involving these gene segments encoding the variable regions that occur during the development of the immune system result in the generation of diverse families of polypeptides. Constitutive expression of various proteins encoded for by genes such as RAG-1 and RAG-2 (recombination-activation-genes) are required for this immunoglobulin gene rearrangement (Oettinger et al., 1990). Further diversification of the immunoglobulin molecule is generated by a high rate of somatic mutation within the immunoglobulin V-region genes that occurs within the B cell (Kim et al., 1981).

Class (isotype) switching can also occur in activated B cells. Recombination between switch regions of C-region genes results in deletion of intervening coding DNA, allowing expression of different C-region isotypes with the same V-region (and hence same antigenic specificity) (Cory et al., 1980).

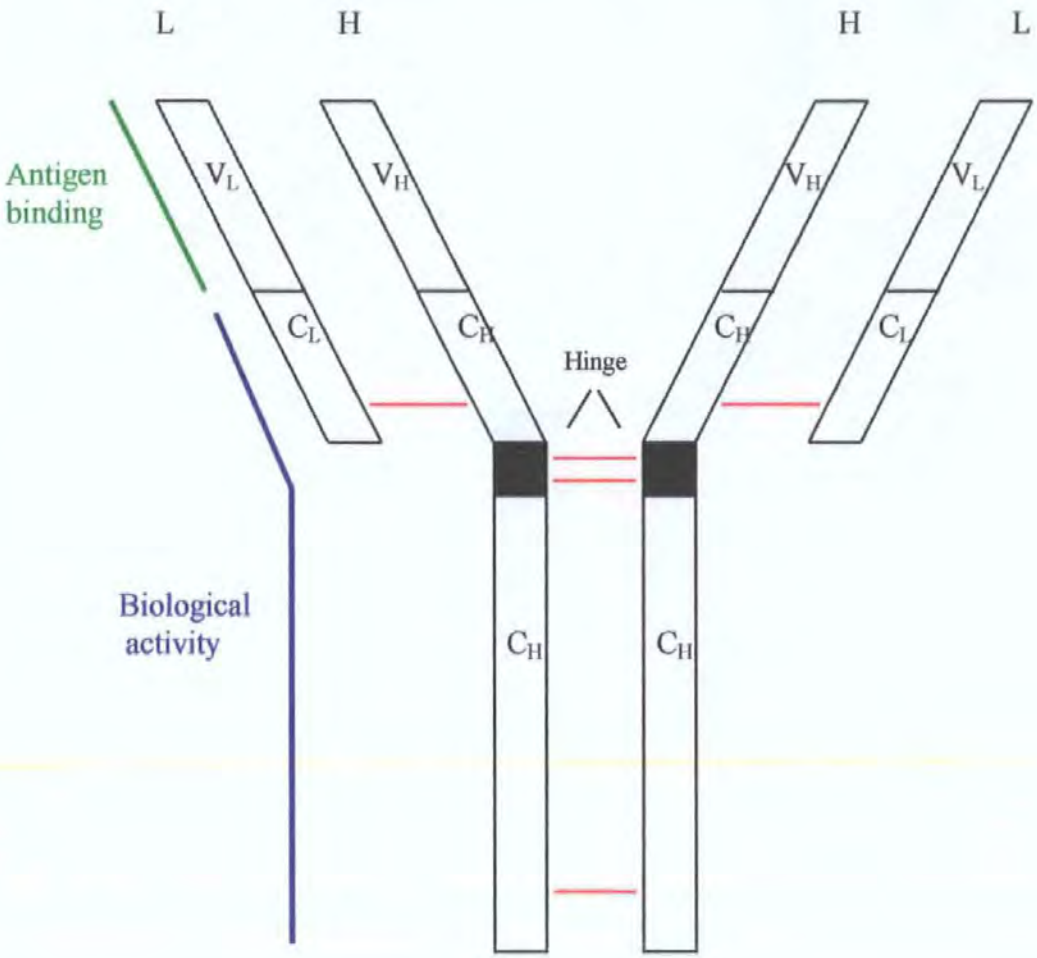

Fig. 2.1 Structure of Immunoglobulin (Ig)

Each heavy (H) and light (L) chain in an immunoglobulin molecule has a variable (V) region, which differs between individual Ig molecules, and a constant (C) region that has limited variability. In some Ig molecules there is also a hinge region. Disulphide bonds link the chains. The chains are in fact folded into domains, held with a disulphide bridge (not shown). The regions involved in antigen binding and in the mediation of biological activity are shown. Biological activity includes binding with cell surface receptors and activation of complement. This region (the F_C fragment), is also the region where joining (J) chains bond to form the polymeric forms of IgA (dimeric) and IgM (pentameric).

—— Disulphide bond

2.3.5.3 T cells

T cells develop into two major subsets, expressing different cell surface markers, cluster of differentiation (CD)4 and CD8. The thymus is required for efficient T cell development and regeneration, and although extrathymic sites of differentiation (e.g. bone marrow) have been described in the adult (Dejbakhsh-Jones et al., 1995), these are not as efficient.

Cells expressing CD4 recognise peptide presented with class II MHC molecules, whilst those with CD8 recognise peptide associated with class I MHC molecules. The expression of CD4 and CD8 also allows differentiation of T lymphocytes into their two major functional subsets. CD4+ cells function as T helper (T_H) cells and CD8+ cells function as cytotoxic T cells (T_C).

Following recognition of an peptide associated with a class II MHC molecule T_H cells exhibit clonal proliferation, specific to the same antigen/MHC class II complex. The T_H clones function as facilitators of the immune response by activating B cells, T_C cells, and other effector cells of the immune response by the secretion of a variety of cytokines. The cytokine profile that is secreted by the T_H cells divides them into two populations (T_H1 and T_H2) producing different effects upon the immune system (Mosmann et al., 1986).

The T_H1 response consists of a cytokine profile including IL-2 and IFN-γ, which results in the activation of a cell mediated immune response involving T_C cells and macrophages. In contrast the T_H2 response (cytokine profile includes IL-4 and IL-5) activates a B cell response. However, this functional division cannot be absolute, as cytokines such as TNFα, GM-CSF, and IL-3 are produced by both populations, and both T_H1 and T_H2 responses are required to deal with many antigens (Allen and Maizels, 1997).

T_C cells (CD8+) interact with an peptide/class I MHC molecule complex, and produce IL-2 by stimulation of CD28, a molecule expressed on T cells (Fraser et al., 1991), which promotes activation into it's effector form, the cytotoxic T lymphocyte (CTL). The CTL, which acts to eliminate altered 'self' cells, does so by activation of soluble (e.g. perforins)

and cell membrane effectors (e.g. Fas ligand), to induce lysis or apoptosis. Because CTL's are CD8+, and therefore class I MHC dependent (which is expressed on all nucleated cells), they can recognise and mediate the destruction of any altered body cell.

2.3.5.4 T cell receptor

The T cell receptor (TCR) family recognises peptide held within the groove of MHC molecules (Allison et al., 1982). This results in the initiation of signal transduction events and cellular activation. As the T cell receptor is specific for both the peptide and an MHC molecule, there are significant structural differences in the TCR between different clones of T cells. Therefore, like the immunoglobulin family, the TCR has an enormously wide range of specificity for different peptides, and is probably the major determinant of response to antigen.

The TCR has a similar structure to immunoglobulin (Fig 2.1). α and β chains (or γ and δ chains) are linked by disulphide bonds. The genes encoding the α and β chains, expressed only in T cells (Hendrick, 1994), have a similar arrangement to the immunoglobulin genes, and undergo similar rearrangement during T cell maturation to form a massively diverse family of antigen specific TCR's.

2.3.5.5 Null cells

These are cells that do not express the T and B cell membrane markers previously described. Natural Killer (NK) cells belong to this group.

NK cells have an F_C receptor for bound antibody, and can also be activated by antibody independent means, via certain cytokines (e.g. IL-2, IL-12, and IL-15).

NK cells play an important role in immune surveillance for altered cells either not exhibiting constitutive expression of class I MHC molecules or expressing altered class I molecules. They have receptors on their surface, killer cell inhibitory receptors (KIR's), which bind to polymorphic class I MHC molecules (Colonna and Samaridis, 1995). If the MHC class I molecule acts as a KIR ligand and is so recognised as self, NK cell function is suppressed.

As with the TCR, KIR and related molecules are diverse, as evidenced by polygenicity and polymorphism of the loci encoding them (Uhrberg et al., 1997).

2.3.5.6 MHC Molecules (Histocompatibility antigens)

There are three classes of major histocompatibility complex (MHC) molecule, class I, II, and III, which are also termed histocompatibility antigens. All three classes of molecule are encoded for by three corresponding regions in the major histocompatibility complex (MHC), on chromosome 6. This is discussed in Chapter 4.

The class III molecules include a group of proteins involved in immune functions, but not in the presentation of antigen or recognition of self. Class III molecules include components of the complement system and members of the tumour necrosis factor family, which will be discussed in section 2.4.

Class I and II molecules are cell surface glycoproteins. They were first characterised as the molecules involved in recognition of self and discrimination from non-self. Subsequently it was found that they play a key role in the generation of an immune response by binding with antigenic peptide to create a ligand for the T-cell receptor.

Class I MHC molecules are expressed on all nucleated cells except neurones and mature trophoblast. Class II molecules, in contrast, are distributed only on B cells, activated T lymphocytes, and APC's, including macrophages and dendritic cells.

Class I molecules bound with peptide are recognised primarily by T_C (CD8+) cells, whilst class II molecules bound with peptide are recognised by the T_H (CD4+) subset. This separation of distribution and function between the two classes is also reflected in their manner of antigen presentation. Class I molecules generally bind and present antigenic peptide (originally endogenous) produced in response to cell injury or infection, or bind cytosolic pathogens (e.g. Virus components). Binding of peptide with MHC class 1 molecule occurs in the ER of the cell. Specific peptide transporters, named TAP 1 and TAP 2 (for transporter associated with antigen presentation) span the ER membrane (Colonna and Samaridis, 1995), and transport peptides to the pre-Golgi region of the cell, where they

combine with the class I molecule, prior to migration to the cell surface.

Class II molecules, in comparison, present peptides which come from exogenous proteins (e.g. bacterial pathogen) which have been internalised and processed within the antigen presenting cell.

There are a limited number of different MHC molecules within each class (e.g. class II molecules identified are the HLA-DR, DQ, DP and DNA/DOB complex). MHC molecules are highly polymorphic and therefore diverse. But this diversity is different from that seen in Ig and TCR's. An individual does not express a broad diversity of MHC molecules, rather the diversity occurs within a species, between individuals. This means class I and II MHC molecules have broad antigenic specificity, each molecule being able to bind and present many different antigens. This is due to the structure of both classes of MHC molecule, resulting in binding specificities to the parts of antigens which are commonly found between diverse antigen species. The structure of class I and II molecules is reviewed elsewhere (Janeway and Travers, 1996).

The diversity of MHC molecules occurring within a species is the basis of discrimination of self and non-self, presenting major obstacles to allogeneic tissue transplants. The reason for this diversity is due to the high polymorphism of the genes encoding for MHC molecules. The MHC is located on chromosome 6 and will be discussed in section 4.1.

2.4 (Soluble and membrane bound) mediators and effectors of immune responses

Various soluble mediators act in conjunction with the cellular immune response. Indeed, some act as important cell to cell signalling molecules. The liver produces proteins which help in the generation of immune responses, the acute phase proteins. Mediators present in normal plasma include the kinins and the complement, clotting and fibrinolytic systems. Histamine, nitric oxide, oxygen free radical species, and the large family of cytokines are secreted or produced by cellular components of the immune system.

2.4.1 Acute phase proteins (APP's)

Pro-inflammatory cytokines such as IL-1, IL-6, and TNF stimulate production of acute phase proteins by hepatocytes (Baumann and Gauldie, 1994). Acute phase proteins include the complement component, C3, C-reactive protein (CRP), and mannose-binding protein (MBP). CRP and MBP act as opsonins and with C3 can activate the complement cascade.

2.4.2 Plasma mediators

Four enzymatic mediator systems are present in the normal plasma. These are the kinin, fibrinolytic, clotting, and complement systems. They all exist as inactive precursors in the plasma and are activated following tissue injury, and, in the case of complement, by interaction with antigen-antibody complexes.

Activation of the kinin system results in the formation of bradykinin which is a potent agent that causes vasodilatation and increased vascular permeability. A precursor of bradykinin, kallikrein, can also activate complement.

Activation of the clotting system and subsequent breakdown of fibrin by the fibrinolytic system results in the formation of peptides chemotactic for neutrophils.

The complement system is now discussed in more detail.

2.4.2.1 Complement activation

Complement is a system of plasma proteins with an important function in acquired and innate immune systems. Activation of complement is via classical (antigen antibody complex) and alternative (pathogen surfaces) pathways. Recent identification of serine proteases which bind to pathogen surface mannose in association with lectin (Matsushita et al., 1998) has helped characterise a third pathway of complement activation, the lectin pathway, which may be an intermediate between the classical and alternative pathways . In innate immunity activation is via the alternative, or related lectin pathway.

Activation of the complement system generates C3 convertases which activate C3 to C3b and C3a. C3b clusters on the surface of foreign surfaces function as an opsonin, and activate other components of the complement system including C5. C5 is split into C5a and

C5b which with C3a, activate mast cells and basophils, leading to the recruitment of additional inflammatory cells and proteins (Kanbe et al., 1996). A reaction sequence is also initiated which leads to the formation of a membrane attack complex (MAC) by complement components, which disrupts cell surface membranes, leading to cell death.

In acquired immunity, activation of the complement system is by the classical pathway. The first component of this pathway, C1, is composed of three subunits which combine to bind to the antibody F_c receptor. This leads to the generation of a C3 convertase. The primary component of the C1 complex, C1q, has been found to be synthesised by dendritic cells (antigen presenting cells) and monocytes within the lymphoid compartment (Schwaeble et al., 1995). Activation products of C3, bound to antigen, are known to enhance phagocytosis and antigen presentation, and it has recently been shown that they affect antibody production by B cells (Dempsey et al., 1996).

2.4.3 Nitric oxide

Nitric oxide (NO) is synthesised from the amino acid, L-arginine, by a family of enzymes named nitric oxide synthase (NOS). This was first found in endothelial cells (Palmer et al., 1988), but is known to occur in other cell types including macrophages (Hibbs et al., 1988). The actions of NO in inflammatory processes are complex (Schmidt and Walter, 1994), reflecting it's effects on different tissues.

NO can produce oxidant mediated tissue injury (Radi et al., 1991), which is probably the effect macrophages and other phagocytic cells produce by it's synthesis. NO also mediates vascular smooth muscle contraction (Palmer et al., 1988), and whilst it inhibits leucocyte-vascular endothelium adhesion in vitro (Kubes et al., 1991), it has been postulated that it may promote leucocyte extravasation by it's effects upon microvascular dynamics and permeability (Mantovani et al., 1997).

2.4.4 Oxygen free radical species

The potential for cellular oxidative stress is from three, highly reactive, oxygen free radicals (OFR's), superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl free radical

(OH•), which are produced as products of normal cell metabolism. Activated neutrophil polymorphs and macrophages are a potent source of OFR's, produced during a metabolic process known as the 'respiratory burst'. Cellular protection against oxidative stress is from endogenous enzymatic pathways involving superoxide dismutase, catalase, and glutathione peroxidase, and endogenous free radical scavengers such as Vitamins E and C, and β-carotene (Sweiry and Mann, 1996). Failure of these detoxification mechanisms or excess production of free radical species results in a disturbance in cell haemostasis, including lipid peroxidation. This leads to increased membrane permeability, which ultimately causes cell death (Slater, 1984). As well as these direct effects upon tissue, free radicals induce the generation of chemotactic factors which leads to accumulation of polymorphonuclear leucocytes in the damaged tissue (Petrone et al., 1980). Increased arachidonic acid metabolism is also stimulated, resulting in enhanced local production of other inflammatory mediators such as prostaglandins.

2.4.5 Cytokines

The term cytokine is used to describe low molecular weight peptides and lipids released from immune cells and other cells which function as mediators of inflammation, regeneration and remodelling. In the immune response cytokines control the intensity and duration of both specific and acquired components by involvement in immune cell activation, development, proliferation and differentiation. They can act by autocrine, paracrine and endocrine means. Cytokines have specific receptors, and binding of their ligand results in signal transduction events that alter target cell gene expression, and therefore function.

Many cytokines are named Interleukins (IL), but others include the tumour necrosis factor (TNF) family, and the interferons (IFN). Classification is an arbitrary system, and cannot be done on the basis of structural similarity, as cytokines have few sequence similarities (Cohen and Cohen, 1996). Many cells secrete cytokines, but the two which are the main producers are the T_H lymphocyte and the macrophage. As there are a multitude of cytokines, Table 2.1

summarises the origin, target cell(s), and actions of principal cytokines. In the following sections the pro-inflammatory cytokine, TNF, and the regulatory cytokine, IL-10 are discussed in detail as their genes have been studied in this work. Two other pro-inflammatory cytokines, IL-1 and platelet activating factor (PAF), are also discussed as their role in the pathogenesis of acute pancreatitis has been extensively investigated in recent years.

Cytokine	Cell(s) of secretion	Target cell/tissue	Summary of known effects
IL-1	Macrophage, Neutrophil, NK cell, T and B lymphocytes, APC's	Macrophages/Monocytes	Production of other cytokines, Increased migration & cytotoxicity.
		T lymphocytes	Production of other cytokines, Stimulation of activity, expression of IL-2 receptors.
		B lymphocytes	Production of immunoglobulin, activation, growth, development.
		NK cells	Production of other cytokines, activation.
		Hepatocytes	Production of acute phase proteins
IL-2	T_H l cells, T_c cells	B cells	Production of Immunoglobulin, growth
		T_c cells	Activation, cytokine production (IFN-γ)
		NK cells	Growth, cytokine production (IFN-γ)
IL-3	T_H cells, NK cells	Stem cells	Growth and differentiation
	Mast cells	Mast cells	Growth and histamine secretion
IL-4	T_H2 cells	B lymphocytes	Activation, proliferation, differentiation, increase class II MHC expression, IgE production
	NK cells, mast cells	T lymphocytes	Proliferation
		Macrophage	Increase class II MHC expression, reduce secretion of pro-inflammatory cytokines
		Mast cells	Growth

Table 2.1 Selected cytokines, source, target cell/tissue, and known effects **continued.....**

IL-	Interleukin	GM-CSF	Granulocyte-macrophage colony stimulating factor
TNF	Tumour necrosis factor	TGF	Transforming growth factor
IFN	Interferon	PAF	Platelet activating factor

Cytokine	Principal immune cell(s) of secretion	Target cell/tissue	Summary of known effects
IL-5	T_H2 cells, mast cells	B cells Eosinophils	Proliferation and differentiation, IgA production Growth and development
IL-6	Monocytes, macrophages, T_H2 cells	B cells Plasma cells Stem cells Hepatocytes	Differentiation to plasma cells Antibody secretion Differentiation Acute phase protein production
IL-9	T_H cell	T_H cell	Proliferation
IL-10	T_H 2 cell, macrophage, B cells	Macrophage T_C cells/NK cells B cell/Mast cell	Suppresses activation and cytokine release Suppress function and cytokine release Proliferation and differentiation
IL-12	Macrophages, B cells	T_C cells/NK cells	Activation
IL-13	T_H cells	Macrophage	Suppresses activation and cytokine release
IL-15	T cells	T cells/NK cells B cells	Proliferation Proliferation and differentiation
IL-16	T_C cells	T_H cells	Chemotaxis, increased expression of class II MHC, cytokine synthesis, suppress antigen induced proliferation

Table 2.1 Selected cytokines, source, target cell/tissue, and known effects **continued.....**

IL-	Interleukin	GM-CSF	Granulocyte-macrophage colony stimulating factor
TNF	Tumour necrosis factor	TGF	Transforming growth factor
IFN	Interferon	PAF	Platelet activating factor

Cytokine	Principal immune cell(s) of secretion	Target cell/tissue	Summary of known effects
TNF-α	Macrophages/Monocytes, T_H cells	Macrophages	Stimulation
		T cells	Activation and cytokine release
		B cells	Antibody production
		Endothelial cells	Expression of cytokines and adhesion molecules
TNF-β	T cells	As TNF-α	As TNF-α
IFN-α	T cells and viral infected cells	All cells	Induce MHC class I expression and antigen presentation
		NK cells	Induce killing of viral infected cells
IFN-γ	T cells/NK cells	Normal cells	Inhibit viral replication
			Induce class II MHC expression
		Macrophages	Increase activity
		APC's, T and B cells	Increase MHC class I & II expression
GM-CSF	T cells	Macrophage, Granulocytes	Activation and proliferation
TGF-β	Macrophages, T and B cells, platelets	Macrophages	Attract, Increase IL-1 production
PAF	Endothelial cells, macrophages, platelets	Macrophages	Activation and attraction
		Platelets	Activation
		Neutrophils	Activation and attraction
		Endothelial cell	Leucocyte adherence, increased permeability

Table 2.1 Selected cytokines, source, target cell/tissue, and known effects

IL- Interleukin GM-CSF Granulocyte-macrophage colony stimulating factor IFN Interferon
TNF Tumour necrosis factor TGF Transforming growth factor PAF Platelet activating factor

2.4.5.1 Tumour necrosis factor (TNF)

Tumour necrosis factor (TNF) is the name given synonymously to TNFα, one of the members of the TNF family. Others are TNFβ (also known as lymphotoxin α, LTα), and LTβ, which share similar effects. 7 other related ligands, including the Fas ligand and nerve growth factor, have been identified, and are reviewed elsewhere (Bazzoni and Beutler, 1996).

TNF and LTα were first isolated in 1975, when their ability to kill tumour cells in vitro and in mice transplanted with tumours was noted (Carswell et al., 1975). A decade later the genes encoding the two proteins were cloned, and their homology was noted (Pennica et al., 1984). Studies also demonstrated the stimulatory effects of TNF in leucocyte adherence to endothelial cells (Gamble et al., 1985), and it's role as a central mediator of systemic shock (Tracey et al., 1986; Beutler et al., 1985).

TNF is considered to be predominantly secreted by the macrophage/monocyte lineage, although other immune cell sources include T and B lymphocytes, neutrophils, activated NK cells, and endothelial cells (Jones et al., 1989; Cohen and Cohen, 1996). Furthermore it is becoming apparent that TNF is expressed by many non-immune cells, both in health and disease. For example, normal pancreatic acinar cells (Gukovskaya et al., 1997), muscle cells (Saghizadeh et al., 1996), fibrocytes (Chesney et al., 1998), and adipose tissue cells (Hotamisligil et al., 1995) all express TNF.

It is unclear whether TNF is detectable in the plasma of normal healthy humans , as conflicting data has been produced (McLaughlin and Davies, 1990; Eigler et al., 1997). Likewise, although it is often detectable in acute inflammatory conditions such as sepsis and acute pancreatitis (Exley et al., 1992), this is by no means a consistent finding , probably reflecting the short half life (14-18 minutes) of TNF, difficulties with it's measurement in serum, and the fact that TNF can function both via autocrine and paracrine routes.

2.4.5.1.1 Structure

TNF is a heterotrimer, consisting of three identical polypeptide chains (Jones et al., 1989). Likewise LTα is a heterotrimer of three LTα molecules, but one of the three molecules of the LTβ heterotrimer is a LTα subunit (Bazzoni and Beutler, 1996).

TNF is synthesised as a membrane bound precursor protein, and cleavage by a specific metalloprotease (TNFα converting enzyme, TACE) produces a molecule 157 amino acids (AA) in length (Rink and Kirchner, 1996). The mature molecule forms into the heterotrimer after membrane cleavage. TNF and LTα are predominantly secreted in soluble form, although TNF does exist in a membrane bound active form which is also capable of TNF receptor (p75) binding (Grell et al., 1995). LTβ and the other members of the TNF ligand family are transmembrane proteins and do not exist in free (soluble) form (Crowe et al., 1994; Bazzoni and Beutler, 1996).

The genes for TNFα, LTα, and LTβ are tandemly arranged within the MHC on chromosome 6. The TNF gene complex will be discussed in section 4.1.4.

2.4.5.1.2 Receptors

TNF has two receptors, a 55 kDa receptor, and a 75 kDa receptor. They have been designated p55 (or TNFR55, or TNFRI, now called CD120a), and p75 (or TNFR75, or TNFRII, now called CD120b) (Bazzoni and Beutler, 1996). It shares these with the LTα heterotrimer, explaining the similar effects of the different proteins. LTβ has it's own specific receptor (Crowe et al., 1994).

The TNF receptor family are transmembrane proteins consisting of two identical subunits. As two different proteins share two different receptors, the extracellular domains are similar, variation being in the cytoplasmic domain. Crystallisation studies of the extracellular domain of the p55 receptor reveal that it is dimeric (Bazzoni et al., 1995).

The exact mechanism of interactions between heterotrimer ligand and dimeric receptor are unknown, and theories are reviewed elsewhere (Rink and Kirchner, 1996; Bazzoni and

Beutler, 1996).

The TNF receptors p55 and p75 are expressed on nearly all nucleated cell types. Both types of TNF receptor are shed from the cell membrane and exist in soluble form as biologically active receptors, binding free TNF and thereby limiting it's action (Hale et al., 1995).

The p55 receptor binding with soluble TNF ligand was regarded as the dominant functional receptor/ligand unit in TNF responses. However, it has been shown that activation of the p75 receptor is maximal when binding membrane bound TNF, and this is more important in T cell activation and proliferation than the receptor binding of soluble TNF (Grell et al., 1995). This suggests that the p75 receptor has an important physiological role in local inflammatory responses.

Different functions of the two TNF receptors are also suggested by 'gene knockout' studies in mice subsequently challenged with lipopolysaccharide (LPS) and soluble TNF.

Deletion of the p55 receptor results in resistance to the lethal effect of low doses of LPS in D-galactosamine sensitised animals, but not to high doses in unsensitised groups. Animals also become highly susceptible to infection by *Listeria monocytogenes,* which is an intracellular bacterium that requires both initial non-specific resistance and later specific T cell mediated immunity for resolution of infection. The same p55 knockout mice also showed no systemic effects on the administration of (soluble) TNF compared to controls in whom high lethality was observed (Rothe et al., 1993).

Studies of deletion of the p75 receptor did not show the same protection from the effects of LPS, but showed decreased lethality (<10% of animals) to the effects of TNF administration compared to controls (100% of animals died) (Erickson et al., 1994).

The cytoplasmic proteins used by the TNF receptors for signal transduction are an ever increasing group, and are reviewed elsewhere (Bazzoni and Beutler, 1996). Expression of transcription factors such as NFκB results from TNF receptor signal transduction.

Expression of NFκB has many known effects in the immune system, including increasing the

transcription rate of various cytokine genes (Baeuerle and Henkel, 1994).

The multiple functions of TNF (and LTα) are summarised in Fig. 2.2. Effects upon immune functions are now discussed.

2.4.5.1.3 Functions of TNF in immune and inflammatory responses

As TNF and LTα function through the same receptors, they are generally considered to have the same effects in acute immune responses. However studies on mice rendered deficient in LTα by gene targeting of immune stem cells, show that LTα may have a function separate from TNF, as it is necessary for the development of lymph nodes and Peyer's patches (De Togni et al., 1994). This may, however, only reflect the fact that an LTα molecule is required for the formation of the LTβ heterotrimer, which has it's own specific receptor that is thought to be important in lymphoid organ development (Crowe et al., 1994).

TNF (and LTα) is regarded as an initiator of the cytokine network, causing other pro-inflammatory cytokines such as IL-1 (Tracey et al., 1989), IL-6, (Van der Poll et al., 1992), and IL-8 (Van Deventer et al., 1993) to be released from and expressed by many immune cell types including macrophages, monocytes, T cells, and endothelium. This will result in a generalised upregulation of the inflammatory response (Cohen and Cohen, 1996), but it is interesting to note that TNF is also known to increase the secretion of IL-10 from monocytes (Platzer et al., 1995). The transcription of genes encoding for such cytokines is thought to be via the activation of the transcription factor NFκB (NFκB/REL unit), which can itself be activated by TNF (Baeuerle and Henkel, 1994).

The endothelial cell is affected in many ways by TNF, which explains early observations of the effect of TNF on leucocyte adherence to endothelial cells (Gamble et al., 1985).

As previously mentioned TNF induces release of other pro-inflammatory cytokines from the endothelial cell, which with TNF affect endothelial cell-leucocyte interaction by different pathways (Mantovani et al., 1997). Expression of members of adhesion molecule families

such as the selectins are transcriptionally upregulated by TNF (also IL-1) (Panes and Granger, 1998) as is the expression of MHC molecules (Pober et al., 1987). Nitric oxide (NO) also has effects upon endothelial cell-leucocyte interaction (see section 2.4.4) . TNF affects the production of NO by increasing the activity of NOS (Mantovani et al., 1997). The endothelial cell is also a site for TNF induced activation of thrombus forming pathways, causing activation of pro-coagulant activity, and inhibiting anticoagulant and fibrin pathways (Mantovani et al., 1997).

TNF activates many other inflammatory cells such as the neutrophil (Van der Poll et al., 1992), and induces T cell mediated cytotoxicity as well as T cell proliferation(Grell et al., 1995). B cells are also affected, production of immunoglobulin and cellular proliferation are both enhanced .

The expression of MHC class I (Pober et al., 1987) and II (Pujol-Borrell et al., 1987) molecules are induced by TNF in association with IFNγ. This not only occurs on cellular components of the immune response, but also on non immune cells such as pancreatic islet cells (Pujol-Borrell et al., 1987).

As well as affecting cellular effectors of the innate immune response, TNF has been identified as having effects upon other soluble mediators of the non-specific immune reaction. The expression of APP's and components of the complement system (e.g. CRP and C3) are enhanced by TNF (Baumann and Gauldie, 1994). Although the effect is thought to be by direct action upon hepatocytes, TNF could induce APP's through it's effect as an endogenous pyrogen (Selby et al., 1987). Glucocorticoids, released in response to fever can stimulate APP production.

2.4.5.1.4 Involvement of TNF in cell death

There are two forms of cell death, necrosis and apoptosis. Necrosis is the type of cell death that occurs with hypoxia, or cell membrane damage caused by complement and antibody. It is characterised by ballooning of the cell membrane and lack of nuclear disintegration. Apoptosis is characterised by nuclear disintegration and shrinking. Apoptosis is important in

CTL mediated cytotoxicity and also in tissue development.

As expected from it's activating and stimulatory effects upon T and NK cells, TNF plays a major role in the mediation of cell death. However TNF can induce both types of cell death without the help of cellular mediators in many cell types (Gukovskaya et al., 1997; Laster et al., 1988).

This involvement of TNF in cell death has relevance to it's inflammatory effects, and evidence indicates a physiologic role for TNF induced apoptosis in different tissues. These include pancreatic acinar cells (Gukovskaya et al., 1997), adipose cells (Prins et al., 1997), and myocytes (Meldrum, 1998). Indeed TNF receptors and ligands are related to the Fas receptor and ligand, and their signal transduction proteins and receptor intracellular domains have structural homology (Bazzoni and Beutler, 1996). Fas is known to be associated with physiological apoptosis occurring in developmental cell lineages such as haemopoetic stem cells and gut endothelium.

2.4.5.1.4 Other effects of TNF

Some of the earliest studies on the functions of TNF showed that when administered in quantities similar to those produced endogenously in response to LPS, TNF induces tachypnoea, hypotension, metabolic acidosis, and acute tubular necrosis in the kidney (Tracey et al., 1986). This led to it being identified as a central mediator of shock.

As well as mediating apoptosis in adipocytes (Prins et al., 1997), TNF is known to suppress the synthesis of lipoprotein lipase in adipocytes , which may partly explain it's effects in producing cachexia (Tracey et al., 1989). TNF is also involved in glucose haemostasis in adipocytes, stimulating glucose uptake by itself , whilst inhibiting insulin dependent uptake (Wang et al., 1998).

TNF is also known to have a role in the regulation of connective tissue and bone remodelling and has been suggested to have a pathophysiologic role in related disorders. TNF suppresses collagen production from fibroblasts (Sato et al., 1998), and induces osteoblasts to stimulate osteoclastic bone resorption (Thomson et al., 1987). Recent

evidence indicates that TNF plays an important role in the bone resorption that accompanies periodontal disease (Assuma et al., 1998).

The evaluation of TNF in the circulatory system has grown apace in recent years. It is produced by cardiac myocytes as well as cardiac macrophages. It's exact role in the pathogenesis of cardiac disease is not clear at present. TNF has been found to depress myocyte and left ventricular contractility (Meldrum, 1998), whilst recent evidence from studies upon isolated myocytes indicates that it protects the heart from hypoxic injury (Nanako et al., 1998).

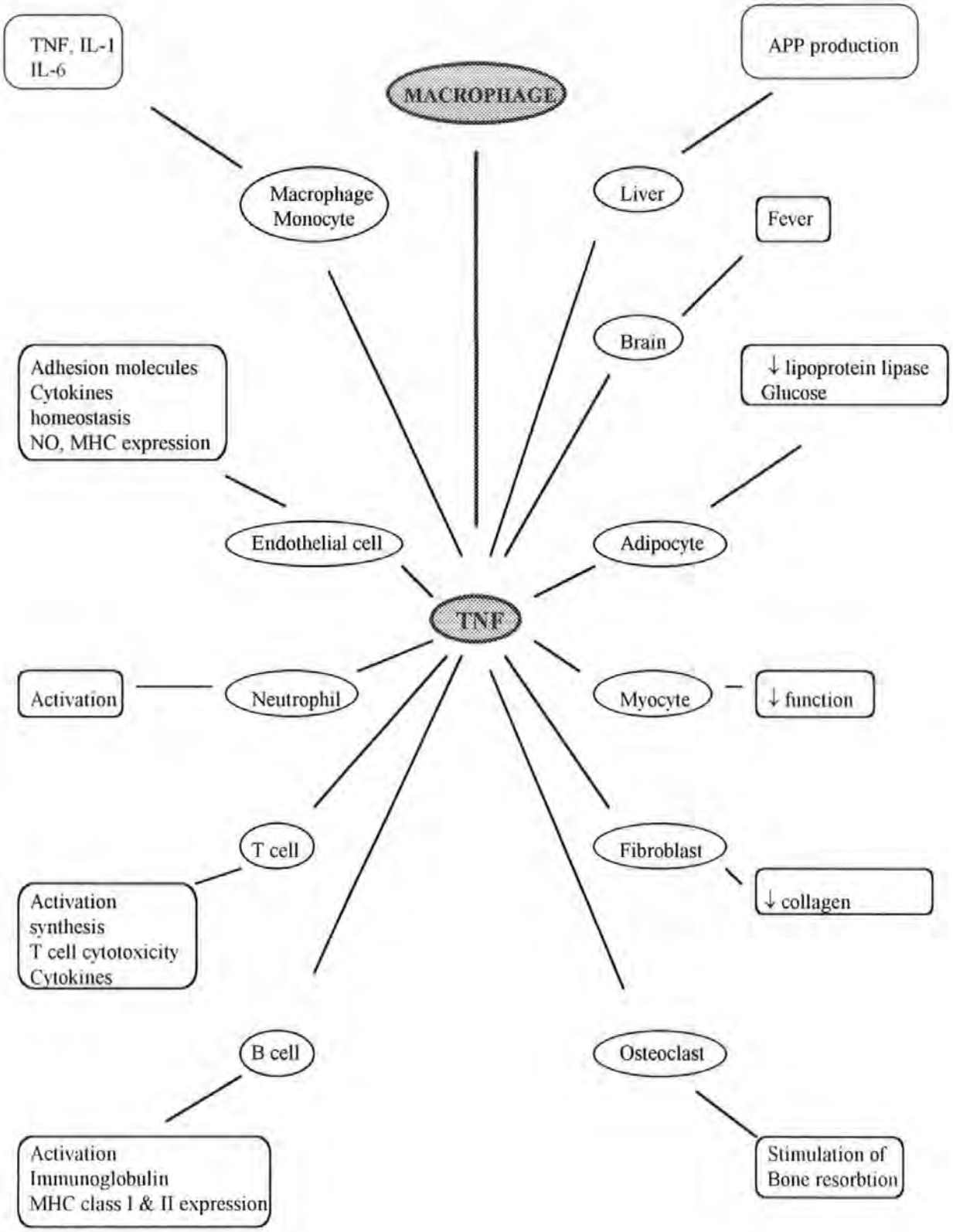

Fig. 2.2 Summary of biological effects of TNF on immune cells, selected organs, and cells of the body.

Although many cell types produce TNF, the main source of the cytokine is the macrophage. Many of the cell types illustrated express TNF themselves, where it may act in an autocrine or paracrine fashion. TNF also mediates apoptosis in some cell types illustrated, e.g. Adipocyte, Myocyte.

2.4.5.2 Interleukin-1 (IL-1)

IL-1 is the name given to two structurally related molecules, IL-1α, and IL-1β. The two molecules are 26% homologous at the amino acid level, and share common receptors (IL-1RI and IL-1RII) , so display identical biological properties (Platanias and Vogelzang, 1990). There exists a naturally occurring antagonist to IL-1, the IL-1 receptor antagonist (IL-1ra), which also is a ligand for both IL-1 receptors. Both IL-1molecules, IL-1ra, and the two receptors are encoded for on the long arm of chromosome 2 (Copekland et al., 1991; Nicklin et al., 1994).

Like TNF, the major cell of production is the macrophage, but it is also produced by other immune cells, connective tissue cells, and many other nucleated cells of the body (Platanias and Vogelzang, 1990).

IL-1α and IL-1β are synthesised as precursor molecules, and in a similar fashion to the TNF ligands are cleaved by specific proteases to produce their active forms.

The IL-1 receptors are located on many cell types, and share 28% homology in their ligand domains, the main structural differences being within their cytoplasmic domains. As is the case with TNF, the transcription factor NFκB is an important mediator of IL-1 induced gene transcription (Baeuerle and Henkel, 1994).

Functionally, IL-1 shares many functions with TNF (Cohen and Cohen, 1996). In the immune system resultant macrophage activation results in the release of more pro-inflammatory cytokines such as TNF and IL-6. Phagocytic cell activity is enhanced, T cells are activated, and B cells are stimulated to produce immunoglobulin (Platanias and Vogelzang, 1990). Endothelial cells are activated (in conjunction with TNF) by IL-1. This results in MHC expression on endothelial cells (Pober et al., 1987), activation of coagulation pathways, induction of NO production (Mantovani et al., 1997), and expression of adhesion molecules (Panes and Granger, 1998). IL-1 is also an endogenous pyrogen and increases the production of APP and complement components from the liver (Baumann and

Gauldie, 1994).

As previously described, TNF is an important mediator of cell death in normal states and disease. IL-1 also plays a role in such events. As well as the activation of cells which have a cytotoxic role, IL-1 has direct cytolytic effects, first demonstrated on tumour cells (Onozaki et al., 1985).

IL-1 is also involved in connective tissue and bone biology. IL-1 decreases collagen secretion in fibroblasts (Chesney et al., 1998), and stimulates bone resorptive activity, both similar to effects seen with TNF.

2.4.5.3 Platelet activating factor (PAF)

Platelet Activating Factor (PAF) is a potent phospholipid mediator which is involved in a wide range of physiological and pathological events.

Whilst it's name implies a specific role (as does TNF), PAF is synthesised and released in numerous cell types and tissues upon which it also exerts it's multiple effects. PAF is synthesised by similar immune cell types that secrete IL-1 and TNF. Endothelial cells, neutrophil polymorphs, monocytes and macrophages all produce PAF (Braquet et al., 1987), and it is also produced by other tissue cells such as the pancreatic acinar cell (Solling and Fest, 1986).

In spite of the fact that PAF is a phospholipid and can simply be incorporated into the membrane, in common with other cytokines it does act upon specific receptors which exist upon the surface of responsive cells (Chao and Olson, 1993). The receptor has been cloned and evidence seems to indicate that GTP-binding proteins are involved in signal transduction (Honda, 1991).

PAF functions in normal physiological processes including haemostasis, inflammation, pressor activity and reproduction (Venable et al., 1993). PAF *in vitro* causes activation of platelets, polymorphonuclear leucocytes, monocytes and macrophages, and *in vivo* PAF causes hypotension, increased vascular permeability, decreased cardiac output , acute bronchoconstriction, and increased leucocyte adherence to endothelial cells (Braquet et al.,

1987), presumably by similar mechanisms to the effects produced by IL-1 and TNF (Mantovani et al., 1997).

It is through this enhancement of transmigration of activated polymorphonuclear leucocytes from postcapillary venules into the interstitium of organs such as the lung, kidney, myocardium and liver that is believed to be one of the principal mechanisms through which PAF exerts it's pathobiological effect. Once within the tissue spaces of these organs the activated polymorphs release proteolytic enzymes, elastases and superoxide ions, and other tissue damaging substances such as Cathepsin B. This results in the release of potent tissue damaging substances such as proteolytic enzymes, elastases and superoxide ions(Braquet et al., 1987).

2.4.5.4 Interleukin 10 (IL-10)

Interleukin-10 (IL-10) is a cytokine that plays an important role in the regulation of inflammatory and immune responses through it's influence on several cells of the immune system. It was first identified a decade ago as a mediator secreted by T_H2 cells that inhibited cytokine production and subsequent effector functions of T_H1 cells (Fiorentino et al., 1989). Subsequent studies of the IL-10 ligand and receptor have revealed that IL-10 is secreted and has effects on many more immune cell types. It mediates suppression of cell mediated immunity (CMI) and inflammation, and promotes the humoral response by playing a supporting role in the development of T_H2 responses.

The ability to secrete IL-10 has been demonstrated in T_H cells (CD4+), T_C cells (CD8+) (Ho and Moore, 1994), B cells (O'Garra et al., 1992), the monocyte/macrophage lineage (Moore et al., 1993), neutrophils (Cassatella and Meda, 1994), NK cells (Mehrota et al., 1998), and keratinocytes (Enk and Katz, 1992). As can be expected immune cells within tissues also secrete IL-10. Kupffer cells in the liver (Knolle et al., 1995)and T cells of the intestinal mucosa (Braunstein et al., 1997) are such examples. However non-immune cells (hepatocytes and intestinal epithelial cells) of these organs have also been shown to express and secrete IL-10 (Napolitano et al., 1997; Ishizaka et al., 1996).

2.4.5.4.1 Structure

Human IL-10 is a homodimer with a molecular weight of 39kD (de Waal Malefyt et al., 1992). The protein structures were derived from cDNA clone sequences and indicate a 178 amino acid polypeptide (Ho and Moore, 1994). Unlike the TNF ligand it does not have a transmembrane domain precursor (Gallea-Robache et al., 1997), so may be produced in active form. Recent evidence seems to indicate that the IL-10 molecule has two functional domains. One region activates the anti-inflammatory properties of IL-10, inhibiting release of pro-inflammatory cytokines (including TNFα and IL-1) and chemokine and MHC class II expression on mononuclear cells, whilst the other domain seems to be involved in regulation of mast cell proliferation (Gesser et al., 1997).

The gene for IL-10 is located on chromosome 1, and will be discussed in section 4.2.

2.4.5.4.2 Receptor

IL-10 has one receptor, designated IL-10R, which is of the size 90-120kD. It is a trans-membrane protein, and in solution interacts with it's ligand by forming a complex of two IL-10 dimers and four receptor monomers (Tan et al., 1995). The mechanism of interaction between ligand and receptor on the cell surface is unknown.

IL-10R has been identified on a number of haemopoetic cells, including monocytes/macrophages and T cells (Liu et al., 1994). In stark contrast to the situation with TNF receptors (see section 2.4.5.1.2), expression has mainly been identified on the haemopoetic cell lineage.

IL-10R is structurally related to the IFN-γ receptor (IFNR). As IL-10 inhibits IFN-γ activation of macrophages, and inhibits release of pro-inflammatory cytokines (e.g. IL-12) that are induced by IFN-γ (Trinchieri, 1997), this has raised the possibility that IFN-γ and IL-10 share a receptor or components of signal transduction pathways.

Pathways of IL-10R signal transduction are under investigation at present. Present evidence seems to indicate two different pathways of signal transduction, involving separate

series of different cytoplasmic proteins, for the proliferative and inflammatory effects of IL-10 (Crawley et al., 1996; O'Farrell et al., 1998).

2.4.5.4.3 Functions of IL-10

The functions of IL-10 are summarised in figure 2.3.

IL-10 was first identified as a mediator secreted by T_H2 cells that inhibited cytokine production from T_H1 cells (Fiorentino et al., 1989). Subsequent investigations have confirmed similar effects upon other cell types including monocytes/macrophages and neutrophils, and NK cells.

T_H1 cells show diminished secretion of cytokines including IFN-γ . T_H1 production of other pro-inflammatory cytokines such as TNF is also slightly affected (Gesser et al., 1997).

In contrast, macrophage secretion of TNF is profoundly suppressed by IL-10 (Hart et al., 1995; Fiorentino et al., 1991). The same is true for other pro-inflammatory cytokines such as IL-1 (Schreiber et al., 1995; Fiorentino et al., 1991), IL-6 (Fiorentino et al., 1991), and IL-12 (Trinchieri, 1997). Interestingly, in line with the general anti-inflammatory effect, macrophage production of the endogenous IL-1 antagonist (IL-1ra) is increased by IL-10 (Gesser et al., 1997).

TNF receptor expression on macrophages in response to IL-10 has also been studied. The most recent evidence indicates that cell surface levels of the TNF receptor (p75) increase initially on the cell surface (Dickensheets et al., 1997; Hart et al., 1996). This however is accompanied by an increase in the levels of soluble TNF receptor, shed from cell membranes, seen in supernatants of cultured macrophages (Hart et al., 1996). This may indicate that IL-10 promotes limitation of the activity of TNF on other cells by the binding of free TNF with the shed (soluble) receptor. The significance of these findings is not clear, as the same cells subsequently cultured with TNFα increase their IL-1α production, which would be expected to have the same pro-inflammatory effects as TNF. Studies of the effect of IL-10 upon p55 (TNF) receptor expression on mononuclear cells have produced

conflicting data, so limiting interpretation.

PAF production from monocytes and neutrophils is also affected by IL-10. Lipopolysaccharide (LPS) stimulates an early release of PAF from these cell types, but sustained production is dependent upon stimulation by pro-inflammatory cytokines (TNF, IL-1). IL-10 blocks this cytokine-dependent sustained release of PAF in mononuclear cell cultures, but conversely it increases early production of PAF in the presence of LPS (Bussolati et al., 1997). The unexpected finding may be due to the fact that initial production of PAF does not require protein synthesis, only cleavage from a membrane precursor (Braquet et al., 1987), and IL-10 acts only to reduce protein synthesis by effects upon gene transcription.

Similar effects upon OFR production by the same cell types were seen in the same series of investigations. The authors postulated that the superoxide production was secondary to PAF release, as it was blocked by a PAF antagonist (Bussolati et al., 1997).

NK cells also show reduced pro-inflammatory cytokine (IFN-γ) secretion upon incubation with IL-10 (de Waal Malefyt et al., 1992). This effect is not seen in the presence of the pro-inflammatory cytokine IL-2, indicating that suppression of cytokine production by IL-10 in NK cells may require other cellular or soluble effectors . It also serves to remind us that regulation of immune responses by cytokines is a complex affair, and *in vitro* studies do not necessarily relate to the *in vivo* situation.

As well as affecting cytokine synthesis by T cells and producing effects upon them through the reduction in macrophage derived cytokines, IL-10 inhibits antigen specific T cell proliferation. This appears to be due to the diminished function of APC's, as IL-10 reduces MHC class II expression on monocytes (de Waal Malefyt et al., 1992; Gesser et al., 1997; de Waal Malefyt et al., 1991).

Expression of other cell surface molecules involved in immune responses is also affected by IL-10. The expression of chemokines (e.g. IL-8) on macrophages is suppressed, resulting in reduced chemotactic activity (Gesser et al., 1997).

IL-10, in conjunction with it's modulation of T_H cell cytokine production, which results in reduction of the T_H1 response, encouraging a T_H2 mediated antibody response (see section 2.3.5.3), also directly affects B cell function by working as a co-stimulator of proliferation and antibody production, and a co-regulator of B cell apoptosis.

Memory B cells are stimulated to develop into functional plasma cells by IL-10 when acting in conjunction with IL-2 and IL-3, but this effect requires that the cell surface molecule CD40, expressed on the B cell, is ligated (Kindler and zubler, 1997; Rousset et al., 1992). CD40 and it's ligand, which is expressed on the surface of T_H cells, are members of the TNF family of ligands and receptors (Bazzoni and Beutler, 1996). Immunoglobulin production is also seen to be increased upon ligation of CD40. This effect is also enhanced by the presence of IL-10 (Ho and Moore, 1994). CD27 is another cell surface molecule which when ligated promotes the differentiation of B cells into plasma cells. IL-10 is again seen as a co-stimulator of this process. When CD27 ligation occurs in the presence of IL-10, increased numbers of plasma cells are seen (Agematsu et al., 1998).

Proliferation of the B cell and plasma cell is in part controlled by the rate of apoptosis. IL-10 has been shown to act as a modulator of this process. This appears to be a bi-phasic phenomenon, as studies show that when IL-10 is present upon initial activation of the B cell in response to antigen, apoptosis is facilitated. However when IL-10 doesn't appear until 72 hours after the initial activation, perhaps a more accurate reflection of the *in vitro* situation, B cell apoptosis is seen to be reduced (Itoh and Hirohata, 1995).

IL-10 also acts to reduce apoptosis in T cells, so allowing proliferation in response to IL-2. Similar effects are also seen on neutrophil and macrophage apoptosis (Arai et al., 1995; Keel et al., 1997). The effect in T cells may be due to IL-10 mediated upregulation of Bcl-2 expression (Cohen et al., 1997). Bcl is an oncogene with an established role in the regulation of lymphoid cell apoptosis.

There has also been interest in the role of IL-10 in allergic inflammation. IL-10 is known to act upon mast cells to reduce the secretion of pro-inflammatory cytokines and eosinophilic

chemokines, and reduces expression of CD40 upon the eosinophil, which increases their rate of apoptosis (Pretolani and Goldman, 1997). These effects occur in addition to suppressive effects upon IgE synthesis.

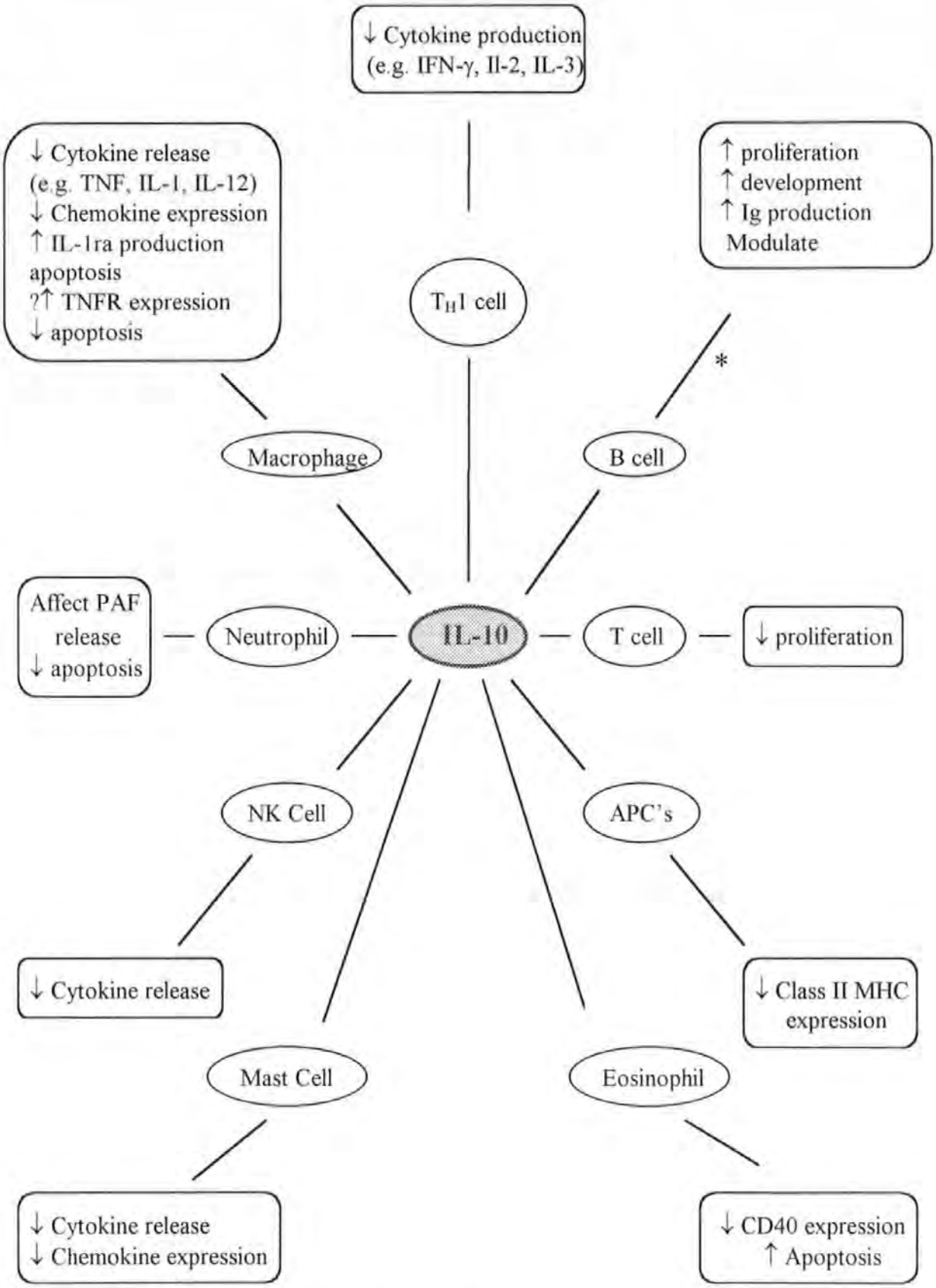

Fig 2.3 Summary of the functions of IL-10

As can be seen, the overall effect of IL-10 is to mediate suppression of cell mediated immunity (CMI) and inflammation, and promote the humoral response by playing a supporting role in the development of T_H2 responses.

* These effects are by facilitating effects of other cytokines and CD cell surface molecule ligation upon the B cell (see text for details).

2.4.5.5 Chemokines

The chemokines are a group of chemoattractant cytokines involved in the activation and trafficking of inflammatory cells. They are a very large family, to date around fifty related proteins are known, classified into 2 broad groups, α (C-X-C) or β (C-C) chemokines depending upon their structure (Premack and Schall, 1996). The α (C-X-C) group has two cysteine residues separated by another amino acid, whilst the β (C-C) group has two adjacent cysteine residues. Most α chemokines are chemoattractant for neutrophils but not monocytes, and most β chemokines are chemoattractant for monocytes and lymphocytes, but not neutrophils. Chemokine ligands and receptors are expressed by many cells involved in migration towards inflammation (monocytes/macrophages, neutrophils, T cells) and by platelets and endothelial cells (Mantovani et al., 1997). Evidence is emerging that chemokines are also expressed by other cells involved in inflammatory processes, such as the pancreatic acinar cell in acute pancreatitis (Grady et al., 1997).

IL-8 is a well characterised (α) chemokine. It is known to affect endothelial cell-leucocyte interaction, mediating the adherence of leucocyte to the endothelial cell, and promoting their subsequent extravasation. This is via activation of adhesion molecules such as the integrins (Panes and Granger, 1998). Chemokines not only mediate cellular extravasation, but are also able to attract inflammatory cells to exact sites of inflammation by the mediation of binding not only to inflammatory and endothelial cells but also to the extracellular matrix (Lloyd et al., 1996).

2.5 Upregulation of the Inflammatory response: progression to the systemic inflammatory response syndrome (SIRS)

Inflammation is a complex, initial, non specific response produced by the body in response to tissue injury produced by mechanical, physical, or infectious stimuli. By it's nature inflammation is rapid in onset and is highly amplified, mediated and effected by humoral and

cellular components of the immune system previously described. The inflammatory response is at first a local response, but may become more widespread, with distant organ systems becoming involved by inflammatory processes.

2.5.1 Inflammation

Inflammation traditionally consists of four classical symptoms, *rubor* (erythema), *tumor* (swelling/oedema), *calor* (heat), and *dolor* (pain). These reflect major events in the inflammatory process; vasodilatation, increased microvascular permeability, cellular activation/adhesion, and activation of complement, coagulation, fibrinolytic, and kinin systems. As previously described, the cells activated in the initial phase are the endothelial cell, neutrophil and macrophage. Activation results in their increased metabolism, with consequent release of inflammatory (e.g. TNF, IL-1) and regulatory cytokines (e.g. IL-1ra, IL-10). Adhesion molecules and receptors are expressed, and secondary inflammatory mediators and effectors including prostaglandins, OFR's, nitric oxide, and proteases are also released. Mediators trigger release of APP's from the liver, recruit T cells and increase class II MHC expression, resulting in the involvement and activation of T cells in the inflammatory process.

The local inflammatory response is physiologically protective, promoting healing and repair of damaged tissue. However, if inflammation is not controlled locally or is exaggerated, systemic inflammation ensues.

2.5.2 Systemic inflammatory response

The upgrading of inflammation to involve systemic organ systems is well recognised. The clinical picture produced is called the systemic inflammatory response syndrome (SIRS), the sequelae of which is multiple organ dysfunction syndrome (MODS).

It has been proposed that there are three stages in the development of SIRS (Bone, 1998). Stage I involves initiation of the local inflammatory response. Stage II is either a controlled amplification of the process, or a loss of homeostatic control. Stage III is the development of SIRS with the advent of MODS. It is summarised in Figure 2.4.

The reason for this loss of local control is not fully understood. One might hypothesise that persistence or increased severity of the initial insult would cause SIRS, but this cannot always be the case, as infection is not always found to persist in patients with MODS after sepsis (Goris et al., 1985), and severity of pancreatic damage does not always correlate with the development of MSOF in acute pancreatitis (Banks et al., 1996).

Although it does not explain the precise mechanism of the development of SIRS, it is commonly thought that the process occurs due to the (inappropriate) systemic release of locally produced cytokines (specifically pro-inflammatory cytokines such as TNF and IL-1) which initiate inflammatory processes in other organs (Kusske et al., 1996b; Kingsnorth, 1997b; Davies and Hagen, 1997b).

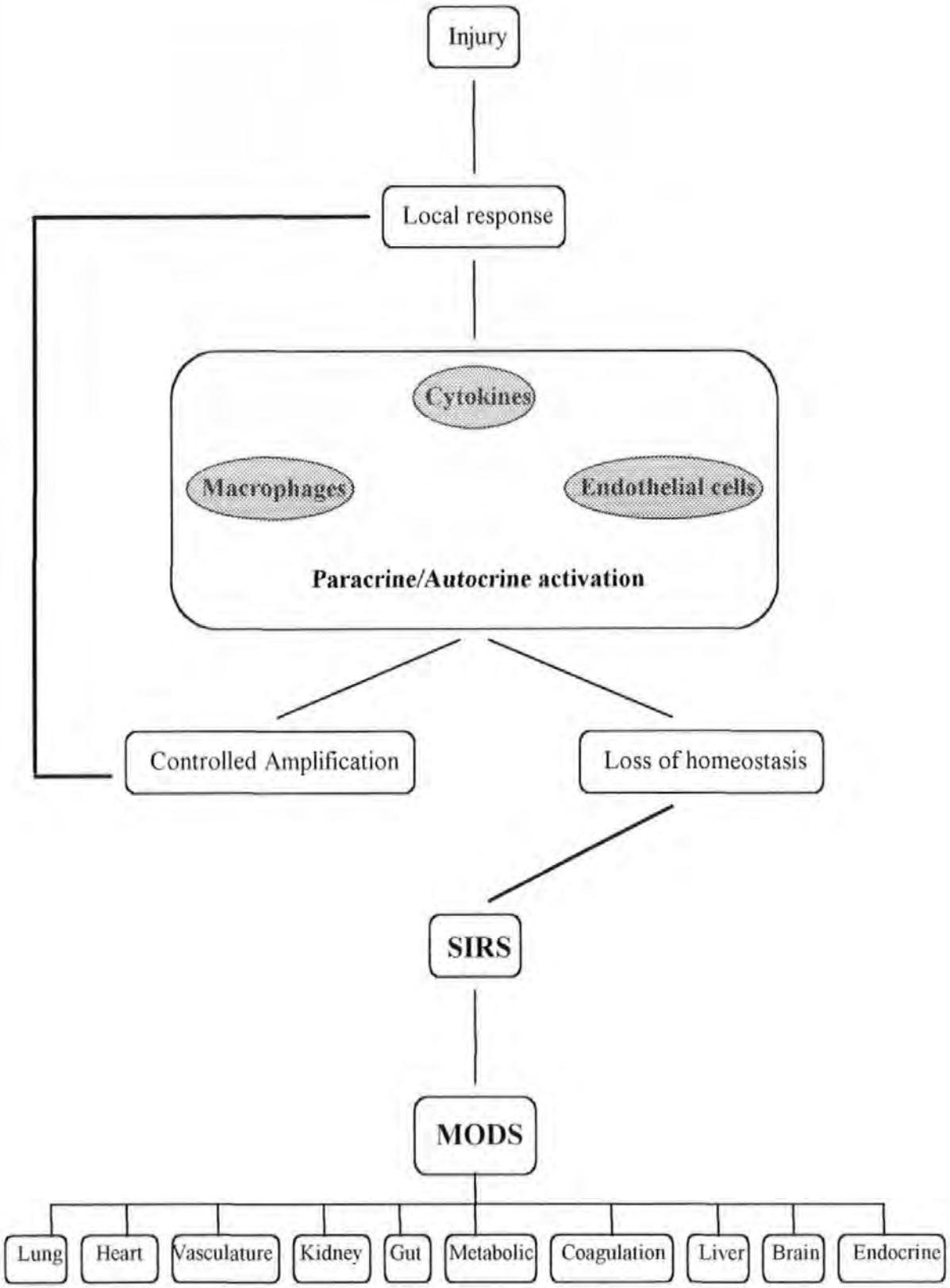

Figure 2.4 Stages in the development of SIRS

Stage I involves initiation and propagation of the local inflammatory response. Stage II is either a controlled amplification of the process, or a loss of homeostatic control. Stage III is the development of SIRS with the advent of MODS.
SIRS Systemic inflammatory response
MODS Multiple organ *dysfunction* syndrome
Adapted from (Davies and Hagen, 1997) and (Bone, 1998)

The involvement of TNF and IL-10, the cytokines studied in this work, in the development of SIRS can be inferred from the multiple functions of both these cytokines which have already been discussed in this chapter. However, their role in the development of SIRS will briefly be summarised with discussion of evidence resulting from clinically based studies.

2.5.3 TNF and IL-10 in the development of SIRS

As previously discussed TNF (along with IL-1) is heavily implicated as a pivotal cytokine in the regulation of inflammation. Administration of TNF to animal models results in the pathophysiological events associated with SIRS (Van der Poll et al., 1992; Tracey et al., 1986), and passive immunisation with anti-TNF antibody confers protection against manifestations of SIRS and death seen in bacterial sepsis (Tracey et al., 1987) or endotoxin administration (Beutler et al., 1985).

There is however evidence in animal models that TNF is also required for normal functioning of immune responses, and is indeed protective against bacterial infection. Mice show increased early resistance to *Listeria* infection when pre-treated with TNF (Havell, 1989), and recombinant TNF administration has been shown to ameliorate systemic effects of peritoneal gram negative sepsis (Echtenacher et al., 1990; Alexander et al., 1991), when given early in the disease process. Similar results have already been described for models studying the effect of TNFR p55 deletion in mice (Rothe et al., 1993) in section 2.4.5.1.2. These results confirm that the functions of TNF are complex, for as well as having deleterious effects, TNF seems to be required for normal immune system functioning.

Studies measuring serum levels of TNF have been done in septic patients. However, results vary widely. The measurement of serum cytokine levels can be problematic (McLaughlin and Davies, 1990), and studies have used different anti-TNF antibodies in their varied immunoassays. Also TNF has a short half life in serum of 14-18 minutes (Davies and Hagen, 1997), and many effects are by paracrine and autocrine routes. Correlation has been made between higher median plasma TNF measurements and mortality in heterogeneous groups of septic patients (Damas et al., 1989; Debets et al., 1989), but conclusions are

based on evidence which is subject to some criticism. For example, serum TNF was detected in only 25% of patients in one study (total =43), but presence of detectable TNF did correlate with mortality (Debets et al., 1989). In contrast, in a smaller group of patients with comparable severity of sepsis (both studies used the same scoring system) serum TNF was detectable in all patients. This study also correlated mortality with mean TNF levels, the mean being raised by 17% (n=3) of patients with levels up to ten times higher than in others who died. The opposite conclusion has been arrived at by a recent study, which suggests that survival, not mortality, correlates with high serum TNF levels. Studying a slightly less heterogeneous group of patients with abdominal sepsis, no association in serum TNF levels was seen between survivors and non-survivors. Again, wide variability in levels was seen, and a significantly higher number of survivors did have a serum TNF level greater than 200pg ml^{-1} (Riche et al., 1996).

Evidence reviewed in section 2.4.6.4 implicates IL-10 as a key regulatory cytokine, modulating release and effects of pro-inflammatory cytokine release. Animal models show that IL-10 confers protection against pro-inflammatory cytokine release and the lethal effects of SIRS (Gerard et al., 1993; Howard et al., 1993; Bean et al., 1993). One group of investigators noted, however, that IL-10 did not diminish activation of components of the coagulation or fibrinolytic systems and only modestly affected neutrophil recruitment and degranulation (Van der Poll et al., 1997). Serum IL-10 is noted to be raised in 57% of patients with septicaemia, whilst only being present in 12% of patients without sepsis or inflammatory disease (post-surgical, cancer, and cirrhotic patients). Also, levels are significantly higher in septic patients with circulatory failure (Systolic BP<90 mmHg) than in septic patients without (Marchant et al., 1994).

In the clinical situation, although it is generally believed that TNF up-regulates systemic inflammation, and IL-10 functions to modify it's release and effects, evidence has been produced that indicates that high levels of serum IL-10, and a higher IL-10 to TNF ratio (both serum measurements) are associated with death a in large, n=464, group of febrile

patients (mostly, 86%, due to varied causes of local and systemic sepsis) (van Dissel et al., 1998). In the study, no significant differences were seen in mean serum TNF levels on their own. This is interesting data, and although it is possible that the cytokine profile detected in this study may indicate the body's (unsuccessful) attempts to deal with the inflammation, the same authors have produced evidence with comparable conclusions in studies of relatives of patients with meningococcal disease (Westendorp et al., 1996). This study first demonstrated a strong genetic influence upon TNF and IL-10 production, and showed that low TNF production in first degree relatives of patients increased the risk of death from meningococcal sepsis 10-fold. Similarly high IL-10 production in relatives increased the risk of death in patients 20-fold. This study will be discussed further in chapter 4.

2.6 Important concepts of immune system function

Previous sections have described cells and their surface molecules involved in immune responses and the mediators that affect those responses. The basis of an immune response is recognition of exogenous and endogenous antigens, recognition of self and discrimination from non self, and subsequent response. To be able to discriminate between self and non self (foreign cells/tissues or altered self), and protect itself from it's own potentially self reactive effector cells, the immune system has to develop tolerance to the body's own antigenic determinants. The ability to produce a highly effective immune response to re-challenge with non-self antigens depends upon the immune system being capable of memory. Breakdown of tolerance to self antigenic determinants is the basis of autoimmunity. A detailed description of the mechanisms of these concepts is outside the scope of this work; however, a general overview is given.

2.6.1 Memory

As previously discussed both B cells, through immunoglobulin diversity, and T cells, via TCR diversity, can recognise and react to the enormous range of potential antigens. Naive B and T cells, already induced with tolerance during foetal development, bind with

previously unencountered (presented) antigen, and mount a response after a lag phase during which clonal expansion of lymphocytes specific to the antigen occurs. This clonal expansion results in the formation of effector and memory lymphocytes. Memory cells have a long life, possibly due to the fact that exposure to antigen has somehow programmed them for long life, so are able to persist in lymphoid tissues, and because they are already clonally expanded are able to mount an effective and rapid secondary response.

2.6.2 Tolerance

Tolerance to self is acquired by clonal deletion of developing B and T lymphocytes. Their natural diversity to antigen recognition includes ability to recognise self antigens, whether they are bound (in the case of T lymphocytes) to MHC molecules, or not (B cell recognition). In the developing foetus selection of lymphocytes non-reactive to self antigens occurs. In this stage of immune system development the exposure of lymphocytes to abundant self antigen results in clonal deletion of the cells recognising that particular antigen. The MHC molecules themselves act as antigens, and clonal deletion of cells reactive to self MHC molecules without bound antigen also occurs. Once the immune system has developed, before birth in the human, further tolerance cannot develop.

2.6.3 Autoimmunity (Inappropriate immunity)

Autoimmunity, which should perhaps be best called inappropriate immunity, can be considered as a dysfunction of the immune system. It has been regarded as break down of tolerance to self, but may be due to the alteration of self by pathogenic organisms or agents, resulting in body cells recognised as foreign, to which a response is mounted.

Autoimmunity can be organ specific, where response is directed against a specific cellular component of an organ (e.g. type I, insulin dependant, diabetes mellitus), or systemic, where autoantibodies are produced against a wide range of antigenic determinants in multiple organs or tissues and/or immune complexes produce damage in a number of organs (e.g. systemic lupus erythematosus, SLE). Antibody or effector T cells can cause the damage seen in autoimmune disease.

There are a number of possibilities as to how autoimmunity can arise:

a) Antigenic similarities may occur between antigenic components of pathogens and self antigens. This is termed molecular mimicry. A good example is rheumatic fever, where antibodies to group A streptococci react with cardiac myosin (Dale and Beachey, 1985). Viral T cell epitopes also have similarities to self peptides (Wucherpfennig and Strominger, 1995), and could lead to an autoantigen directed T cell response.

b) Self antigens not previously encountered by the immune system, could be released following damage to cells by toxic influences. This cannot be regarded as a break down of tolerance, but rather an immune response to an antigen recognised as foreign.

c) The MHC has been found to be associated with the development of autoimmune disease. A good example is the association between HLA-DR3 and DR4 and type I diabetes (Wolf et al., 1983). Presumably such associations occur because different MHC molecules may vary in their affinity for antigenic peptides, be they self or exogenous. Indeed one single variation (polymorphism) in an amino acid residue of the HLA-DQ molecule β chain confers susceptibility to type I diabetes (Morel et al., 1988; Todd et al., 1987). These associations, however, are not by themselves causative, and imply the presence of other (environmental) factors in the development of autoimmunity.

d) MHC molecules may be aberrantly expressed on non-APC tissue cells, allowing antigen from internal cellular components to be combined with and presented by the secreted MHC molecule. There is evidence for aberrant MHC expression in both thyroid autoimmune disease and type I diabetes (Pujol-Borrell et al ., 1986). IFN-γ is known to induce expression of class II MHC molecules on a number of non immune cells (Janeway and Travers, 1996), including pancreatic β cells, thyroid acinar cells and intestinal epithelial cells. It has been hypothesised that infection and trauma, both resulting in local IFN-γ production, cause MHC expression on non APC cells, resulting in inappropriate T cell activation. IFN-γ would also induce the production of other pro-inflammatory

cytokines, that would help activate effector cells.

In spite of the presentation of these possible mechanisms as separate entities it is unlikely that any occur in isolation, more that the development of autoimmunity is a multifactorial process.

3 Pathogenesis of acute pancreatitis

3.1 Pathological features in acute pancreatitis

3.1.1 Pancreas

The gross and microscopic appearance of the pancreas is variable depending upon the severity of the disease. When the local inflammatory process is only mild the pancreas appears swollen and oedematous. If the local inflammation is severe the gland structure appears very disordered, with varying degrees of necrosis and haemorrhage. Fat necrosis appears as yellow plaques and nodules in the pancreas, throughout the mesentery, and dotted around the peritoneal fat. Ascites is often an accompanying feature, being turbid or haemorrhagic if the gland is severely diseased (Rosai, 1998).

Microscopic appearances in the early stages of human acute pancreatitis are assumed to be similar to those seen in experimental animals. Acinar cells lose their polarity and zymogen granules accumulate. Vacuoles form after fusion of these granules with lysosomes (Steer and Meldolosi, 1987). Other early changes involve interstitial oedema and intravascular margination of leucocytes. An inflammatory cell infiltrate of neutrophil polymorphs and monocytes is also seen (Formela et al., 1994; Schoenberg et al., 1990), being especially evident at areas of acinar cell necrosis. Haemorrhage appears and is associated with fat and parenchymal necrosis (Lerch et al., 1992). The Islets of Langerhans appear to be more resistant to damage, although most become necrotic (Senninger et al., 1986).

3.1.2 Other organs

The lung, often the first systemic organ to be affected in severe acute pancreatitis, is affected by a diffuse alveolar injury, the clinically defined Adult Respiratory Distress Syndrome (ARDS). Oedema, intra-alveolar haemorrhage and fibrin deposition occur early, and macrophages and polymorphs accumulate in the interstitial space (Murakami et al., 1995). Hyaline membranes form to line alveolar ducts and alveoli.

Damage to the kidney can also occur, often occurring secondary to hypotension and vascular depletion. The kidneys become pale and enlarged as cellular components become

ischaemic, swell, and die. The tubular epithelial cells and interstitium are most susceptible to ischaemic damage, and inflammatory cells infiltrate the interstitium.

Cardiovascular failure is also common. Failure is secondary to physiological events, so no gross pathological changes are seen acutely.

3.2 Pathogenesis of acute pancreatitis

Following the initiation of the disease, resulting in acinar cell damage, the pathophysiological process is understood to progress in three phases. Local inflammation is followed by a varying degree of systemic inflammatory response, which can progress to overwhelming sepsis with the translocation of bacteria from the gastrointestinal tract.

In spite of decades of investigation, elucidation of the pathogenic mechanism of acute pancreatitis remains a difficult challenge. Major obstacles include the inaccessibility of the human pancreatic gland, technical difficulties in experimentation with pancreatic enzymes and tissues, and the lack of correlation between experimental animal models of acute pancreatitis and human disease which may limit the clinical application of laboratory research (Banerjee et al., 1994; Steinberg and Schlesselmann, 1987).

3.2.1 Experimental models used to study the pathogenesis of acute pancreatitis

Various animal models of acute pancreatitis have been used to study the pathogenesis of acute pancreatitis. In this chapter, evidence is presented which has been produced in models which differ from each other in mode of disease initiation and severity, both at morphological levels in the pancreas and in effects on systemic organ function and animal mortality. Basic methodological details of the five *in vivo* animal models used in studies described in this work are presented in Table 3.1, which also gives information on the severity of disease produced, both local and systemic. A more comprehensive review has been published in recent years (Banerjee et al., 1994).

In view of the different pathophysiological mechanisms which must exist in the development of acute pancreatitis, no single experimental method produces the ideal model, and this must

be taken into account when studying the results of experimental studies and deciding upon their application to the human and clinical situation.

Type	Agent	Administration	Animal	Effects on pancreas	Systemic effects
Secretagogue	Caerulein (CCK analogue)	Intraperitoneal Intravenous or subcutaneous	Rat	Oedema	Lung injury (ARDS like)
			Mouse	Necrosis	Lung injury (ARDS like)
Diet-induced	CDE Diet	Oral	Mouse (female)	Haemorrhage and peripancreatic fat necrosis	Acidosis, hypoxia, and hypovolaemia
Duct injection	Bile/Taurochlorate	Pancreatic duct	Rat, Dog, Cat	Oedema and necrosis	Low systemic complication rate
Duct ligation	N/A	N/A	Rat, Oppossum	Necrosis and haemorrhage	Lung, kidney failure High mortality
Microvascular	Microspheres	Artery supplying pancreas (branch of splenic)	Rat	Oedema, haemorrhage, and fat necrosis	Lung injury

Table 3.1 Animal models of acute pancreatitis: Overview of methodology and local and systemic effects
CDE Choline-deficient ethionine-supplemented
CCK Cholecystokinin
ARDS Adult respiratory distress syndrome

3.2.2 Disease Initiation

The precise mechanisms which lead to initial acinar cell damage are not fully understood. As already outlined, acute pancreatitis is a disease with multiple different aetiologies, so different mechanisms may operate to initiate damage and subsequent inflammation in the acinar cell. This first phase in the pathogenesis of the disease has been most investigated in relation to the two main aetiologies, alcohol and gallstones, but other mechanisms of initiation are apparent with other less common aetiologies and will also be discussed.

3.2.2.1 Alcohol

Clinically, pancreatitis caused by excessive consumption of alcohol occurs in both acute and chronic forms. Acute alcoholic disease can be defined as an episode of acute pancreatic inflammation occurring without evidence of chronic pancreatitis (e.g. calcifications and cysts). The first attack of alcoholic acute pancreatitis is often considered to be a step in the development of chronic pancreatitis (Lankisch and Banks, 1998c), and a recent epidemiological study provides evidence to support this view (Lankisch et al., 1998). Therefore evidence supporting hypotheses relating to the pathogenic mechanism of alcohol in acute and chronic pancreatitis will be discussed.

3.2.2.1.1 Ethanol as an acinar cell toxin

Ethanol has been postulated to have a direct damaging effect upon the acinar cell (Meier, 1995). The evidence supporting this is provided by a number of observations describing different effects of ethanol or it's metabolites.

Chronic ethanol ingestion by rats causes fatty changes in the acinar cell, which may be a result of disordered intracellular lipid metabolism, as cells have an increased cholesteryl ester content. Ethanol exposure was also shown to decrease secretory capacity of acinar cells and increase their lipase content (Wilson et al., 1988), which may lead to intracellular damage.

This leads to the theory that increased acinar cell enzyme production and accumulation may override endogenous protection mechanisms and has been supported by the recent

demonstration of increased intrapancreatic mRNA levels for digestive enzymes (lipase, trypsinogen, chymotrypsinogen) in rats fed ethanol for four weeks (Apte et al., 1998). Metabolites of ethanol have also been shown to injure pancreatic acinar cells. Fatty acid ethyl esters (FAEEs), non-oxidative products of ethanol metabolism found in high levels in the pancreas in intoxicated humans, infused into rats at levels comparable to that found in the human, produce pancreatic oedema, trypsinogen activation, and vacuolisation of acinar cells (Werner et al., 1997a).

3.2.2.1.2 Effects on ducts and ductules

Precipitates of proteins or 'plugs' have been demonstrated at high levels compared to controls in pancreatic juice of alcoholics with pancreatitis (Guy et al., 1983). These are hypothesised to cause obstruction and subsequent damage. Formation of these will depend upon concentration of proteins within the ducts. Lithostathine, a pancreatic secretory protein, is a major component of protein plugs. In the rat pancreas mRNA levels for lithostathnine increase after four weeks of ethanol exposure in the diet (Apte et al., 1996). Direct measurement of the same protein, previously called pancreatic stone protein (PSP), in the pancreatic juice of alcoholics with chronic pancreatitis has however produced conflicting results (Schmiegel et al., 1990; Multigener et al., 1985).

Duct permeability is also altered by ethanol exposure in a cat model, which may trigger autodigestion of the gland by allowing macromolecules including digestive enzymes to escape into the interstitium (Wedgwood et al., 1986).

3.2.2.1.3 Effect upon Sphincter of Oddi tone

Alcohol may interfere with Sphincter of Oddi motor activity, causing reflux of duodenal contents culminating in acinar cell damage (Goff, 1993). In the presence of a common channel (see Section 1.1.1) bile could also reflux into the pancreatic duct. However, the application of the common channel theory to explain human disease pathogenesis has been questioned, as a common channel does not always exist (Singh and Simsek, 1990).

3.2.2.1.4 Other effects of ethanol

Intragastric ethanol given to cats has been shown to cause a reduction in pancreatic blood flow and interstitial pH, an index of pancreatic ischaemia. This effect was also seen in animals with induced chronic pancreatitis (Toyama et al., 1997). The ethanol was given to produce levels in the cat similar to the seen in intoxicated humans. Ischaemia is postulated by the same authors to explain bouts of acute pancreatic inflammation.

Recently, expression of transcription factors NF-κB and AP-1, both involved in regulation of cytokine expression, have been demonstrated in acinar cells treated with ethanol in vitro (Gukovsky et al., 1998). Metabolites of ethanol, not ethanol itself, caused this expression, and while it is a finding which deserves further investigation, the effect may of course be non specific.

Oxygen free radicals (OFR's) are postulated by many to be involved in the initial cellular damage seen in acute pancreatitis, and there is some supporting circumstantial evidence (discussed in section 3.2.4.3.1). Proponents of this theory (Braganza, 1991), have not attempted to explain how aetiological agents such as ethanol could cause generation of OFR species. One study has however provided evidence that this is the case. Rats enterally fed ethanol for a period of 4 weeks not only had evidence of scattered pancreatic necrosis, but also had evidence of OFR species generation in the pancreas, stimulated specifically by ethanol (Iimuro et al., 1996).

In chronic pancreatitis, including disease caused by excessive ethanol production, a consistent pathological finding is some degree of lymphocytic infiltrate (Lankisch and Banks, 1998d). T cells, a lymphocyte sub-population, require antigen presentation in conjunction with MHC molecule expression on target cells. Whilst MHC expression on β cells in the pancreatic islets has been extensively investigated (van Dissel et al., 1998), only one group of investigators have explored the question of MHC expression upon pancreatic cells in pancreatitis. In pancreatic specimens from patients requiring operation for chronic

pancreatitis, MHC class I and II expression was found in 57% of patients. These included patients with alcohol induced disease. Interestingly expression was confined to ductal and ductular epithelium (Jalleh et al., 1993). One could postulate that MHC expression may also be increased in acute pancreatitis initiated by alcohol. This, however has not been investigated. Even so, cell mediated cytotoxicity is undoubtedly a factor in the pathogenesis of alcoholic chronic pancreatitis. Perforin mRNA, an activation marker for cytotoxic cells, is expressed by cells with a high frequency in the pancreata of human subjects with alcoholic chronic pancreatitis compared to controls (Hunger et al., 1997). The relevance of these findings to the pathogenesis of the acute form of the disease is unable to be tested in humans due to the unavailability of tissue specimens.

As can be seen from the large number of hypotheses concerning the early pathogenesis of alcohol induced pancreatitis, each supported by some evidence, the exact mechanism is not yet defined. It may be that several mechanisms act in synergy.

3.2.2.2 Cholelithiasis

The early pathogenesis of acute pancreatitis caused by cholelithiasis is generally thought to be due to hypertension in the pancreaticobiliary duct system, secondary to the passage of a gallstone or obstruction (Steer and Meldolosi, 1987). The first description of acute pancreatitis caused by an obstructing stone at the duodenal papilla was by Opie in 1901. He provided a full pathological description of a case of severe acute pancreatitis where a biliary calculus was found lodged at the duodenal papilla occluding the termination of both the biliary and main pancreatic duct ('common channel'). He also described a series of experiments upon dogs where bile had been injected into the pancreatic duct. All the seven dogs so treated developed differing degrees of pancreatitis evident upon pathological examination after one to seven days (Opie, 1901). He developed two theories, the first that obstruction so caused resulted in impaired flow from the pancreas which initiated damage, the second that obstruction in the presence of a common channel would allow reflux of bile into the pancreatic duct, so causing pancreatitis.

Gallstones are not often found impacted at the ampulla, either at operation or autopsy (Wilson et al., 1988). The opposite viewpoint is often quoted, supported by evidence that states that impaction occurs in 63% of patients with biliary pancreatitis (Acosta et al., 1980). However this data is heavily biased as it only included patients with clinical evidence of biliary obstruction.

As impaction is uncommon, the transient passage of gallstones past the confluence of pancreatic and bile ducts and the ampulla may initiate pancreatitis. This is supported by evidence demonstrating that 95% of patients with acute biliary pancreatitis have gallstones present in their faeces (Acosta and Ledesma, 1974).

Whether the passage of stones causes bile reflux into the pancreatic duct depends upon whether there is a 'common channel' formed by the confluence of pancreatic and biliary duct. This is not always the case (Lankisch and Banks, 1998b). Also the pancreatic duct is known to have a higher physiological pressure, so precluding the theoretical reflux of bile into the pancreas (Lerch et al., 1994). Therefore it may be that the initiation of inflammation in the pancreas is by obstructed pancreatic flow. This view is supported by an animal study where pancreatitis occurred on concomitant obstruction of the pancreatic and bile duct, whether or not bile reflux was present (Senninger et al., 1986).

Whatever the exact mechanism of disease initiation in the presence of total or partial obstruction, the acinar cell shows early signs of injury in experimental models (Lerch et al., 1992).

3.2.2.3 Viral infections

Mumps virus, Coxsackie, Hepatitis B, Cytomegalovirus, and the Herpes simplex I & II viruses are some of the agents believed to initiate pancreatitis (Parenti et al., 1996). Although the pathogenesis of viral acute pancreatitis is not discussed in standard texts and reviews, the pathogenic mechanism can be presumed to be similar to that observed in viral infection of any cell in the body.

Viruses increase MHC class I expression and initiate inappropriate class II expression on cells (Maudsley, 1995), resulting in the presentation of viral or previously unencountered self antigen, stimulating immune mediated damage to the host cell. Viruses can also directly injure cells, and infection of adjacent cells (of same or different type) may cause damage via increased local cytokine production, mediating MHC independent and/or dependent cell damage.

3.2.2.4 Drugs

There are a number of drugs associated with the development of acute pancreatitis. The reader is referred to other texts for a comprehensive list (McArthur, 1996), and the most well known are listed in the footnotes to table 1.1. The mechanism of action is thought to be direct cytotoxicity upon the pancreatic acinar cell or individual hypersensitivity (McArthur, 1996). There is no experimental evidence regarding this.

3.2.3 Events in the acinar cell

Irrespective of the nature of the aetiologic agent or it's exact mechanism of promoting disease initiation, the acinar cell is now believed to be the site of the early steps in the pathogenesis of acute pancreatitis (Lerch et al., 1992). Disruption of the mechanism of acinar cell secretion is believed to occur, leading to intracellular activation of pancreatic enzymes, and subsequent autodigestion. Evidence supporting this theory has recently been published. Trypsinogen activation, assessed by measurements of TAP, is known to occur prior to the appearance of detectable morphological or functional damage to the acinar cell (Frick et al., 1997).

Previous belief that pancreatitis was associated with acinar cell hypersecretion led to unsuccessful clinical trials with agents known to reduce output from the exocrine portion of the gland (Gjrup et al., 1992). However, animal models show that pancreatic exocrine secretion is reduced early in the course of acute pancreatitis, and recovers on resolution of disease (Neiderau et al., 1990).

In both *in vivo* and *in vitro* models of acute pancreatitis, it has been shown that an apparent

blockade to zymogen granule exocytosis occurs (Leach et al., 1990; Steer and Meldolosi, 1987). Zymogen granules accumulate within the acinar cell, and undergo abnormal fusion with lysosomes. This results in the formation of large vacuoles which contain both digestive enzymes and lysosomal hydrolases, an event termed 'co-localisation' (Steer and Meldolosi, 1987).

In the normal acinar cell, lysosomal enzymes are mostly segregated when processing occurs at the Golgi apparatus. The segregation is not complete as a considerable fraction of lysosomal enzymes escape processing in the Golgi (Hirano et al., 1991). However, studies evaluating the subcellular fractionation of the lysosomal enzyme Cathepsin B (known to activate trypsinogen (Greenbaum and Hirschkowitz, 1961)), indicate that in the early stages of acute pancreatitis the already incomplete segregation becomes more pronounced; half of the total cellular free Cathepsin B is recovered from the zymogen granule fraction of the cell (Watanebe et al., 1984; Saito et al., 1987).

Both incomplete segregation of enzymes and co-localisation are thought to lead to the activation of proenzymes, an event which may account for the fragility of the cytoplasmic vacuoles observed in animal models at the time that co-localisation occurs (Saluja et al., 1987; Saluja et al., 1989).

How the aetiologic agent causes this disruption within the acinar cell is the subject of ongoing investigation. Recently, increased levels of cytoplasmic calcium have been implicated as a mediating factor that may lead to zymogen granule activation (Ward et al., 1995). This does seem to occur prior to trypsinogen activation, as studies in animal models of acute pancreatitis show that therapeutic blockade of calcium influx reduces TAP release (Frick et al., 1997).

As described, disruption of intracellular trafficking and processing and resulting autodigestion are the events that are first detectable in the pathogenesis of acute pancreatitis. This initiation of disease leads to the generation of local inflammation.

3.2.4 Local inflammation - The role of leucocytes, cytokines, and other inflammatory mediators

The onset of local inflammation is seen morphologically as oedema, haemorrhage, necrosis, and further disruption of acinar cell architecture. Cellular infiltrates of inwardly migrated leucocytes are seen in the tissue interstitium. Inflammation is followed by death of acinar cells. This can occur by necrosis or apoptosis, mediated by cellular and soluble effectors. Chemoattraction of macrophages and neutrophils and propagation of a macrophage derived cytokine response are subjects covered in depth in this work, however, as a number of other factors may also play important roles in the propagation of local inflammation, they will also be discussed here. These include generation of oxygen free radicals (Sweiry and Mann, 1996), changes in microvascular perfusion mediated by the kallikrein-kinin system (Weidenbach et al., 1995), and complement activation (Weiser et al., 1996).

Most experimental studies evaluate the role of individual cellular infiltrates, cytokines, and other mediators of inflammation as key mediators playing the predominant role in the disease pathogenesis. It is important to realise that as all may play some part in upregulating local inflammation, it is likely that to some degree they all act in synergy.

Early in the course of disease there is inward migration of neutrophils and macrophages. The mechanisms of inflammatory cell recruitment and activation, including the role of cytokines in this process will now be discussed.

3.2.4.1 Leucocytes

Neutrophils migrate into the pancreas early in the course of the disease process. In experimental pancreatitis in rats, intravascular margination and tissue infiltration is seen as early as 3-3.5 hours after the induction of disease (Folsch et al., 1998; Schoenberg et al., 1990), and interstitial tissues are heavily infiltrated when assessed at 12 hours (Schoenberg et al., 1990). Neutrophils are shown to predominate over mononuclear cells in the inflammatory cell infiltrate early in the course of disease (Sandoval et al., 1996). The effect of neutrophil depletion in has been assessed in models of mild experimental pancreatitis. In

one study neutrophil depleted animals demonstrated a reduced inflammatory cell infiltrate and pancreatic oedema (Fink and Norman, 1996), whilst two other studies demonstrated both these aforementioned effects plus a reduction in acinar cell vacuolisation (Sandoval et al., 1996; Fujimoto et al., 1997), and acinar cell necrosis (Sandoval et al., 1996). A corresponding increase in the number of acinar cells undergoing apoptosis is also seen (Sandoval et al., 1996; Fujimoto et al., 1997), which suggests that neutrophils and their released products (e.g. NO, OFR's) are capable of regulating cell death response, playing a key role in the development of necrosis.

Macrophages are also seen in the inflammatory cell infiltrate in the course of acute pancreatitis, although they are not the most predominant cell early in the course of disease (Sandoval et al., 1996). Using immunohistochemical techniques it has been demonstrated that although macrophage infiltration starts soon after the induction of acute pancreatitis, maximal proliferation occurs 2 days after induction of disease (Goto et al., 1993). Intraperitoneal injections of a specific macrophage activating agent have been used to evaluate the effect of macrophage activation on the pancreas in rats. The experiments showed that macrophages alone can induce pancreatic damage. Although it is a crude estimate of damage, pancreatic water content was shown to be increased. Unfortunately the authors did not report histological effects (Andersson et al., 1997). Macrophage depletion in mice with mild pancreatitis results in significant attenuation of oedema, but no reduction in acinar cell vacuolisation, necrosis, or inflammatory infiltrate (Fink and Norman, 1996). Whilst not being the most prominent cell type in the inflammatory cell infiltrate seen in acute pancreatitis, macrophages are considered to play a crucial role in the pathogenesis of acute pancreatitis by their production of pro-inflammatory and regulatory cytokines (Kusske et al., 1996b; Kingsnorth, 1997b). This is based upon evidence evaluating the effects of cytokines (considered to be predominantly produced by the macrophage) in disease pathogenesis (see section 3.2.4.2).

It is pertinent to point out at this point that depletion of cellular components of the immune system will also cause depletion of the products of those cells, e.g. cytokines, chemokines, OFR's.

Lymphocytes are not considered to be a predominant pancreatic infiltrating cell in acute pancreatitis, being seen in chronic pancreatitis (Lankisch and Banks, 1998d). However, recently the effect of acute pancreatitis upon lymphocyte proliferation has been assessed. Studies indicate that lymphocytes, T lymphocytes, and CD4 and CD8 subsets are reduced in the early course of acute pancreatitis (Widdison and Cunningham, 1996; Pezzilli et al., 1997). This will be discussed in section 3.2.5.1.

3.2.4.2 Cytokines

Cytokines play a central role in the mediation of both the local and systemic inflammatory response seen in acute pancreatitis, causing activation of polymorphonuclear leucocytes, monocytes and macrophages and promoting their migration into tissue interstitium. Many of the recent advances in the understanding of the pathophysiology of acute pancreatitis have come from a greater understanding of the role of cytokines in the local and systemic manifestations of the inflammatory process.

This section will discuss the role of specific cytokines in the local inflammatory disease process in acute pancreatitis , and review evidence that suggests that certain cytokines are pivotal mediators of disease progression.

3.2.4.2.1 Tumour necrosis factor (TNF)

As discussed in section 2.4.5, TNF is a key cytokine with many pro-inflammatory effects of it's own. It also induces the release of other pro-inflammatory cytokines.

In rats, TNFα mRNA is expressed, and TNFα protein is expressed, produced, and released by pancreatic acinar cells in health. Both the p55 and p75 TNF receptor are expressed (Gukovskaya et al., 1997). The same series of investigations revealed that one of the effects that TNF has upon normal acinar cells is the activation of NFκB, so it is possible that the

effects of TNF are mediated, at least partially, by the activation of this transcription factor. As well as this possible role in the mediation of the effects of TNF, the blocking of NFκB activation is also shown to prevent increased expression of TNF mRNA in whole pancreatic mRNA extracts from rats with pancreatitis (Dunn et al., 1997), illustrating that NFκB may be a regulator of the production as well as effects of TNF. Controlled experiments also indicate that an effect of TNF upon normal pancreatic acinar cells *in vitro* is to mediate apoptosis, and this effect is also seen *in vivo* during experimental acute pancreatitis (Gukovskaya et al., 1997).

In contrast to the investigations of Gukovskaya *et al*, TNFα mRNA or protein could not be detected in healthy mouse pancreas (Norman et al., 1995a).

However, investigators agree that TNF expression in the pancreas is induced by (Fu et al., 1997; Norman et al., 1995), or increased by (Gukovskaya et al., 1997) the onset of experimental pancreatitis. This occurs as soon as 30 minutes after the induction of disease. It seems that increased TNFα is produced by both acinar cell and infiltrating macrophage (Gukovskaya et al., 1997). Markedly increased serum levels of TNFα have also been detected after the onset of acute pancreatitis (Hughes et al., 1995). These investigators also observed similar results in confirmed 'sterile' germ -free rats, so that the rise in TNF could be attributed to the pancreatitis, rather than the presence of endotoxin, as had been previously postulated.

To assess whether TNF has a pivotal role in the generation of local inflammation, antagonism of TNF by administration of anti-TNF polyclonal antibody has been investigated in animal models of acute pancreatitis. Studies demonstrate that prophylactic administration significantly reduces gross and microscopic pathology associated with pancreatitis including a reduction in inflammatory cell infiltrate(Gukovskaya et al., 1997; Hughes et al., 1996), whilst another study demonstrated minimal effect upon markers of local inflammation (Grewal et al., 1994). Treatment using TNF antiserum raised in rabbits increased rather

that attenuated pancreatic oedema in one rat model (Guice et al., 1991), however evidence that the antiserum used contains a blocking antibody to TNF is lacking.

Whilst there is no evidence that treatment using anti-TNF antibody after the initiation of the disease process is beneficial, the antagonism of TNF by recombinant soluble TNF receptor has been tested therapeutically and prophylactically in an animal model. Reductions in pancreatic oedema and serum markers of inflammation were noted, but no effects were seen upon the degree of necrosis, vacuolisation or inflammatory cell infiltrate in histological specimens of pancreas (Norman et al., 1996b). TNFR1 (p55) receptor knockout mice also show reduced pancreatic damage in necrotising acute pancreatitis (Denham et al., 1997b). Similar effects are seen with IL-1 receptor deficient animals, but no additive effect is seen with loss of both TNF and IL-1 receptor.

Recently, the effect of blocking macrophage production of TNF has been assessed using a specific synthetically produced molecule (CNI-1493) that inhibits TNFα mRNA translation (Denham et al., 1997a). This approach significantly reduces pancreatic necrosis in mice with mild and severe experimental disease. It must be noted that these effects may also be due to the reduction in IL-1, as both TNF and IL-1 mRNA levels were significantly reduced in treated animals, compared to diseased controls.

On the basis of the experimental evidence reviewed, it can be seen that as TNF is produced in the pancreas by both acinar cell and macrophage, and seems to contribute in no small part to the development of acute pancreatitis, it is possible to conclude that TNF plays a major role in the induction and propagation of local injury.

3.2.4.2.2 Interleukin-1 (IL-1)

IL-1 has many similar effects to TNF and as such is also a key cytokine in the regulation of immune responses (section 2.4.5). Although IL-1 can be produced by many non-immune cells, it's constitutive expression in the pancreas has not been shown (Fink and Norman, 1996; Fink and Norman, 1997). Conversely it appears that IL-1 receptors, IL-1ra, and IL-1 converting enzyme (ICE) are all expressed by normal healthy pancreatic tissue (Fink and

Norman, 1997). Studies show that IL-1 is produced in the pancreas early in the course of acute pancreatitis (Fu et al., 1997; Fink and Norman, 1997; Norman et al., 1997), and that the cells of origin are the infiltrating macrophages and neutrophil (Fink and Norman, 1996). By using IL-1 receptor gene 'knockout' mice that were unable to recognise or respond to IL-1, it has been shown that the presence of IL-1 receptor is required for maximal progression of acute pancreatitis, as evidenced by a reduction in acinar cell vacuolisation and necrosis, and reduction in parenchymal oedema and inflammatory cell infiltrate (Norman et al., 1996a). As already discussed, similar effects are seen with TNFR1 (p55) knockout animals, but the effect is not additive when both receptors are absent (Denham et al., 1997b).

An endogenous method of IL-1 regulation occurs as part of molecular interaction in the IL-1 system by a naturally occurring IL-1 receptor antagonist (IL-1ra) (Dower et al., 1994). Down regulation of IL-1 can also be produced by inhibition of ICE. Both these methods of IL-1 blockade have been tested in models of acute experimental pancreatitis, mostly from the same group of investigators.

In models of mild and severe acute pancreatitis administration of IL-1ra reduced serum amylase levels, pancreatic oedema, inflammatory cell infiltrate, and histological severity of necrosis in a dose dependant manner (Fink et al., 1997; Norman et al., 1995; Norman et al., 1995). Treatment also reduced serum levels of TNFα and IL-6 (Norman et al., 1995c). The development of disease seems not to be by a direct action of IL-1 on pancreatic acinar cells, as *in vitro* viability of acinar cells was not affected by incubation with IL-1 (Fink et al., 1997). These beneficial effects were seen when IL-1ra was given as prophylaxis and treatment. In contrast another group of investigators using a different model of experimental pancreatitis showed that administration of IL-1ra had no effect upon local consequences of inflammation. However, both the initial dose of IL-1ra used, and subsequent maintenance infusion dosage were below those shown to have beneficial effect in the previously described experiments (Tanaka et al., 1995).

Irreversible inhibition of IL-1 synthesis by administration of a novel ICE inhibitor, VE-13045, has been shown to have similar beneficial effects upon experimental disease. Again, cytokine production and amylase levels were reduced, whilst morphological changes in the pancreas were also significantly improved (Norman et al., 1997).

As discussed in the previous section, reduction in IL-1 (and TNF) production by blockade of mRNA processing reduces severity of pancreatic necrosis (Denham et al., 1997a).

It can be seen from the presented evidence that IL-1, like TNF, plays a major role in the upregulation of the local inflammation seen in the pathogenesis of acute pancreatitis.

3.2.4.2.3 Platelet activating factor (PAF)

In 1986 it was shown that the mammalian pancreas could synthesise PAF, by demonstrating the incorporation of radiolabelled acetate into the PAF molecule by isolated Guinea pig pancreatic lobules stimulated by the CCK analogue, cerulein. PAF released into the culture medium was shown to have incorporated radioactivity, indicating that the isolated lobules were synthesising PAF rather than releasing the stored form (Solling and Fest, 1986).

Involvement of PAF in acute pancreatitis was demonstrated *in vivo* in 1989 by injection of PAF into the superior pancreatoduodenal artery of rabbits. It resulted in dose dependent elevations of serum amylase and morphologic changes associated with acute pancreatitis, including oedema, neutrophil infiltration, and acinar cell vacuolisation and necrosis. Electron microscopy showed the damage at ultrastructural level, first zymogen granules accumulating at the acinar portion of the cell, then vacuoles developing containing cellular debris. The damage induced progressed over the studied time, from 3-72 hours (Emmanuelli et al., 1989). In the same series of investigations it was also shown that the administration of a PAF antagonist inhibited these effects, demonstrating that the changes were due to the specific effect of PAF. While this may suggest that the local inflammatory response induced may be dependant upon a direct effect of PAF upon the acinar cells, it is also possible that secondary mediators released by activated inflammatory cells within the pancreas could play a key role.

Systemically administered PAF has also been shown to have an aggravating effect upon established experimental disease. Animals treated with PAF show an increase in inflammatory cell infiltrate and development of intrapancreatic haemorrhage as compared to those with animals with acute pancreatitis treated with placebo (Yotsumoto et al., 1994). Intraperitoneal injection of exogenous PAF also causes biochemical and morphological changes similar to that seen in acute pancreatitis. The same study demonstrated that a PAF antagonist given prophylactically gave a protective effect, reducing tissue damage (Tomaszwska et al., 1992).

It has also been shown that therapeutic PAF antagonism reduces histological pancreatic damage. The antagonist was given 30 minutes after the induction of pancreatitis, when early histological changes in the pancreas are already occurring (Formela et al., 1994) .

PAF is therefore also considered to have a major role in the mediation of local pancreatic damage (Kingsnorth et al., 1995).

3.2.4.2.4 Interleukin 10 (IL-10)

As discussed in section 2.4.5, IL-10 plays an important role in regulating immune and inflammatory responses though it's effect upon several cellular components of the immune system. IL-10 is known to be secreted by many immune cells, and hepatocytes and intestinal epithelial cells (Napolitano et al., 1997; Ishizaka et al., 1996). However it's expression by any healthy constituent cells of the pancreas has not been assessed. As previously discussed, macrophages infiltrate the pancreas in acute pancreatitis, and macrophages are a predominant secretor of IL-10.

Recently there have been well designed experimental studies which have assessed the role of IL-10 in the pathogenesis of acute pancreatitis and evaluated it's potential as a therapeutic agent.

Pre-treatment with intraperitoneal IL-10 in mice with caerulein induced pancreatitis caused reduction of serum amylase rise and the extent of acinar necrosis (Van Laethem et al., 1995). Other histological parameters were not affected. Both effects were seen after a delay

of 6 hours. In another study treatment after the induction of caerulein induced pancreatitis in rats produced similar effects, significantly reducing pancreatic oedema compared with controls (Rongione et al., 1997). Both studies also looked at TNFα mRNA and protein within pancreatic tissue and found their expression to be significantly attenuated in treated animals, consistent with the assumption that IL-10 down-regulates the inflammatory response at least in part by reducing expression of TNFα.

A beneficial effect of IL-10 was also demonstrated in a model of severe necrotising pancreatitis induced with a CDE diet in mice (Kusske et al., 1996a). Again serum enzymes and local pancreatic damage were seen to be attenuated, when assessed at 54 and 80 hours post disease onset. Oedema, inflammation, necrosis, and haemorrhage were all significantly reduced in treated animals compared to controls. Again, although significant effects were seen earlier in the course of disease, a greater reduction was seen after a delay. These effects were regardless of whether IL-10 was given as pre-treatment or after disease commenced.

These results suggest that IL-10 plays an important role in the down regulation of the local inflammatory response in acute pancreatitis as revealed by the attenuation of it's deleterious effects upon the pancreas.

3.2.4.2.5 Chemokines

As discussed in section 2.4.5.5 chemokines are a group of chemoattractant cytokines involved in the activation and trafficking of inflammatory cells. As the inflammatory cell infiltrate is a predominant feature of local inflammation in acute pancreatitis, chemokines must be involved in disease pathogenesis. To date, there has been a limited amount of useful experimental work in this area. Serum IL-8, a C-X-C or α-chemokine (chemoattractant for neutrophils), has been measured in patients with acute pancreatitis, and is found to be significantly higher in patients with complicated and uncomplicated disease compared to healthy controls (Gross et al., 1992; De Beaux et al., 1997). Whilst this implicates the

involvement of IL-8 in disease pathogenesis it gives little detailed insight into it's exact role, which can only be inferred. Recently two chemokines, *mob-1* (a C-X-C, α-chemokine), and *mcp-1*, (a C-C, β-chemokine, chemoattractant for macrophages) have been identified as being involved in acute pancreatitis. mRNA from both chemokines was identified in rat pancreas after induction of acute pancreatitis by caerulein. The source of both was identified by immunocytochemistry as the acinar cell (Grady et al., 1997). Acinar cells did not express these two chemokines in healthy animals, although some expression in ductular cells was identified. The same investigators provided some evidence that *mob-1* and *mcp-1* were important in disease pathogenesis by showing that blockade of their expression *in vivo* prevented the increases in pancreatic oedema seen with this model. However, this may not have been a direct effect, as the agent used to block their expression (Pyrrolidine dithiocarbamate, PDTC) is a potent inhibitor of NFκB activation, so expression of other cytokines known to be important in inflammation could also have been affected . The expression of TNF, for example, is known to be reduced by blockade of NFκB activation (Dunn et al., 1997). PDTC also an antioxidant, so reduction of OFR's may have reduced inflammation also (see section 3.2.4.3.1). If blockade of chemokine expression is shown to be the main factor in reducing the pancreatic inflammation, then this may be due to reduced chemoattraction for neutrophil and macrophage produced by both chemokines.

Another study, using mice with deleted β-chemokine receptors, showed that although no effects on diseased pancreas morphology were seen, reduced neutrophil influx occurred, as reflected by decreased levels of the leucocyte enzyme, myeloperoxidase (Gerard et al., 1997).

Both these studies, as could be expected from the known functions of chemokines, demonstrate that chemokines may play an important role in the pathogenesis of acute pancreatitis.

3.2.4.3 Other inflammatory mediators/effectors

3.2.4.3.1 Oxygen free radicals (OFR's)

The exact source of OFR species in acute pancreatitis is considered to be varied. Different pathological mechanisms have been hypothesised. These include the activation of the xanthine oxidase system by disease, production by damaged mitochondria, cytochrome P450 oxidation of products of cell damage, and production by activated PMN leucocytes (Schoenberg et al., 1995). The latter must be a major source of OFR's as production by neutrophils is well known, and there is always a heavy infiltrate of these cells in the pancreas affected by inflammation.

Evidence for the involvement of oxidative stress in the early pathogenesis of AP has been provided by numerous studies, mostly producing indirect evidence of free radical excess by the detection of depleted glutathione or increased products of lipid peroxidation in animal (Dabrowski and Chwiecko, 1992; Schoenberg et al., 1990; Nonaka et al., 1989), and human disease (Braganza et al., 1995).

In attempts to provide further evidence regarding the role of OFR species in disease pathogenesis, and to assess possible therapeutic benefits, agents have been used in experimental models to inhibit the formation of free radicals, or reduce their tissue levels. Some agents show beneficial effects as evidenced by a reduction in histological damage, gland oedema, and serum pancreatic enzymes (Wang et al., 1995; Wisner et al., 1988; Neiderau et al., 1992; Wisner and Renner, 1988; Nonaka et al., 1991; Schoenberg et al., 1994), while others show minimal or no benefit (Hotter et al., 1995; Furukawa et al., 1994; Lankisch et al., 1989; Steer et al., 1991).

The evidence that oxidative stress is a key step in local disease pathogenesis is indirect and conflicting, although this area of investigation is at an early stage.

3.2.4.3.2 Kallikrein-kinin system

Kallikrein is normally present in the pancreas in it's active precursor form. Once activated it produces active bradykinin and Lys-bradykinin by cleaving them from their inactivating α-2

macroglobulin, to which they are physiologically bound. Kinins are known to increase microvascular permeability and vasodilatation , as well as causing the release of a number of other mediators of inflammation, including leukotrienes, prostaglandins, and thromboxanes. Kinins are released during experimental acute pancreatitis (Ryan et al., 1964), and are known to aggravate biochemical and histological markers of local disease progression when infused into animals with pancreatitis (Yotsumoto et al., 1993). This type of evidence led to the hypothesis that kinins play a key role in disease pathogenesis.

However, evidence from studies using kinin inhibitors in experimental acute pancreatitis is less conclusive. In a study where disease is induced by an ischaemia-reperfusion type injury administration of a kinin antagonist has shown a beneficial effect on the pancreatic microcirculation (Hoffmann et al., 1996a). In other studies the effect upon oedema and histological damage has not been consistent. Reduction in oedema by blocking bradykinin has been seen (Griesbacher et al., 1993), although most studies indicate that kinins do not play a large role in disease pathogenesis as local oedema and injury are either not affected (Lerch et al., 1995), or are worsened by kinin antagonism (Weidenbach et al., 1995).

The evidence for the involvement of kinins in the pathogenesis of acute pancreatitis is conflicting and at best, circumstantial. Earlier interest in kinins as key mediators of pancreatic inflammation doesn't seem to have been sustained.

3.2.4.3.3 Complement activation

Complement is an important component of immune responses, in particular, components of non-specific immunity (see section 2.4.2.1). Several investigators have suggested a key role for various components of the complement system as pivotal mediators of local inflammation in acute pancreatitis. This has led to the testing of complement inhibition in experimental acute pancreatitis, and one study in mice deficient in the complement component C5.

However, in a model of mild disease, administration of soluble complement receptor type I (sCR1), a potent inhibitor of the classical and alternate complement pathways, failed to

ameliorate pancreatic oedema or serum markers of disease (Weiser et al., 1996). Another inhibitor of the complement cascade, plasma C1 esterase inhibitor (on it's own or in combination with Antithrombin III) also had no effect in mild or severe models of disease (Yamaguchi et al., 1997).

In a recent study in mice with pancreatitis induced by duct ligation the authors compared mice with mutations in the Hc gene on chromosome 2 with wild type mice. Mice with Hc mutations lack circulating levels of C5. Although the authors demonstrated a significant decrease in pancreatic oedema and in leucocyte infiltration in the mutant animals, differences were not striking, and the mutant animals still has significantly higher levels of oedema and histologically evaluated leucocyte infiltration in their pancreata than sham operated animals .

There is no strong evidence that complement, by itself, is a key factor in disease pathogenesis, but components of the complement system have effects on neutrophil migration and induction of other inflammatory mediators such as Interleukin-1 (Frank and Fries, 1991), so the complement system must be involved to some degree in the development of local inflammation.

3.2.5 Systemic Inflammation - The role of leucocytes, cytokines, and other inflammatory mediators

In the natural course of acute pancreatitis the disease may progress no further than a local inflammatory process of the pancreas. However in cases of severe acute pancreatitis, a systemic inflammatory response can occur, resulting in distant organ dysfunction and failure. This process appears to be much the same process as seen in severe sepsis and burns and has been termed the Systemic Inflammatory Response Syndrome (SIRS). Dysfunction and failure of the respiratory system, renal system, cardiovascular system, and coagulation system can occur.

A number of mechanisms of this upregulation of the inflammatory response have been proposed and investigated. These include cellular activation and pro-inflammatory cytokine

release, regulated by anti-inflammatory cytokines released by the macrophage and T-cells (Kusske et al., 1996b; Kingsnorth, 1997b). However, as with the mediation of local inflammation, a number of other mediators may also play a role in the generation of systemic inflammation, and these warrant mention here, as they are likely to act in conjunction with other inflammatory pathways, and have been investigated as having key roles in the pathogenesis of the systemic inflammation that occurs with severe acute pancreatitis.

3.2.5.1 Leucocytes

Neutrophil infiltration and activation in organs distant from the pancreas is considered to be an important mediating step in the development of pathological damage on those organs. The metabolic activity of circulating neutrophils is demonstrated to be increased in both mild and especially in severe acute pancreatitis (Widdison and Cunningham, 1996). Neutrophils are also known to infiltrate the lung in models of acute lung injury which are similar to ARDS (Takahashi et al., 1990). Indeed in the lung injury produced secondary to necrotising pancreatitis in rats, neutrophils are found heavily infiltrating the lung tissue, as soon as three hours after induction of disease (Folsch et al., 1998; Murakami et al., 1995). In 1982 it was shown that depletion of neutrophils prevented the increased permeability normally seen in sheep lung vasculature after the induction of experimental pancreatitis (Barie et al., 1982). Rats depleted of neutrophils by anti-neutrophil serum (ANS) have no histologically detectable lung injury and demonstrate reduced lung surface chemolumninescence attributable to OFR production by activated neutrophils (Murakami et al., 1995). Another study using polyclonal and monoclonal anti-neutrophil Ab has shown similar results upon the lung injury, and in addition demonstrated a significant improvement in survival rate of treated animals, evident in rats treated prophylactically and therapeutically (Inoue et al., 1995). Reduction in lung neutrophil sequestration following experimental acute pancreatitis has been demonstrated using prostaglandin (Yamanaka et al., 1997). This also results in reduced lung injury as demonstrated by a reduction in lung

oedema. It was postulated by the authors that the effect of the prostaglandin treatment was due to it's depletion of neutrophils, although of course, it may be via another mechanism of action of prostaglandin, such as affecting the permeability of pulmonary vasculature.

Indirect evidence of the involvement of neutrophil activation in severe acute pancreatitis complicated by systemic inflammatory injury is provided by studies of serum markers of neutrophil activation in patients. One such marker, polymorphonuclear elastase, is found to be increased in patients with severe disease associated with lung injury and sepsis (Mora et al., 1997).

This study also provided indirect evidence of macrophage activation in pancreatitis associated with systemic complications, by the measurement of a marker of macrophage activation, Neopterin, which was found to be increased in patients with severe disease.

Although there is some evidence that blood monocytes, precursors of macrophages, have impaired phagocytic activity in patients with acute pancreatitis associated with organ failure (Larvin et al., 1993), it is difficult to reconcile this with other evidence indicating that other aspects of macrophage function are enhanced and that immune function is increased early in acute pancreatitis (Widdison and Cunningham, 1996). It could be that the capacity of phagocytic cells, which remove particles including bacteria and protease-antiprotease complexes, becomes saturated during severe acute pancreatitis.

Alveolar macrophages isolated from the lungs of animals induced with experimental pancreatitis show a strong cytotoxic effect upon endothelial cells *in vitro*, an effect thought to be due to macrophage NO production (Tsukahara et al., 1996). Also, bronchoalveolar macrophages from rats with lung damage following acute pancreatitis show increased expression of cytokine induced neutrophil chemoattractant (CINC), indicating macrophages are activated systemically during severe acute pancreatitis.

The main role of the macrophage in the development of a systemic inflammatory response is considered to be it's release of pro-inflammatory and regulatory cytokines (Kusske et al., 1996b; Kingsnorth, 1997b). Isolated peripheral blood mononuclear cells (PBMC's), a

mixed cell population comprised largely of monocytes and lymphocytes have been shown to increase their *in vitro* release of pro-inflammatory cytokines in patients with severe disease (McKay et al., 1996; De Beaux et al., 1997). Evaluation of the evidence indicates that PBMC production of TNFα and IL-6 are both increased in patients with systemic complications. One of the studies did not actually find that levels of TNFα were increased in their patients with severe disease, but the patients they characterised as severe were a mixed group consisting of patients with local complications and reversible renal impairment (De Beaux et al., 1997), rather than with properly defined organ failure as in the other study (McKay et al., 1996).

Lymphocyte subsets are shown to be altered in patients with severe acute pancreatitis. Significant reductions in CD4 and CD8 counts are seen (Widdison and Cunningham, 1996; Curley, 1996), which return to normal after resolution of disease (Curley, 1996). Similar results have been noted with the SIRS associated with burns, sepsis, and trauma. The significance of these findings is unclear, as circulating lymphocytes are only a small proportion of the total lymphocyte population, and redistribution to lymphoid and other tissues would reduce the circulating fraction measured in these studies. This could though be taken as evidence of immune compromise during the systemic inflammatory response, especially as further evidence of reduced T cell function is provided by the demonstration of reduced IL-2 production by T cells *in vitro* from mice with severe, CDE diet induced acute pancreatitis (Curley et al., 1996). However, a study in patients with acute pancreatitis shows that the CD4:CD8 ratios in both severe and mild acute pancreatitis are unchanged, indicating that host immunity may not be impaired; in immunosuppressed patients CD4 lymphocytes are depleted and the CD4:CD8 ratio increases. In addition the same study (Widdison and Cunningham, 1996) demonstrates that IL-2 receptor expression is increased on lymphocytes, indicating that lymphocytes are activated and undergoing clonal proliferation, a normal immune response to (processed) antigen.

3.2.5.2 Cytokines

The pro-inflammatory and regulatory role of the cytokines TNF, IL-1, PAF, and IL-10 in the pathogenesis of systemic complications associated with acute pancreatitis are now discussed.

3.2.5.2.1 Tumour necrosis factor (TNF)

As discussed above PBMC secretion of TNFα is increased in patients with organ failure associated with acute pancreatitis (McKay et al., 1996). Levels of the two soluble TNF receptors, shed from cell membranes, are also shown to be increased in patients with manifestations of organ failure (De Beaux et al., 1996), implicating TNF as having a central role in the pathogenesis of the systemic complications of acute pancreatitis.

TNF is known to increase pulmonary vascular permeability, oedema, and decrease oxygen diffusion across the alveolar basement membrane (Stephens et al., 1988). It may do this by decreasing gene transcription of lung surfactant C (Rink and Kirchner, 1996), reduction of which will increase lung compliance and reduce oxygen transfer. When administered in quantities similar to those produced endogenously in response to LPS, TNF is also known to induce tachypnoea, hypotension, metabolic acidosis, and acute tubular necrosis in the kidney (Tracey et al., 1986).

The data outlined above serves to implicate TNF as a central mediator of severe systemic disease in humans. More direct evidence is supplied by animal studies. Reducing endogenous TNF production or using TNF antagonists is shown to improve clinical recovery and survival rates, which can be regarded as crude indicators of the severity of systemic complications.

Confirmed blockade of TNFα expression at the gene level by inhibiting NFκB is shown to improve (unspecified by authors) clinical recovery of experimental animals with severe acute pancreatitis (Dunn et al., 1997).

Antagonism of TNF by using polyclonal anti-TNF antibody also improves survival of

experimental animals, prophylactic administration improving survival from 15 to 85% (Hughes et al., 1996). Similar improvements in mortality rates are observed using a recombinant form of the type I receptor to decrease TNF levels (Norman et al., 1996b), and using mice devoid of the type I receptor gene (Denham et al., 1997b).

One study in the literature has supplied evidence to contradict some of the above. Antagonism using rabbit derived antimurine TNF antiserum was shown to increase rather than attenuate pulmonary oedema in rats with caerulein induced acute pancreatitis (Guice et al., 1991). Although the anti-serum was shown to neutralise TNF and cross react with rat TNF it cannot be excluded that the other elements of the antiserum had some effect, calling into question the interpretation of the data.

3.2.5.2.2 Interleukin-1 (IL-1)

IL-1 antagonism produces similar results in experimental animals. Prophylactic and therapeutic administration of IL-1ra to mice with severe (CDE diet induced) acute pancreatitis, results in significant reductions in mortality (Norman et al., 1995b). Experiments in rats have confirmed this finding (Tanaka et al., 1995). Interestingly both investigations demonstrated an improvement in histological parameters of lung damage normally seen in the models studied, and an improved urine output was also seen with treatment in the studies in rats, indicating that haemodynamic shock was ameliorated. Levels of other pro-inflammatory cytokines, TNF and IL-6, were also seen to be reduced. Blockade of IL-1 synthesis by the IL-1 Converting enzyme (ICE), and deficiency of the IL-1 receptor also improves mortality and reduces associated cytokine levels in rats with severe pancreatitis (Fu et al., 1997; Norman et al., 1997).

3.2.5.2.3 Platelet activating factor (PAF)

The involvement of PAF in the pathogenesis of the systemic sequelae of severe acute pancreatitis has also been assessed using specific PAF antagonists in animals and in humans. In experimental animals a modest but significant improvement in survival rates were seen on treatment with a PAF antagonist. A 66% improvement in a low survival rate (30%) was

noted at 48 hours in rats with severe acute pancreatitis (Dabrowski et al., 1995). Lung injury is also ameliorated by treatment with a PAF antagonist given before (Zhou et al., 1992) and after the induction of disease (Galloway and Kingsnorth, 1996).

A platelet activating factor antagonist, Lexipafant ®, which was the only PAF antagonist that had been shown to be effective after induction of acute pancreatitis has been tested in two phase II trials in human disease (McKay et al., 1997; Kingsnorth et al., 1995).

In both studies clinical outcome measures and inflammatory markers were used to assess effect upon the disease. Organ failure scores were used to assess the effect of the PAF antagonist in downregulating the systemic inflammatory response.

Both studies were double blind and placebo controlled, and both showed that the PAF antagonist reduced incidence and severity of organ failure in the treated groups. Levels of serum IL-8 and IL-6 were also reduced in the treated cohort (Kingsnorth et al., 1995). Numbers however were relatively small, only 60 patients in the studies combined having organ failure. Nevertheless, results were deemed encouraging and a Phase III trial was undertaken (Kingsnorth for the British Acute Pancreatitis Study Group, 1997). Patients with predicted severe acute pancreatitis, received an infusion of PAF antagonist for up to 7 days after study entry.

Again, effects upon organ failure score were seen. Mortality was reduced in the treated cohort, especially in the group of patients (n=205) treated within 48 hours of the onset of their disease, it being reduced from 20.4% to 9.3% ($p<0.04$). These results are subject to confirmation by a further Phase III trial which is at present ongoing.

3.2.5.2.4 Interleukin-10 (IL-10)

IL-10 is postulated to have an important role as a regulatory mediator in the systemic complications associated with severe acute pancreatitis through it's ability to affect various components of the inflammatory response (see section 2.4.5.4).

Levels of serum IL-10, when measured in patients with acute pancreatitis, have been shown

to be different in patients with severe as opposed to mild disease. In a group of 45 patients admitted with acute pancreatitis (mostly studied from the onset of the disease), levels were shown to be significantly lower on day 1 in those with severe disease as compared to those with mild disease, and whilst levels in those with mild disease rapidly decreased over the next 3 days of their illness, levels in the patients with severe disease did not change significantly until the 4^{th} and 5^{th} days (Pezzilli et al., 1997b).

Work in animal models has provided limited data on the effect IL-10 therapy has upon the development of systemic complications. Only one of the three published studies has used an animal model of sufficient disease severity to study mortality. In mice with CDE diet acute pancreatitis both prophylactic and therapeutic administration of IL-10 reduced mortality to zero in study animals, compared to 40% in control animals (Kusske et al., 1996a).

This data suggests that IL-10 plays a key role in the regulation of the inflammatory response seen in severe acute pancreatitis.

3.2.5.2.5 Chemokines

Serum levels of the C-X-C (α) chemokine, IL-8, have been compared in patients with severe disease associated with systemic complications and patients with mild disease. The one study with sufficient numbers and patient characterisation reported that levels of IL-8 were raised in those with systemic complications compared to those with mild disease (McKay et al., 1996).

Only one study has assessed the role chemokine expression has upon the systemic inflammatory response in severe acute pancreatitis. In mice with caerulein induced acute pancreatitis, genetic deletion of the β-chemokine receptor (CCR1) is associated with protection from the pulmonary damage associated with the disease, as assessed by lung oedema, tissue myeloperoxidase activity, and alveolar membrane thickening and permeability (Gerard et al., 1997).

3.2.5.3 Other inflammatory mediators/effectors

3.2.5.3.1 Oxygen free radical species

As well as having effects upon local disease, OFR's are postulated to be involved in the pathogenesis of the systemic inflammatory response (Braganza et al., 1995; Sweiry and Mann, 1996). The mechanisms are presumed to be the same as discussed in section 3.2.4.3.1. Superoxide radicals have been detected in the lung of rats with experimental acute pancreatitis, and this evidence seems to indicate that they are produced, at least in part, by neutrophils (Murakami et al., 1995). Lung injury can be ameliorated by reducing OFR species during the course of experimental disease (Guice et al., 1989). Data in other experimental models has shown that mortality rate, a crude indicator of multiple organ failure resulting from SIRS, is reduced in some experimental models by the administration of similar therapy (Schoenberg et al., 1994; Nonaka et al., 1991).

Evidence that this situation is applicable to the pathogenesis of the human form of the disease is also scanty. There has been no evidence produced to indicate OFR excess in severe forms of the disease, and one report that experimental treatment with detoxifying enzymes reduces severity of disease (Braganza, 1991). Patient numbers were very small, limiting the conclusions that could be drawn.

3.2.5.3.2 Kallikrein-kinin system

The involvement of kinins in the upregulation to a systemic inflammatory response has been suggested for many years, and Trysalol, an inhibitor of Kallikrein, the precursor of kinins, was tested as a therapeutic agent in the 1950's (Kellermeyer and Graham, 1968). Recent evidence has not confirmed that kinins play a major role in the development of systemic complications of acute pancreatitis. However, animals induced with a severe form of experimental pancreatitis do show maintenance of haemodynamic function (Kanbe et al., 1996), and improved survival rate (Hoffmann et al., 1996b; Frank and Fries, 1991b) on administration of a kinin antagonist. This effect of kinin antagonism can be predicted as it is known that kinins cause systemic vasodilatation. Inhibition of Kallikrein by a C1 esterase

inhibitor is discussed below (section 3.2.5.3.5).

3.2.5.3.3 Nitric oxide

Endogenous nitric oxide (NO) is involved in immunomodulation, mediation of endothelium-dependent relaxation of blood vessels, and functions as a neurotransmitter (Schmidt and Walter, 1994). The first two of these three classic functions of NO are probably important in the pathogenesis of acute pancreatitis, but it's precise role is at an early stage of investigation. In the local inflammation produced by acute pancreatitis, NO treatment seems to mostly improve markers of disease severity (Werner et al., 1997b; Molero et al., 1995b). However, it has been advocated that NO functions as a mediator to worsen the systemic inflammation associated with acute pancreatitis (Tsukahara et al., 1996). In animals induced with severe pancreatitis, alveolar macrophages expressed high levels of NO synthase (NOS) mRNA, high levels of NO, and demonstrated NO mediated cytotoxicity (Tsukahara et al., 1996). Conflicting evidence has been produced by other investigators (O'Donovan et al., 1995), and it seems unlikely that NO functions as a mediator to upregulate systemic inflammation when NO has excited considerable interest as an adjuvant treatment for the adult respiratory distress syndrome (ARDS) (Joillet et al., 1997).

3.2.5.3.4 Proteolytic enzymes

Since it was first advocated by Chiari in 1896 that the systemic complications of acute pancreatitis could be explained by systemic spillage of pancreas-derived proteolytic enzymes (Chiari, 1896), this theory has maintained considerable interest, and is still quoted in textbooks and authoritative reviews upon the subject (Mergener and Baillie, 1998). However therapies based upon this theory have had little success, and it is clear that pancreatic enzyme secretion is diminished during acute pancreatitis (Neiderau et al., 1990). Evidence that an aberrant leucocyte response occurs (Larvin et al., 1993; Barie et al., 1982), has led to the more attractive suggestion that activated macrophages, and inflammatory mediators play the major role in systemic complications of acute pancreatitis (Kusske et al., 1996b; Kingsnorth, 1997b; Rinderknecht, 1994b; Rinderknecht, 1988b).

Recently, the role of the pancreatic enzyme trypsin in the development of the systemic inflammatory response, has again been investigated (Acioli et al., 1997). It relates to it's ability to cleave components of the complement system, C3 and C5, resulting in the generation of the chemotactic chemokines C3a and C5a (Frank and Fries, 1991). Serum trypsin levels were seen to increase as complement activity reduced, producing a negative correlation. This was taken as evidence that trypsin plays an important role in complement mediated neutrophil accumulation in the lungs-see below (Acioli et al., 1997). This only suggests a relationship, a point acceded to by the authors.

3.2.5.3..5 Complement activation

As with the mediation of local inflammation, it would be surprising if complement didn't have some role to play in the development of the systemic inflammation seen in severe acute pancreatitis, due to it's multiple effects such as those involving neutrophil chemotaxis.

The only data regarding it's function in the systemic inflammation seen in severe acute pancreatitis is when complement inhibitors have been used in animal models of the disease. Complement inhibition fails to affect the pulmonary damage of experimental acute pancreatitis as assessed by oedema and vascular permeability (Acioli et al., 1997; Weiser et al., 1996), although it does reduce myeloperoxidase levels and neutrophil sequestration in the lung (Acioli et al., 1997). This latter effect conforms with the known function of complement as a neutrophil chemotactic agent.

Complement inhibition by inhibiting the function of C1 esterase is shown not to affect survival of taurochlorate induced pancreatitis in the rat, however when used in combination with Antithrombin III, survival is increased when measured at 72 hours (Yamaguchi et al., 1997). No difference was seen in the pathological damage to lungs, kidneys or livers, and the reason for this effect is unknown, although the authors speculate that it is due to inhibition of kallikrein by the inhibitor (C1 INH).

3.2.5.3.6 Endotoxaemia

Endotoxin, the lipopolysaccharide associated with the cell membranes of gram negative organisms, causes hypotension and organ failure similar to that seen in acute pancreatitis when injected into animals (Parillo, 1993). Endotoxaemia can be detected in the serum of patients with acute pancreatitis (Curley, 1996; Exley et al., 1992), and is present in higher levels in those with severe disease, which is associated with organ failure. Endotoxaemia without bacteraemia is well recognised in acute pancreatitis (Foulis et al., 1992), and may result from local sepsis or translocation of gut derived endotoxin (see below).

3.2.6 Sepsis

The SIRS in severe acute pancreatitis can in itself cause significant morbidity and mortality, but progression of the disease to involve sepsis often heralds irreversible organ failure and death (Isenmann and Buchler, 1994).

Infectious complications occur in patients who have SIRS (Runkel, 1996), and are most common in patients who have developed pancreatic necrosis (Isenmann and Buchler, 1994).

3.2.6.1 Bacterial translocation

The bacterial spectrum of colonising organisms is similar to that of gut flora, and it is widely accepted that the gastrointestinal tract is the source of infection (Banks, 1997; Isenmann and Buchler, 1994).

Various investigators have studied sepsis occurring in animal models of severe acute pancreatitis. All confirm that pathogens are intestinal in origin. Severe acute pancreatitis has been shown to increase the rate of bacterial contamination of intestinal lymph nodes and pancreas (Runkel et al., 1995; Runkel et al., 1990). Pancreatitis also promotes bacterial translocation into mesenteric lymph nodes (Widdison et al., 1994; Runkel et al., 1991). This evidence suggests that sepsis in acute pancreatitis is due to bacterial translocation from the gut, occurring via haematogenous or lymphatic routes.

The exact mechanism of bacterial translocation may be due to disruption of the intestinal flora, loss of gut-barrier function, impairment of host defence, or a combination of all three.

3.2.6.2 Disruption of intestinal flora

Studies in animal models show that the intestinal flora is altered following the onset of acute pancreatitis. The bacterial flora changes in as little as two days following the onset of acute pancreatitis. A greater proportion of pathogenic gram negative aerobic bacteria are noted first in the large bowel , then the jejunum and ileum (Widdison et al., 1994). This change in enteric flora is followed by bacterial translocation (Muncy et al., 1993). Pancreatitis also significantly reduces gut transit time (Muncy et al., 1993; Runkel et al., 1991). This may promote the alteration of bacterial flora and subsequent translocation, as pharmacological depression of gut motility produces a similar sequence of events (Runkel et al., 1993).

3.2.6.3 Gut-barrier function

Loss of the physical gut barrier function provided by the mucosa is suggested by experiments demonstrating that intestinal permeability to both fluorescent beads (Medich et al., 1993) and enteric bacteria (Widdison et al., 1994; Kazantse et al., 1994) is increased during acute pancreatitis. The mechanism of this is unclear, but it may be secondary to impaired gut wall blood supply which is impaired by acute pancreatitis, or the fact that reduced gut motility provides increased contact time between luminal contents and mucosa, thus increasing the chance of permeation.

3.2.6.4 Impairment of host defence

The reduction in the ability of the host immune system to deal with bacteria may also play a role in the development of sepsis. The evidence that this is important is circumstantial, but it is an attractive concept. CD3, CD4, and CD8 positive lymphocytes are reduced (Curley, 1996), and monocyte phagocytosis is impaired (Larvin et al., 1993) in patients with severe acute pancreatitis, with restoration of normal levels of lymphocytes on recovery.

Impairment of the local clearance of bacteria from the pancreas has been noted in an animal model of acute pancreatitis, this could be countered by the administration of levimasol, indicating that the impairment of clearance was due to impaired phagocytic activity (Widdison et al., 1992).

3.3 Hereditary pancreatitis (HP)

To complete the discussion on the pathogenesis of acute pancreatitis, recent developments in the understanding of hereditary pancreatitis will be reviewed. The suggested molecular mechanism involved in the development of HP may have relevance to the pathogenesis of acute pancreatitis.

HP is a rare disorder, as only about 400 patients in 95 kindreds around the world have been reported . It is an autosomal dominant disorder with 80% penetrance and is characterised by recurrent acute attacks of pancreatitis, and subsequent progression to complicated chronic pancreatitis. Many patients with chronic HP will ultimately develop pancreatic cancer.

The gene for HP has recently been mapped to chromosome 7q35 (Whitcomb et al., 1996b). Trypsinogen genes have been located at this position, as the surrounding region has been entirely sequenced due to the presence in the area of the TCRβ locus. Screening revealed that there existed a mutation (G-A transition) in the third exon of the cationic trypsinogen gene. This was confirmed to be present in all individuals affected by, and known to be obligate carriers of HP (Whitcomb et al., 1996a). This mutation produces a amino acid difference in the trypsin molecule, as the arginine (CGC) at codon 117 (Arg117) is replaced by a histine (CAC). Trypsin is central to the pancreatic enzyme cascade. Trypsinogen is activated to trypsin by the cleavage of an octopeptide (trypsinogen activation peptide; TAP). The process is normally initiated in the duodenum by brush border enterokinase, but trypsin can also activate trypsinogen by the cleavage of TAP.

The trypsin molecule consists of two globular domains connected by a single polypeptide chain and although the position of Arg117 is opposite the catalytic site and trypsin inhibitor

binding site (PSTI), it is in the middle of the connecting chain. Arginine is the recognition site for trypsin-like enzymes and studies suggest that Arg117 is the initial hydrolysis site in the trypsin degradation of the trypsin molecule itself, which renders it inactive (Whitcomb et al., 1996a).

Figure 3.1 details the proposed mechanism of HP.

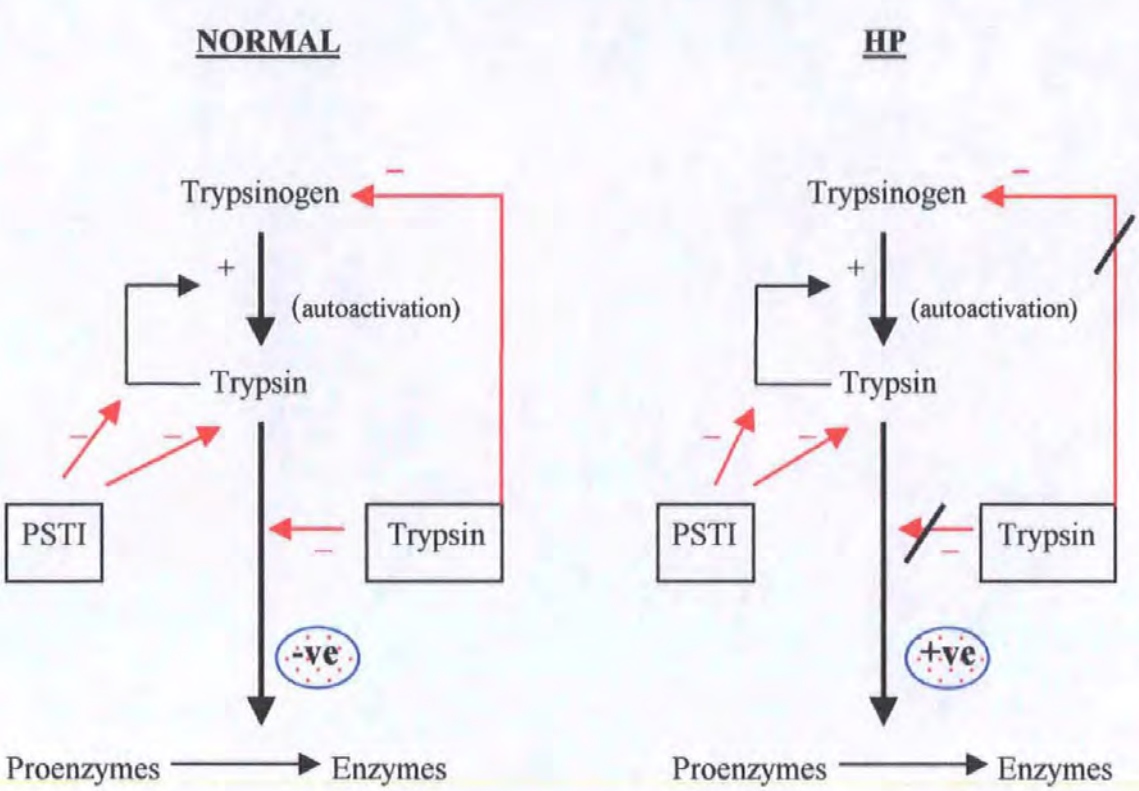

Fig 3.1 Proposed mechanism for the development of hereditary pancreatitis (HP)

In HP, the normal inhibitory influence of trypsin upon trypsinogen/trypsin activation are not functioning as the Arg117 mutation prevents hydrolysis, and therefore inactivation of trypsin and trypsinogen by trypsin.
Trypsin inactivation of the trypsin molecule is only one of the endogenous protective mechanisms preventing uncontrolled trypsinogen/trypsin activation. The trypsin inhibitor, PSTI fits into a pocket on the trypsin molecule and inhibits it's action. Normally PSTI and trypsin hydrolysis of trypsin will prevent trypsin activation in the pancreas. However, when the Arg117 mutation of HP is present and trypsin hydrolysis of trypsin is absent, the function of PSTI can be overridden as it can only activate up to 20% of potential trypsin. It is hypothesised this mechanism explains why attacks of HP are intermittent, an attack only occurs when more trypsin is active than can be inhibited by PSTI.
PSTI - Pancreatic secretory trypsin inhibitor
HP - Hereditary pancreatitis
Negative influences upon trypsin activation are shown in red ——

—/— inhibitory influence doesn't function due to HP mutation at in trypsin molecule

(Adapted from Whitcomb et al, Nature Genetics 1996)

As discussed in section 1.1.3, trypsin inactivation of the trypsin molecule is only one of the endogenous protective mechanisms preventing uncontrolled trypsinogen/trypsin activation. The trypsin inhibitor, PSTI fits into a pocket on the trypsin molecule and inhibits it's action. Normally PSTI and trypsin hydrolysis of trypsin will prevent trypsin activation in the pancreas. However, when the Arg117 mutation is present and trypsin hydrolysis of trypsin is absent, the function of PSTI can be overridden as it can only activate up to 20% of potential trypsin. It is hypothesised this mechanism explains why attacks of HP are intermittent, attack only occurs when more trypsin is active than can be inhibited by PSTI (Whitcomb et al., 1996a).

This proposed mechanism demonstrates the importance of premature trypsin activation in the development of acute attacks of HP. Whitcomb et al (1996) claim it also demonstrates the importance of premature trypsin activation in the development of (non-HP) acute pancreatitis. As can be seen from the abundance of evidence presented in this chapter concerning other proposed mechanisms of the early pathogenesis of acute pancreatitis, it is unlikely that this is the whole story.

3.4 Alcohol and the liver

As well as being an aetiological factor for acute and chronic pancreatitis, prolonged excessive alcohol consumption can cause a variety of other diseases affecting other organs of the body. Liver disease is the most common, but an alcoholic may also develop alcoholic cardiomyopathy.

It will be helpful to briefly discuss alcoholic liver disease in relation to it's pathogenesis as a group of alcoholics with liver disease (alcoholic hepatitis and cirrhosis), but without a history of pancreatic disease, have been used as a 'control' group in this work. It is also useful to be able to compare it with alcoholic pancreatitis.

3.4.1 Alcohol induced liver injury

As with pancreatic disease, severe liver disease develops in only a minority of heavy drinkers. For example, biopsy studies show that established cirrhosis occurs in less than 20% (Derr et al., 1990). It appears the level at which liver disease starts to occur is the daily consumption of > 80 g day^{-1}, and that consumption has to be consistent over five years (Sherlock and Dooley, 1993). Morphological changes in the liver, and the clinical syndromes they produce are of three different types:

1. Fatty liver.

   Diffuse fatty change occurs, with fat accumulating towards the central zone of the liver's functional and anatomical unit, the acinus. Patients are usually asymptomatic, and clinically may show a slightly enlarged liver. Fatty liver is not regarded as a precursor of the other types of alcoholic liver disease, and is present in the majority of chronic, heavy alcohol abusers.

2. Alcoholic hepatitis (Steatohepatitis).

   Hepatocytes are swollen and steatosis usually occurs. With increasing severity of disease, necrosis becomes more conspicuous, and some collagen deposition occurs. Clinical presentation varies from those with anorexia, fatigue, and weight loss to those with pyrexia, anorexia, jaundice, and repeated vomiting with pain from an acutely enlarged liver. Alcoholic hepatitis is regarded as a precursor of cirrhosis, with which it may co-exist.

3. Cirrhosis.

   Fibrous bands divide the liver into 'nodules'. Necrotic areas become replaced by fibrosis, and the amount of steatosis reduces as cirrhosis progresses. Symptoms are those of end stage liver disease; ascites, oedema, malabsorption etc.

3.4.2 Pathogenesis of alcoholic liver disease

Many of the effects of alcohol upon the liver are thought to be related to the toxic effects of alcohol or it's metabolites. Alcohol cannot be stored so oxidation, predominantly in the liver, is obligatory. The main ethanol oxidation pathway is via conversion to acetaldehyde catalysed by alcohol dehydrogenase (Crabb, 1995). Acetaldehyde causes membrane damage to mitochondria and cell necrosis. The acetaldehyde is converted to acetate by aldehyde dehydrogenase. Both these processes of oxidation, by nature of their process, produce hydrogen, carried by nicotinamide adenine dinucleotide (NAD), as NADH. The excess hydrogen generated inhibits protein synthesis and increases lipid peroxidation, both ultimately injurious to the liver (Situnayake et al., 1990). This increased NADH:NAD ratio is also termed 'increased redox potential'. It is also thought to be responsible, at least in part, for the formation of fatty liver, collagen deposition, and impaired gluconeogenesis seen in alcoholic liver disease (Sherlock and Dooley, 1993). The reoxidation of NADH also results in an increased oxygen requirement, possibly causing hypoxic necrosis of liver cells. Alcohol is also metabolised by the mitochrondrial oxidising system, using cytochrome oxidising enzymes such as Cytochrome P450. Oxidation by this pathway also produces acetaldehyde and generates OFR's (Crabb, 1995), which have the potential to cause cellular damage.

Cytokines are also implicated in the pathogenesis of alcoholic liver disease. In alcoholic hepatitis monocyte TNF secretion is increased and serum levels have been found to correlate with severity of liver injury (Bird et al., 1990). Furthermore, reversible hepatitis is a consequence of TNFα administration (Schilling et al., 1992). IL-1 and IL-6 are also found to be raised in chronic alcoholics with liver disease (Sherlock and Dooley, 1993).

3.4.3 Alcohol-induced liver and pancreatic disease: do they co-exist?

It is unknown whether an alcoholic is more likely to develop alcohol-induced acute or chronic pancreatitis, liver cirrhosis, or both conditions. Whatever the relationship, it is certainly true that both conditions do not by nature always co-exist. Reports have produced

data that suggests that alcoholic hepatitis or cirrhosis occurs in from 5 to 20% of patients with chronic alcoholic pancreatitis (Lankisch and Banks, 1998a). There is no data on patients with acute pancreatitis, but as acute and chronic pancreatitic disease due to alcohol are probably a spectrum of disease (see section 3.2.2.1) the same figures can probably be applied.

4 Immunogenetic considerations

This chapter will first consider the gene structure, polymorphism and disease associations of the MHC, the TNF locus within it, and the IL-10 gene located on chromosome 1. Some other cytokine gene polymorphisms will also be discussed, as will the evidence for a genetic component in susceptibility to alcohol related liver and pancreatic disease.

4.1 Major Histocompatibility Complex (MHC)

The major histocompatibility complex (MHC) extends over 4Mb of DNA on the short arm of chromosome 6 in band 21.3. Over 200 genes have been located within the MHC (Trowsdale and Campbell, 1998), mapped within three major gene clusters, the class I, class II, and class III genes (Fig. 4.1).

The class I and II gene products are central to the functioning of the immune system, being the molecules that present processed antigen to T cells, so causing their activation. The class I genes encode for the classical and non-classical class I antigens. The class II region encodes for the HLA-D antigens and other immune response proteins involved in antigen processing. The class III region generally encodes secreted proteins involved in the generation of an immune response, including complement components, TNF, and heat shock proteins (HSP).

The MHC is highly polymorphic, being one of the most variable genetic complexes known. The region is remarkable not only because of the number of polymorphic loci it contains within it's relatively short length of DNA, but for the extraordinary large number of different alleles at many of the loci. The diversity thus generated creates major obstacles to successful organ transplantation.

In addition, alleles at loci within the MHC associate in non-random fashion. This is called linkage disequilibrium, and results in the generation of allelic associations, termed haplotypes. The strength of this phenomenon within the MHC is illustrated by the fact that linkage can occur between alleles which are geographically close, such as alleles within a

12Kb region in the TNF gene cluster (Crouau-Roy et al., 1993), and alleles separated by up to 2Mb of DNA (Jongeneel et al., 1991; Wilson et al., 1993).

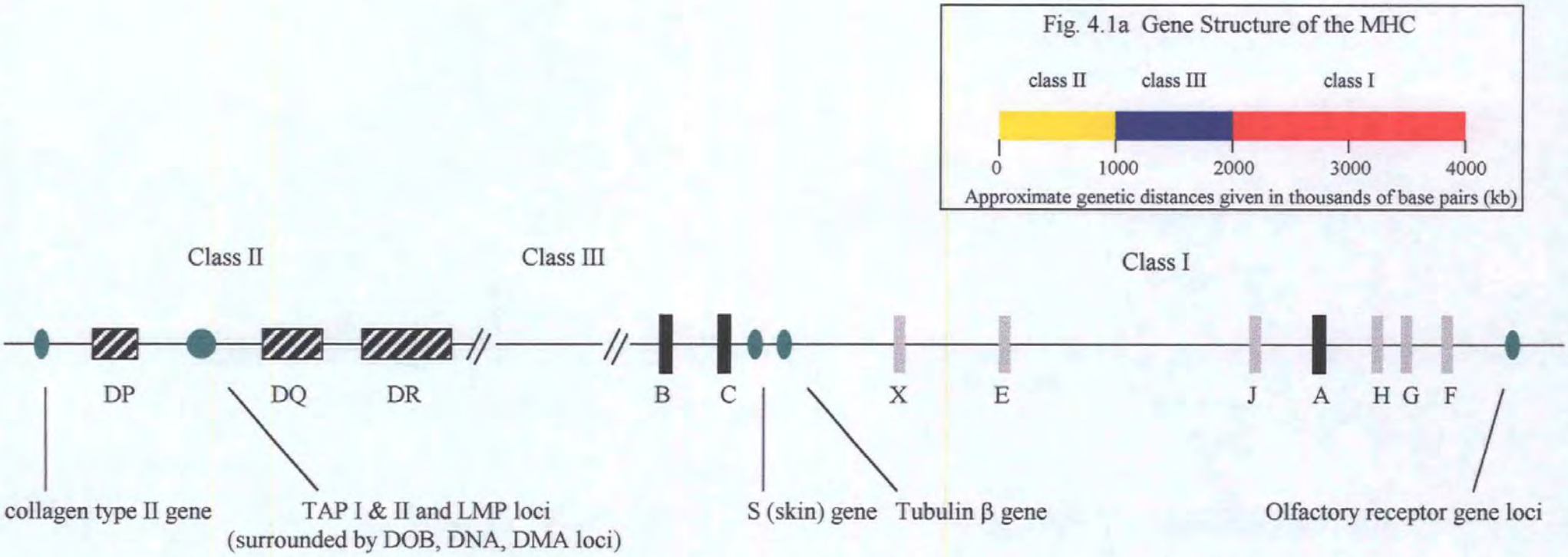

Fig 4.1. Map of MHC; class I and II regions

The classical and non-classical class I loci, the HLA-D loci, and other genes of interest, mentioned in the text, are depicted.
The class III region is shown in figure 4.2.

Classical class I antigen locus HLA-D locus

Non-classical class I locus Loci of other genes of note

4.1.1 Class I region

The class I region is approximately 2Mb in length, lies telomeric to class III (see Fig. 4.1), and contains the polymorphic classical transplantation antigen genes, HLA-A, HLA-B, and HLA-C, and the non-classical class I genes. The non-classical genes include HLA-E, HLA-F, and HLA-G, and a number of pseudogenes, HLA-J and HLA-X, which do not encode protein products. As described in section 2.3.5, HLA-A, B, and C are expressed on almost all nucleated cell types and present peptides from intracellularly degraded proteins to CD8+ (T_C) cells. This provides a mechanism for immune recognition of intracellular proteins.

The non-classical class I genes show limited expression of their class-I like products. Their function is not well established, and some are thought functionless, representing remnants of gene duplication events. HLA-H, for example, is related structurally to HLA-A, but shows limited diversity and alleles with mutations that render the heavy chain of the molecule unable to function in antigen presentation (Zemmour et al., 1990). However, it has recently been shown that HLA-E is the predominant ligand for certain NK cell inhibitory receptors (KIR), implying it has an important role in mediating the lysis of cells by some NK cell subsets (Braud et al., 1998).

The class I region also contains other, structurally unrelated genes. The first to be mapped was the β-tubulin gene (TUBB), telomeric to HLA-C, and others include the S gene (for skin), which is expressed at high levels in differentiating keratinocytes in the epidermis, so may have a function related to skin differentiation and structure (Zhou and Chaplin, 1998). A group of olfactory-receptor-like genes, which exhibit some polymorphism, are situated telomeric to HLA-F (Trowsdale and Campbell, 1998).

As previously mentioned, the class I region is highly polymorphic. Polymorphism of the HLA-A,B and C molecules is well characterised (Parham et al., 1988), and amino acid variations at positions in the MHC molecule chains correspond with genetic variation at the individual loci, producing multiple alleles. The number of sites at which there is nucleotide

variation can be extensive between different alleles, as many as eighty-eight can occur (Le Boutellier, 1994). The HLA-E locus also has well characterised polymorphism (Grimsley and Ober, 1997), and within the class I region are a number of dinucleotide repeat polymorphisms, or microsatellites (Le Boutellier, 1994).

4.1.2 Class II region

Three subregions, DP, DQ, and DR, make up the class II region which spans approximately 1Mb of DNA, located centrometric to the class I and III regions (see Fig 4.1). Each of the subregions has at least one α- and one β-chain pair of genes, whose protein products form the class II molecule heterodimers. Therefore, each subregion, DP, DQ, and DR, contains at least two functional genes, but can have more, which is often dependent upon the haplotype. For instance, the DR region contains between one and four expressed DRB genes, which can associate with the α-chain encoded by the single DRA gene (Ragoussis, 1995). The DQ region in contrast, has only a pair of functional genes, as the additional genes DQA2, DQB2, and DQB3 are not expressed. This is due to the presence of altered gene promoter sequences.

There are also additional separate α and β genes within the class II region. They encode for chains which are similar to the classical class II molecule α and β chains. The DNA and DMA genes encode class II α chains, whilst the DMB and DOB genes encode β chains. The DMA and B genes lie within the same locus (DM) (Ragoussis, 1995). The DM genes are expressed in the same tissues as the classical class II genes and are also inducible by IFN-γ. Although not in the same gene locus it is possible that the DNA and DOB gene products also form a similar heterodimer, as their mouse homologues have been shown to do (Karlson et al., 1992).

There are also a number of other genes within the class II region which encode entirely different classes of protein, but nevertheless have important functions in immune system functioning. The (antigenic) peptide transporter genes TAP1 and TAP2 , also called RING4

and RING11 (for really interesting new gene) are situated within the class II region (Spies et al., 1990) although they have effects upon class I molecule functions. They encode proteins that span the ER and transport degraded intracellular peptide to the pre-Golgi regions (Kleijmeer et al., 1992) where association with class I molecules takes place prior to transport to the cell surface. Defects in these genes result in unstable class I molecules and the loss of presentation of intracellular antigens (Parham, 1990).

The LMP (large multifunctional protease) genes, LMP2 and LMP7 are also situated within the class II region. They encode proteins homologous to a large cytoplasmic protease which degrades intracellular proteins. It has been suggested that LMP2 and 7 gene products bind to this complex, leading to production of peptides suitable for class I and II presentation (Ragoussis, 1995).

Several other genes, including the collagen type II gene, COL11A2, the RING1 gene (which encodes a transcription factor) and RING3 (encodes a nuclear kinase involved in cell cycle control), and other expressed genes of unknown function (e.g. RING9) also map to the class II region (Trowsdale and Campbell, 1998).

As with the class I, the class II region is highly polymorphic, variations in the class II molecule chains being produced by amino acid differences. These correspond with the different alleles caused by polymorphism in the nucleotide sequence, which like the class I region, can be extensive. It is also of interest that polymorphism exists in the TAP genes (Powis et al., 1992), and the LMP2 gene (Ragoussis, 1995). In the rat, polymorphism of the TAP genes affects the repertoire of peptides available for class I binding (Monaco, 1992).

4.1.2.1 Class I and class II disease associations

Several diseases have been found to have associations with certain HLA alleles. Table 4.1 details some of the better known disease associations. It is not surprising that the pathophysiology of all the HLA linked diseases involves immunological processes, with the exception of idiopathic haemochromatosis, which must have an HLA association by virtue

of linkage with it's gene locus.

Disease	**HLA Allele**	Relative Risk
Class I Associations		
Idiopathic haemochromatosis	A3	8.2
Myaesthenia gravis	B8	4.4
Ankylosing spondylitis	B27	87.4
Reiter's disease	B27	37.0
Post-salmonella arthritis	B27	29.7
Post-yersinia arthritis	B27	17.6
Post-gonococcal arthritis	B27	14.0
Uveitis	B27	14.6
Amyloidosis (in Rheumatoid Arthritis)	B27	8.2
Subacute thyroiditis	B35	13.7
Class II associations		
Tuberculoid leprosy	DR2	8.1
Goodpasture's syndrome	DR2	13.1
Thyrotoxicosis (Graves' disease)	DR3	3.7
Chronic active hepatitis	DR3	13.9
Dermatitis herpetiformis	DR3	56.4
Rheumatoid arthritis	DR4	5.8
Multiple sclerosis	DR2,DR6	12
Crohn's Disease	DQ2	250
Coeliac disease	DQ8	14
Insulin dependent diabetes mellitus	DQ2/8	20
	DR3	6.3
	DR3/4	14.3
Narcolepsy	DQ6	38

Table 4.1 HLA class I and II disease associations

Relative risk - Risk of developing disease relative to general population if carry that particular allele
Where allele is given as x/x, e.g. DR3/4 it denotes heterozygocity
The gene for idiopathic haemochromatosis has been mapped within the class I region. It's exact locus has not been determined, but it is situated telometric to HLA-A (Ragoussis, 1995).

4.1.3 Class III region

The class III regions spans approximately 1.1Mb of DNA between the class I and class II regions (fig 4.1 and 4.2). It contains the TNF gene loci which will be discussed in section 4.1.4. It also contains the genes for the complement components C4, C2 (classical pathway) and Bf (alternative pathway) (Ragoussis, 1995). The C2 and Bf genes are less than 2 kb apart and lie approximately 30 kb telomeric to the C4 genes, C4A and B, which are separated by about 10kb. The 21-hydroxylase gene, CYP21B, involved in mineralocorticoid and glucocorticoid synthesis, and a pseudogene CYP21A are immediately centrometric to the C4 genes (Dunham et al., 1987). A duplicated locus, lying telomeric to the C2 and Bf genes, encodes the major heat (inducible) shock proteins (HSP70) (Trowsdale and Campbell, 1998). These are stress proteins expressed in response to heat, oxygen free radicals, toxic metals and other stress stimuli. They bind to denatured or inappropriately folded proteins and are said to act as chaperones in the recovery of cells from stress. Other genes mapped within the class III region include G1-11 (some of which were previously called BAT genes; for HLA-B associated transcripts), and other genes of unknown or speculated function (Trowsdale and Campbell, 1998). It has been suggested various genes within the region encode for protein kinases, transcription factor (REL family) inhibitors; which influence TNF signalling, and a protein like a TNF receptor-associated-protein (Trowsdale and Campbell, 1998).

The class III region, as expected, is also highly polymorphic. TNF polymorphisms will be discussed in section 4.1.4, but it is also known that the C4A and C4B loci are highly polymorphic; 13 alleles are recognised at the C4A locus and 13 at the C4B locus. The HSP70 locus is also known to be polymorphic (Chouchane et al., 1997).

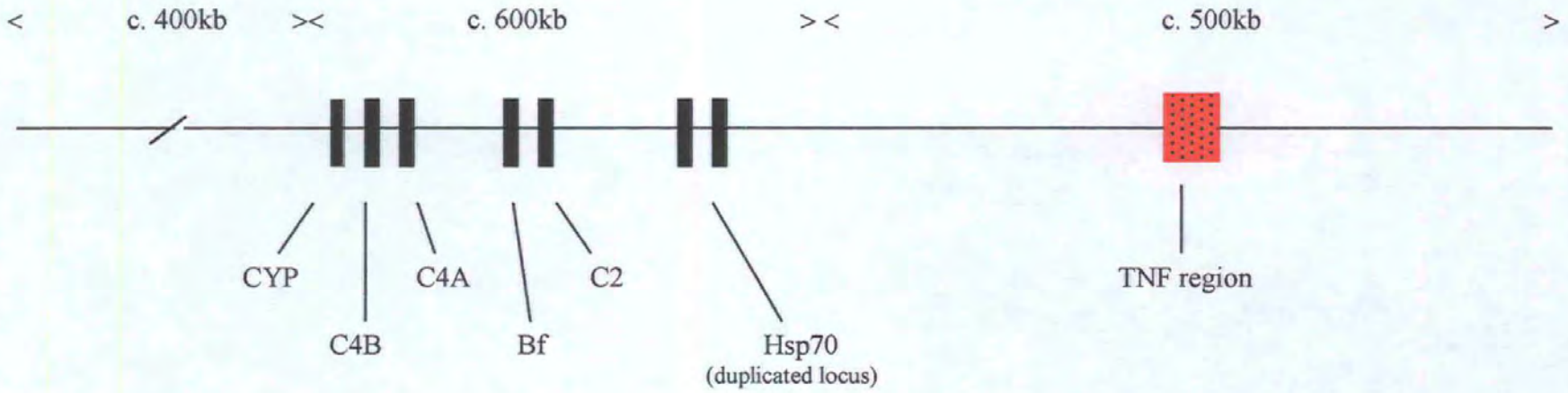

Figure 4.2 MHC Class III region

The TNF region is depicted in more detail in figure 4.3
CYP - 21-hydroxylase gene, CYP21B
C4B, C4A, Bf, & C2 are complement components
Hsp70 - major heat (inducible) shock proteins
TNF - Tumour necrosis factor region

gene locus

class III sub-region

4.1.4 TNF region

The TNF region (Fig.4.3) maps at the telomeric end of the class III region in the middle of the MHC gene cluster (Carroll et al., 1987; Dunham et al., 1987). The TNF locus is usually designated as the 7kb region encompassing the TNFα and TNFβ (LTα) genes, which are tandemly arranged, and separated by about 1.2kb (Udalova et al., 1993; Jongeneel et al., 1991). The LTβ gene also maps 4kb centromeric to these two genes (Trowsdale and Campbell, 1998), as illustrated in Fig. 4.3. Further upstream (4kb) of the LTβ gene is LST-1 (for leucocyte specific transcript). The function of it's protein product is unknown. Likewise the gene mapped telomeric to TNFβ, the NB6 gene, also has no known function. The gene further downstream from this, IkBL, produces Ikβ1, which is speculated to be an inhibitor of the Rel family of transcription factors (Trowsdale and Campbell, 1998). In contrast functions of TNFα and β, and that of LTβ are well described, as discussed in section 2.4.5.

Regulation of TNF production is complex, having been shown to occur at the transcriptional and post-transcriptional (translational) levels. Sequences within the TNFα promoter region control the rate of it's transcription (Goldfield et al., 1990), and it has been shown that there are binding sites for the transcription factors NFκB and AP-1 within this region (Rink and Kirchner, 1996). NFκB binding is known to increase TNF expression (Baeuerle and Henkel, 1994). The effect of LPS upon TNF mRNA processing appears to be to upregulate gene expression, suggesting that TNF production can also be affected at the translational level (Rink and Kirchner, 1996).

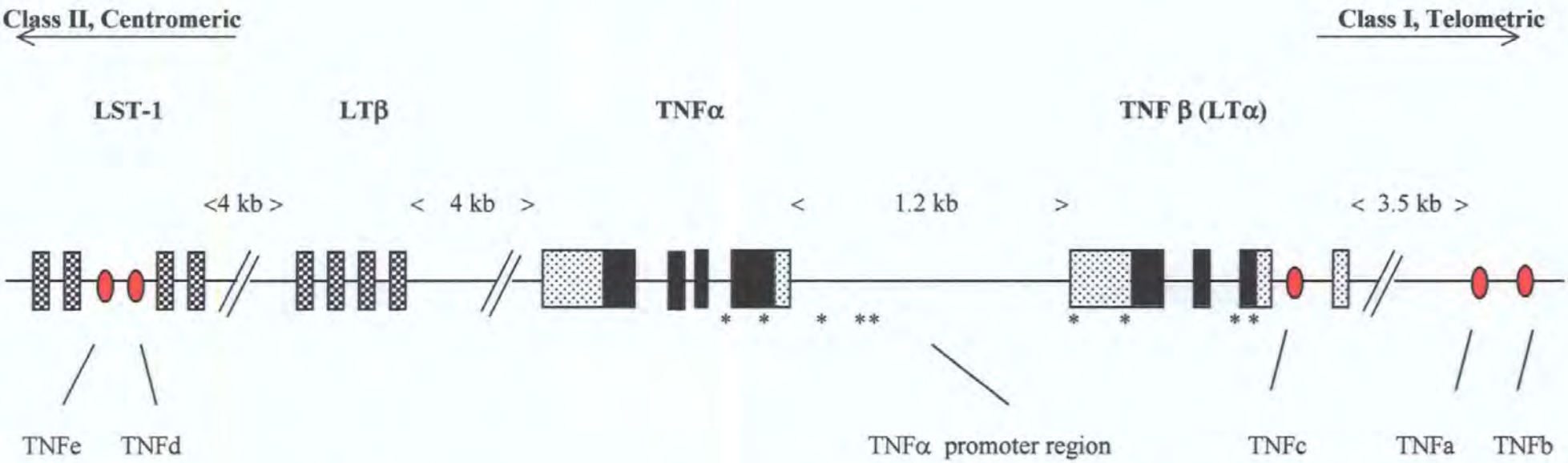

Figure 4.3 TNF region within MHC class III

This shows the intron/exon structure of the four genes in the TNF region. All have 4 exons.
Microsatellites TNFb-e are $(CT)_n$ repeats, TNFa is a $(CA)_n$ repeat.
The TNFα promoter region contains the bi-allelic base substitution polymorphisms at -308, -244, and -238.
Within the first exon of the TNFα gene is the bi-allelic C-insertion site polymorphism.
Within the first intron of the TNFα gene is the bi-allelic base substitution polymorphism at +488.
Within the first intron of the TNFβ gene are the AspHI (+365) and NcoI RFLP (+249) polymorphisms.
Within the third exon of the TNFβ gene is a bi-allelic base substitution polymorphism, and there is an EcoRI restriction site within it's fourth exon.
The LST-1 and LTβ gene structures are unknown.

TNF microsatellite locus

* Site of bi-allelic polymorphism

Translated exon

Untranslated exon

Intron/Exon structure unknown

4.1.4.1 Polymorphism of the TNF loci

Polymorphism of the TNF region is well recognised and is illustrated in Fig 4.3.

Single base substitutions at occur at positions -376, -308, -244, -238, and -163 (G-A transitions) in the TNFα promoter region (Fanning et al., 1997; D'Alfonso and Momigliano Richiardi, 1994; Wilson et al., 1993). There is also a C insertion site at position +70 (within first exon) and a base substitution polymorphism (G-A transition) at position +488 within the first intron of the TNFα gene (Fanning et al., 1997; D'Alfonso and Momigliano, 1994; Messer et al., 1991). All positions are relative to the transcription initiation start site of the TNFα gene. The polymorphisms at positions -308 and -238 were the first described and are the most extensively investigated.

In the region of the TNFβ gene, there are four base substitution polymorphisms, three of which create a restriction site. An NcoI site (A-G transition) is at position +249 from the TNFβ transcription start site, and an AspH1 site (C-G transition) is at position +365. Both these are situated in the first intron (Bouma et al., 1996). The gene also contains an EcoR1 restriction site in it's fourth exon (Rink and Kirchner, 1996), and a C-A transition at position +720 (also called AA26), within it's third exon (Fanning et al., 1997). As they are base transition polymorphisms, all are biallelic, again illustrating the low degree of sequence polymorphism actually within the TNF gene loci.

Around the TNF region exist five polymorphic microsatellite loci (Fig. 4.3). Microsatellites are tandemly repeated arrays of sequence elements (usually a CA or CT dinucleotide) occurring at unique sites within the genome, and are often highly polymorphic, differing in the number of repeats. They are inherited in a normal co-dominant fashion (Weber and May, 1989). The microsatellites within the TNF region are denoted TNFa-e. TNFc is situated within the first intron of the TNFβ gene, in close proximity to the NcoI polymorphism at position +249, and is bi-allelic. However the other TNF microsatellites exhibit a greater degree of polymorphism. TNFa and TNFb are located 3.5 kb telometric of

the TNFβ gene and have at least 13 and 7 alleles respectively (Nedaspasov et al., 1991; Jongeneel et al., 1991). TNFd and TNFe are located *circa* 4kb upstream of the LTβ gene, within intron 3 of the LST-1 (also called B144) gene and have 3 and 7 alleles respectively (Udalova et al., 1993). All except TNFa, which is a $(CA)_n$ repeat, are $(CT)_n$ repeats.

4.1.4.2 Nomenclature

Individual TNF microsatellite loci and alleles are named in this work as proposed by Nedaspasov and colleagues (1991). The allele with the least number of dinucleotide repeats (n=x) is designated allele 1, and subsequent alleles are numbered according to the number of repeats (n=x+1,allele 2; n=x+2, allele 3; etc.).

For the bi-allelic polymorphisms in and around the TNF loci there is no agreed international nomenclature, and individual studies have used different systems. This has the potential to cause confusion, as investigators have named the respective common and uncommon alleles of individual loci typed by PCR-SSP and PCR-SSOP as allele 1 (common) and allele 2 (uncommon), whereas for loci typed by PCR-RFLP alleles 1 and 2 are sometimes named according to whether the fragment is digested (allele 1) or undigested (allele 2). This does not always correspond; for example the undigested fragment of the NcoI polymorphism in the TNFβ gene is the most common in Caucasian populations (Stuber et al., 1996b; Stuber et al., 1996b). Therefore in this work the alleles will be named according to the nucleotide present.

Furthermore, loci will be named according to their position from the gene's transcriptional start site, using TNF for the TNFα gene and TNFB for the TNFβ gene.

E.g. –308 TNF.G and –308 TNF.A; +249 TNFB.A and +249 TNFB.G (NcoI site)

Where the work referred to has typed the polymorphism by PCR-RFLP and have used nomenclature based upon presence or absence of digestion the allelic nomenclature used by that study will also be put in parentheses as shown:

(TNFB*1) = digested fragment

(TNFB*2) = undigested fragment

4.1.4.3 TNF region linkage and extended MHC haplotypes

As expected, there is evidence of much linkage within the TNF region, and between the TNF region alleles and other MHC alleles. Linkage between the microsatellites, producing distinct three locus haplotypes, has been characterised by maximum likelihood estimates in previous population based and family studies (Jongeneel et al., 1991; Nedaspasov et al., 1991; Crouau-Roy et al., 1993) and their existence has been confirmed in homozygous cell lines(Weissensteiner and Lanchbury, 1997; Udalova et al., 1993). Table 4.2 details known 3 locus TNF microsatellite haplotypes so confirmed in North European Caucasian populations. Five locus haplotypes have also been characterised and confirmed in comparable populations (Weissensteiner and Lanchbury, 1997).

An association between individual TNFa, b, and c alleles is also seen with alleles at other loci within the MHC, including class I, II, and III alleles (Weissensteiner and Lanchbury, 1997; Udalova et al., 1993; Jongeneel et al., 1991; Abraham et al., 1993), and three and five locus microsatellite haplotypes associate with HLA class I and II extended haplotypes (Garcia-Merino et al., 1996; Crouau-Roy et al., 1996; Jongeneel et al., 1991; PLevy et al., 1996). Table 4.3 details known TNF microsatellite and HLA class I and II associations in populations of Caucasians.

Likewise, bi-allelic polymorphisms in the region of the TNFα promoter and TNFβ gene also produce distinct haplotypes (Fanning et al., 1997; Bouma et al., 1996), which have been shown to have certain HLA associations (Fanning et al., 1997). Individual alleles of the bi-allelic polymorphisms are also seen to be in linkage with certain HLA alleles and haplotypes. One of the first detected was allele –308 TNF.A of the TNFα promoter which was shown to be linked with HLA-A1, -B8, and -DR3 alleles, and the HLA-A1,B8,DR3 haplotype (Wilson et al., 1993). Many other HLA class I and II associations have since been described, including those between haplotypes produced by 6 of the bi-allelic

polymorphisms (Fanning et al., 1997). Table 4.3 also details known class I and II associations of the -308 polymorphism.

TNFa-b-c microsatellite haplotype
11-4-1
2-1-2
6-5-1
10-4-1
2-3-1
7-4-1
2-5-2
5-5-2
1-5-2
5-5-1
4-7-2
12-4-1
4-5-1

Table 4.2. 3 locus microsatellite haplotypes of the TNF region

These haplotypes have been characterised by allele segregation and maximum likelihood estimates in previous population based and family studies (Jongeneel et al., 1991; Nedaspasov et al., 1991; Crouau-Roy et al., 1993) and their existence has been confirmed in homozygous cell lines(Weissensteiner and Lanchbury, 1997; Udalova et al., 1993).

HLA Association	-308 allele	TNFa allele	TNFb allele	TNFc allele	TNFd allele	TNFe allele	Reference
Class I							
A1	TNF2	-	-	-	-	-	(Fanning et al., 1997; Wilson et al., 1993)
A1	-	a2	-	-	-	-	(Abraham et al., 1993)
A3	TNF1	-	-	-	-	-	(Fanning et al., 1997)
B7	TNF1	-	-	-	-	-	(Fanning et al., 1997)
B8	TNF2	-	-	-	-	-	(Fanning et al., 1997; Wilson et al., 1993)
B8	-	a2	b3	-	-	-	(Abraham et al., 1993)
Cw*0701	TNF2	-	-	-	-	-	(Fanning et al., 1997)
Cw*0702	TNF1	-	-	-	-	-	(Fanning et al., 1997)

Table 4.3 Class I and II associations with TNF-308 and microsatellite polymorphic alleles reported in Caucasians **continued.....**

HLA Association	-308 allele	TNFa allele	TNFb allele	TNFc allele	TNFd allele	TNFe allele	Reference
Class II							
DR1	TNF1	a2	b1	c2	d4	e1	(Weissensteiner and Lanchbury, 1997)
DR2	TNF1	a11	b4	c1	d3	e3	(Weissensteiner and Lanchbury, 1997)
DR2	TNF1	a10	b4	c1	d3	e3	(Weissensteiner and Lanchbury, 1997)
DR3,DQB1*0201	TNF2	-	-	-	-	-	(Deng et al., 1996)
DR4,B62	-	a2	b1	-	-	-	(Monos et al., 1995)
DR4,B44	-	a6	b5	-	-	-	(Monos et al., 1995)
DR3	TNF2	-	-	-	-	-	(Wilson et al., 1993)
DR3	TNF2	a2	b3	c1	d1	e3	(Weissensteiner and Lanchbury, 1997)
DR3	TNF1	a1	b5	c2	d4	e3	(Weissensteiner and Lanchbury, 1997)
DR4	TNF1	a2	b1	c2	d4	e1	(Weissensteiner and Lanchbury, 1997;
DR4	-	a11	b4	c1	d3	e3	Wilson et al., 1993)
							(Weissensteiner and Lanchbury, 1997)

Table 4.3 Class I and II associations with TNF-308 and microsatellite polymorphic alleles reported in Caucasians **continued.....**

HLA Association	-308 allele	TNFa allele	TNFb allele	TNFc allele	TNFd allele	TNFe allele	Reference
DR5	-	a10	b4	c1	d3	e3	(Weissensteiner & Lanchbury,1997)
DR5	-	a6	b5	c1	d3	d3	(Weissensteiner & Lanchbury,1997)
DR5	-	a5	b5	c2	d5	e3	(Weissensteiner & Lanchbury,1997)
DR6	TNF1	-	-	-	-	-	(Wilson et al., 1993)
DR6	-	a6	b5	c1	d3	e5	(Weissensteiner & Lanchbury,1997)
DR6	-	a2	b1	c2	d4	e1	(Weissensteiner & Lanchbury,1997)
DR7	TNF1	a7	b4	c1	d3	e3	(Weissensteiner & Lanchbury,1997)
DR7	TNF1	a2	b5	c2	d4	e3	(Weissensteiner & Lanchbury,1997)
DR8	-	a2	b1	c2	d4	e1	(Weissensteiner & Lanchbury,1997)
DQB1*06	TNF1	-	-	-	-	-	(Fanning et al., 1997)
A1,B8,DR3	TNF2	a2	b3	-	-	-	(Gallagher et al., 1997a; Wilson et al., 1993a)
A2,B44,Cw5,DRB1*0401	-	a6	b5	-	d4	-	(Hajeer et al., 1996)
A1,B8,CW7,DRB1*0301	-	a2	b3	-	d2	-	(Hajeer et al., 1996)

Table 4.3 Class I and II associations with TNF-308 and microsatellite polymorphic alleles reported in Caucasians **continued.....**

HLA Association	-308 allele	TNFa allele	TNFb allele	TNFc allele	TNFd allele	TNFe allele	Reference
A3,B7,DR2	TNF1	a11	b6	c1	-	e1	(Gallagher et al., 1997a)
A1,B8,C4AQ0,C4B1,DR3	-	a2	b3	-	-	-	(Abraham et al., 1993)
B27,C1,DR1,DQ5	-	a6	b5	c1	d3	e3	(Udalova et al., 1993)
B18,DR3	-	a1	b5	c2	-	-	(Crouau-Roy et al., 1996)
B18,C5,DR3,DQ2	-	a1	b5	c2	d4	e3	(Udalova et al., 1993)
B7,SC31,DR2	-	a11	b4	c1	-	-	(Garcia-Merino et al., 1996)
B8,SC01,DR3	-	a2	b3	c1	-	-	(Garcia-Merino et al., 1996)
B62,Cw3	-	a2	b1	c2	d5	-	(Hajeer et al., 1996)
DR1, DQ5	-	a2	b1	c2	d4	e1	(PLevy et al., 1996)
DR3,B8	-	a2	b3	-	-	-	(Monos et al., 1995)
DR3,DQB1*0201	TNF2	-	-	-	-	-	(Deng et al., 1996)
DR4,B62	-	a2	b1	-	-	-	(Monos et al., 1995)
DR4,B44	-	a6	b5	-	-	-	(Monos et al., 1995)

Table 4.3 Class I and II associations with TNF-308 and microsatellite polymorphic alleles reported in Caucasians (Footnotes on next page)

Table 4.3 Class I and II associations with TNF-308 and microsatellite polymorphic alleles reported in Caucasians (Footnotes)

Where a class I or II association with TNF polymorphic alleles occurring as a haplotype is shown, the TNF alleles are underlined
In a Japanese population, TNFa9 and TNFd4 showed strong associations with HLA-B35 and B44, respectively (Asano et al., 1997).

4.1.4.4 Involvement of MHC and TNF alleles in TNF protein production?

Evidence has recently been produced that concludes that approximately 60% of the inter-individual variation in TNF secretion is genetically determined. This is based on a pedigree-based maximum likelihood model, using data on TNF production by whole blood samples after *in vivo* endotoxin stimulation in 190 first degree relatives of 61 families and 26 monozygotic twins (Westendorp et al., 1996).

Evidence that MHC molecules were involved in the secretion of TNF came shortly after the TNF genes were mapped to the MHC gene complex. Anti-class II MHC molecule mAb (anti-DR and -DQ) were shown to inhibit, in a dose dependent manner, the secretion of TNFα and IL-1by cultured monocytes stimulated with LPS (Santamaria et al., 1989). Importantly, the same series of experiments also demonstrated that certain HLA-DR haplotypes secreted more TNF and IL-1 than others. The results from this study indicated that in their North American study population, DR2 and DR4 +ve cells were low secretors of both cytokines, whilst DR3 +ve cells secreted high levels. This association has been investigated in other studies, and partially confirmed. HLA-DR2 was confirmed to be associated with low levels of TNFα secretion, and DR3 was confirmed as being associated with high levels of TNF production in North American and European populations (Garcia-Merino et al., 1996; Poicot et al., 1993; Jacob et al., 1990), but DR4 was found to be associated with high not low levels of production (Poicot et al., 1993; Jacob et al., 1990). An association with TNF secretion has also been observed with TNF microsatellite alleles (Poicot et al., 1993). The study shows significantly higher TNFα secretion by stimulated monocytes in subjects with TNFa2 (~3.4 ng/ml) and TNFc2 alleles, whilst the TNFa6 allele correlated with low TNF secretion (~2.4 ng/ml). The level of secretion was compared with a baseline of 3 ng/ml. Data for the TNFc2 allele was not shown in the paper.

Another study reported that individuals homozygous for the TNFa2 allele have a higher *in vitro* PBMC TNFα secretion than individuals homozygous for the TNFa11 allele (Garcia-

Merino et al., 1996). The difference did not reach statistical significance however ($p<0.1$). Using data inferred from the associations of individual markers in the MHC, which have been shown to be linked to TNF microsatellite alleles and TNF microsatellite haplotypes, it has been suggested that haplotypes containing the alleles TNFa2, TNFb1, TNFb3, and TNFc2 are all associated with higher levels of TNF secretion by LPS stimulated monocytes *in vitro* (Weissensteiner and Lanchbury, 1997). This work classifies many of the known haplotypes as having, 'high', 'low', or 'intermediate' secretor status.

There is disagreement as to whether the bi-allelic polymorphism at position -308 in the TNFα promoter influences TNFα production. Evidence that the –308 TNF.A allele has effects upon TNF levels was largely circumstantial, based on the fact that it is linked with HLA-DR3 (Wilson et al., 1993) and that DR3 typed mononuclear cells exhibit higher production of TNF upon stimulation *in vitro* (Jacob et al., 1990). However, differences in TNF production between individuals could not be explained by the -308 polymorphism in a study which also found no correlation between genotype of the -238 and -376 polymorphisms (Huizinga et al., 1997). Inserted gene constructs with deletion of the promoter region containing the -308 polymorphism and constructs with the -308 variation have no effect upon LPS stimulated TNF secretion in macrophages (Stuber et al., 1996b), and allele specific transcript quantification and reporter gene studies, again using TNFα promoter constructs also did not detect any effect of the G-A transition (Brinkman et al., 1996). Conversely, -308 variation is claimed to have effects upon TNFα production (Wilson et al., 1997). This is based upon more circumstantial evidence produced by these authors, showing that the polymorphism has significant effect upon transcriptional activity as detected by a reporter gene assay. This study did not show any difference in the specific affinity of DNA-binding protein for the polymorphic promoter site with or without the -308 variation, leaving open the question of how the detected alteration in transcription is affected by the polymorphism.

The NcoI restriction site (A-G transition) at +249 in the first intron of the TNFβ gene has also been linked with levels of TNFα and β secretion in vitro by stimulated monocytes. +249 TNFB.A (called TNFB*2 in study) homozygotes are shown to have a significantly higher secretion of TNFα than +249 TNFB.G (TNFB*1) homozygotes. Heterozygotes for this gene have an intermediate level of TNFα secretion. This correlates with the finding of higher mean TNFα levels in sera of patients with sepsis homozygous for the +249 TNFB.A (TNFB*2) allele (Stuber et al., 1996a). Another study has reported higher levels of TNFβ secretion in +249 TNFB.G (TNFB*1) homozygotes, compared to +249 TNFBA (TNFB*2) homozygotes (Biragyn and Nedaspasov, 1995).

4.1.4.5 TNF allele disease associations

On the basis of the hypothesis that TNF alleles have important influence upon TNF secretion, TNF alleles have been characterised in populations of patients with various diseases or pathophysiological states in which TNF is thought to play a key developmental role.

4.1.4.5.1 Base substitution and RFLP biallelic polymorphisms

Details of many studies that have been carried out in this regard are detailed in Table 4.4. Some are further discussed below.

The -308 and -238 polymorphisms in the TNFα promoter are the loci that have been most extensively studied in attempts to find association with disease. Neither allele of the -308 locus has been found to be associated with susceptibility to ankylosing spondylitis (Vejans et al., 1994), alcoholic liver disease (Grove et al., 1997), insulin resistance (Day et al., 1998), or rheumatoid arthritis (RA) (Vinasco et al., 1997c). Nor has association been found with either susceptibility to, or severity of, post-operative sepsis (Stuber et al., 1996b) or multiple sclerosis (MS) (Huizinga et al., 1997). Susceptibility to systemic lupus erythamatosus (SLE) is reported to be associated with the –308 TNF.A allele, although, as detailed in the table, the two studies conclude differently as to whether the association is

independent of known HLA associations (Rudlaweit et al., 1996; Sullivan et al., 1997). Although no association has been found with disease susceptibility, allele TNF.A of the -308 polymorphism is reported to be increased in frequency in patients with fatal meningococcal disease compared to survivors (Nadel et al., 1996), and those with mucocutaneous as opposed to milder forms of leishmaniasis (Cabrera et al., 1995). In Western African patients with malaria, subjects who were homozygotes for –308 TNF.A had an increased risk of the more severe, cerebral form of the disease (McGuire et al., 1994). A study from North Africa reports that –308 TNF.A is more common in patients with various forms of malignant disease, including breast carcinoma and lymphoma, compared to a group of healthy controls with similar ages (Chouchane et al., 1997).

On the other hand a negative association has been reported between this allele of the -308 locus (TNF.A) and Ulcerative colitis (UC) in a population of Dutch patients with inflammatory bowel disease (IBD) (Bouma et al., 1996). In UC patients it is present at a frequency of 0.15, compared with 0.25 in healthy controls ($p=0.44$). The same study reports no difference in allelic distribution of the -238 TNFα polymorphism, or the AspHI (+365) and NcoI (+249) restriction polymorphisms of the TNFβ gene, in the same group of patients with IBD.

The -238 TNFα promoter polymorphism has been investigated in populations of patients with other diseases. Investigators have reported increased frequency of the -238 TNF.A allele with reduced insulin resistance in relatives of patients with insulin-dependent diabetes mellitus (IDDM) (Day et al., 1998), and alcoholic steatohepatits (Grove et al., 1997). Although their respective studies did not report an association between the -238 locus and susceptibility to either RA or MS, TNF.A at -238 is reported to be more common in RA patients with an earlier age of onset of disease (Vinasco et al., 1997) (the difference between mean ages being a decade) and in MS patients with more severe, progressive disease. This was assessed by their being confined to nursing homes (Huizinga et al., 1997).

The NcoI restriction site polymorphism in the TNFβ gene has also been investigated in patients with post-operative sepsis and rheumatoid arthritis. No association between either allele and susceptibility to RA was found (Vinasco et al., 1997), but two studies report associations between this locus and severity of postoperative sepsis (Stuber et al., 1996b; Stuber et al., 1996b). Although the same group of authors report both studies, no mention is made that the groups of patients are the same. The authors report that the more common allele TNFB.A (they called TNFB*2), was reduced in frequency in survivors compared to non survivors, and TNFB.A (they call TNFB*2) homozygotes had a significantly higher mortality rate. This meant heterozygotes had a significantly higher rate of survival, as did TNFB.G (they call TNFB*1) homozygotes. One of the studies reports that the group of patients homozygous for TNFB.A (they call TNFB*2) had significantly higher median plasma TNF measurements (Stuber et al., 1996a).

A recent study that characterised 6 of the bi-allelic polymorphisms associated with the TNFα (-308, -238, +488) and TNFβ (+249, +365, +720) genes in patients with common variable immune deficiency (CVID) found that the +488 TNF.A allele was strongly associated with a subgroup with granulomatous disease (p=0.0005), and that this was independent of known class I and II associations (Mullighan et al., 1997). This subgroup of patients are known to have high serum TNF levels, but it is pre-emptive to reason that the +488 polymorphism is the cause of this as it's functional relevance is unknown.

Disease	-308 locus		-238 locus		NcoI locus		Reference
	Susceptibility	Severity	Susceptibility	Severity	Susceptibility	Severity	
Rheumatoid arthritis	No	Yes[1]	No	Yes[1]	No	No	(Vinasco et al., 1997)
	No	No	No	Yes[2]	-	-	(Brinkman et al., 1997)
	No[3]	-	-	-	No[3]	-	(Field et al., 1997)
	No	No	-	-	-	-	(Wilson et al., 1995)
Ankylosing spondylitis	No	No					(Vejans et al., 1994)
Wegener's granulomatosis	No	-	-	-	No	No	(Mascher et al., 1996)
Systemic lupus erythematosus	Yes[4]	-	-	-	-	-	(Sullivan et al., 1997)
	Yes[5]	-	No	-	-	-	(Rudlaweit et al., 1996)
Psoriasis (types I and II)	No	-	Yes[6]	-	-	-	(Sullivan et al., 1997)
	No	-	Yes[7]				(Arias et al., 1997)
Sarcoidosis	No	Yes[8]	-	-	No	No	(Seitzer et al., 1997)

Table 4.4 Studies of the TNFα -308, -238, and TNFβ NcoI biallelic polymorphisms in immune linked, inflammatory, infective, metabolic and malignant disorders

Continued.....

	-308 locus		-238 locus		NcoI locus		
Disease	Susceptibility	Severity	Susceptibility	Severity	Susceptibility	Severity	Reference
Coeliac disease	Yes[9]	-	-	-	-	-	(Manus et al., 1996)
Liver disease (alcoholic)	No	Yes[10]					(Grove et al., 1997)
Coronary heart disease	No	-	No	-	-	-	(Hermann et al., 1998)
Multiple sclerosis	No	No	No	Yes[11]	-	-	(Huizinga et al., 1997)
Ulcerative colitis	Yes (-ve)[12]	-	No	-	No	-	(Bouma et al., 1996)
Crohn's disease	Yes (-ve)[13]	-	-	-	-	-	(Louis et al., 1996)
Obesity	Yes[14]	-	No	-			(Hermann et al., 1998)
Insulin resistance	Yes[15]	-	-	-	-	-	(Day et al., 1998)*
IDDM	Yes[16]	-	-	-	-	-	(Deng et al., 1996)
Asthma	Yes[17]	-	-	-	Yes[17]	-	(Moffatt and Cookson, 1997)
Malignant disease	Yes[18]	-					(Chouchane et al., 1997)
Chronic hepatitis B	No	-	Yes[19]	-	-	-	(Hohler et al., 1997)

Table 4.4 Studies of the TNFα -308, -238, and TNFβ NcoI biallelic polymorphisms in immune linked, inflammatory, infective, metabolic and malignant disorders

Continued.....

	-308 locus		-238 locus		NcoI locus		
Disease	Susceptibility	Severity	Susceptibility	Severity	Susceptibility	Severity	Reference
AIDS	No[20]	No	-	-	-	-	(Knuchel et al., 1998)
Post-operative sepsis	No	No	-	-	No	Yes[21]	(Stuber et al., 1996a)
Meningococcal disease	No	Yes[22]	-	-	-	-	(Nadel et al., 1996)
	No	No[23]	No	No	-	-	(Westendorp et al., 1996)*
Leishmaniasis	No	Yes[24]	-	-	No	Yes[23]	(Cabrera et al., 1995)
Malaria	No	Yes[25]	-	-	-	-	(McGuire et al., 1994)

Table 4.4 Studies of the TNFα -308, -238, and TNFβ NcoI biallelic polymorphisms in immune linked, inflammatory, infective, metabolic and malignant disorders

TNF is thought to be important in the pathogenesis of all disorders studied
The study populations are all N European Caucasians unless otherwise specified
If an association is claimed by the study, details are given in footnotes, below:

* study in relatives of patients
IDDM Insulin dependent diabetes mellitus
AIDS Acquired immune deficiency syndrome

1. -308 TNF.G/G genotype associated with increased presence of nodular disease compared with TNF.G/A genotype (25.5 vs. 0%, p=0.03); -238 TNF.A/A genotype associated with lower mean age of onset compared with –238 TNF.G/A genotype (49±14.2 vs. 59±8.8, p=0.02). Study population Spanish.
2. -238 TNF.G/A genotype associated with a slightly less frequent occurrence of erosions, although this was not statistically significant (82 vs. 94%). Study also looked at other TNFα bi-allelic polymorphisms at positions -376, -163, and +70 and found no associations.
3. Study also looked at the bi-allelic polymorphism at +365 in TNFβ locus-no association. Likewise with microsatelite alleles.

4. -308 TNF.A allele associated with Systemic lupus erythematosus (SLE) in African-Americans. Association reported to be independent of HLA-DR alleles
5. -308 TNF.A allele increased in incidence in (UK) Caucasians (p=0.04). -308 TNF.A allele strongly linked with DR3 in the Caucasians, so association due to DR3. In native South African patients, DR2 but not DR3 was increased, and the frequency of –308 TNF.A was actually reduced rather than increased. This contrasts with later study (see note 4).
6. -238TNF.A allele associated with juvenile onset psoriasis and psoriatic arthritis compared with controls (38 vs. 7%, p<0.01and 32 vs. 7%, p<0.03 respectively).
7. -238 TNF.G/A genotype associated with type I psoriasis compared to controls (41 vs. 8%, $p<10^{-5}$). N American Caucasians.
8. -308 TNF.A allele associated with subtype of disease with acute onset and spontaneous remission compared with controls (41 vs. 23%, p=0.02).
9. -308 TNF.A allele associated with susceptibility to coeliac disease (CD) compared to controls (50 vs.16%, $p<10^{-6}$). However the association was not independent of the association of CD with HLAB1*0201. Study in N American Caucasians and Taiwanese Chinese.
10. -238 TNF.G/A genotype associated with alcoholic steatohepatitis (ASH) compared with controls or patients with other lesions of alcoholic liver disease (ASH vs. non-ASH 16%vs 4%, p=0.036). No difference in age between patients, therefore total cumulative alcohol consumption claimed likely to be similar.
11. -238 genotypes differently distributed in patients 'hospitalised' in a nursing home compared to those attending outpatient clinic $\chi^2=6.9$, p<0.01). Difference attributed to absence of -238 TNF.A in 'hospitalised cohort. Numbers, however, were small; -238 TNF.A allele frequency in controls/clinic patients/'hospitalised' patients was 0.1/0.03/0.0.
12. -308 TNF.A allele decreased in patients with ulcerative colitis (UC) compared to controls (15 vs.25%, p=0.044). Crohn's disease (CD) and UC patients studied. Study also looked at AspHI RFLP polymorphism within TNFβ gene, no association was found. Haplotypes across the four loci (-308, -238, NcoI, AspHI) also were not significantly differently distributed between patient groups (UC and CD) and controls. -308 association with UC not confirmed by larger study, see note 13.
13. -308 TNF.A allele decreased in patients with CD compared to controls (13.2 vs.21.3%, p=0.044). CD and UC patients studied. Did not confirm -308 association with UC, see note 12.
14. -308 TNF.A allele carriers were more frequently obese than non-carriers (40 vs. 30%, p<0.004). This finding not confirmed in study of Day et al (1998), see note 15.
15. Non-diabetic relatives of non-insulin dependent diabetes mellitus patients carrying -308 TNF.A allele had reduced mean insulin resistance (p=0.03) and increased mean insulin sensitivity (p=0.04) compared with those with only –308 TNF.G allele.
16. IDDM subjects have increased allelic frequency of -308 TNF.A compared to controls. However this association was due to linkage disequilibrium with

HLA-DR3, DQB1*0201 haplotype, and not independent of it.

17. Asthma significantly more common compared to controls in subjects with -308 TNF.A allele ($p=0.004$), and (NcoI) +249 TNFB.G allele ($p=0.005$).
18. Significantly increased carriage of -308 TNF.A allele in patients with malignant disease (lymphoma, breast carcinoma, other tumours) compared to age matched controls (35 vs. 16%, $p<0.005$). Differences confirmed between individual tumour groups. Study also associated hsp70 polymorphism (MHC class III) with same groups. No linkage between 2 loci detected. Study in population of North Africans.
19. -238 TNF.A allele significantly more common in patients with chronic hepatitis B infection compared to controls and those with acute infection (25 vs. 6 and 7% respectively, $p<0.04$ and $p<0.003$).
20. Study in N American Caucasians.
21. (NcoI) +249 TNFB.A (called TNFB*2 in study) heterozygotes had significantly higher survival than homozygotes ($p=0.007$). Also see text, section 4.1.4.2.1.
22. -308 TNF.A allele carriage associated with higher risk of death in childhood meningococcal disease compared with patients surviving (30 vs. 12%, $p=0.03$).
23. -308 and -238 allele frequencies no different in relatives of survivors and non-survivors with adult meningococcal disease
24. -308 TNF.A allele present at significantly higher frequency in mucocutaneous leishmaniasis (MCL) patients compared with healthy control subjects (18 vs.6.9%, $p<0.05$). +249 TNFB.G (TNFB*2) allele also higher in MCL compared to controls. Population of Venezuelan subjects.
25. In Gambian population cerebral malaria associated with increased frequency of -308 TNF.A/A homozygotes vs. mild malaria, 4.5% vs.1.8% ($p=0.04$). In cases of cerebral malaria, those who died or had severe neurological sequelae (99/376) had even higher frequency of homozygotes, 8.1 vs. 1.8% ($p=0.005$). found to be independent of HLA class I and II alleles.

4.1.4.5.2 TNF microsatellite polymorphisms

The TNF microsatellite polymorphisms have also been investigated as regards their possible association with selected diseases. Table 4.5 details many of these studies, and some are now discussed.

In a group of Caucasian IDDM subjects who were DR3/DR4 positive, the allele TNFa2 was significantly more frequent compared to DR3/DR4 controls (Poicot et al., 1993). The same study demonstrates that the allele TNFa6 is correspondingly reduced in frequency in their DR3/DR4 patients compared to controls.

Although differences in allele distribution could not be assigned to any particular allele, another study in a population of South Indians with Non-IDDM (NIDDM) and IDDM noted significant differences in the distribution of TNFa and TNFb microsatellite alleles between IDDM patients and controls and between NIDDM patients with retinopathy as opposed to NIDDM patients with no associated eye disease (Hawrami et al., 1996). This latter association was found to be caused exclusively by NIDDM patients with proliferative retinopathy.

Further characterisation of TNF microsatellite alleles and 3 locus (TNFabc) haplotypes has been carried out in populations of North American patients with IDDM and multiple sclerosis (MS) (Garcia-Merino et al., 1996). The authors also related their TNF microsatellite haplotypes to extended HLA haplotypes. Individual allelic data is not presented, but the haplotype TNFa2b3c1 was found to be significantly increased in patients with IDDM, compared to controls. Also, the TNFa11b4c1 haplotype was found to be significantly increased in frequency in the patients with MS. These associations were not found to be independent of the already identified HLA haplotype associations. The TNFa2b3c1 haplotype was found to be carried exclusively by HLA-B8, SC03, DR3 subjects, a haplotype already identified as being associated with IDDM. Likewise the TNFa11b4c1 haplotype association was 80% attributable to it's HLA-B7, SC31, DR2

linkage, already known to be associated with MS.

Another North American study has studied TNF microsatellite alleles in Caucasians with IBD (Plevy et al., 1996). Characterisation of 3 (TNFabc) and 5 locus (TNFabcde) haplotypes was carried out. They found the 3 locus haplotype TNFa2b1c2 to be significantly increased in patients with Crohn's disease (CD) compared to ulcerative colitis (UC). It was also more frequent in CD patients compared to controls, but this figure did not reach statistical significance. The frequency of the 5 locus haplotype TNFa2b1c2d4e1 was also found to be significantly increased in patients with CD compared to those with UC and the healthy controls. The authors showed that the TNFa2b1c2d4e1 haplotype was associated in 50% of subjects with HLA-DR1/DQ5, an HLA combination they had previously found to be associated with CD. The TNF 5 locus haplotype gave a more significant association with CD than the HLA-DR1/DQ5 association (Odds Ratio 4.4 vs. 2.5).

As can be seen, many of the reported associations of TNF alleles and haplotypes have not been confirmed by similar studies in comparable populations. Furthermore, it is apparent that associations reported are often secondary to, or linked to, known HLA associations.

Disease	Details of TNF microsatellite allele or haplotype association	Reference
IDDM	TNFa2 increased in DR3/4 +ve (p=0.047) , a6 decreased in DR3/4 +ve vs. controls (p=0.051)	(Poicot et al., 1993)
	Distribution of TNFa and b alleles different vs. controls (p=0.016)[1]	(Hawrami et al., 1996)
	TNFa2b3c1 haplotype frequency patients vs. controls, 24.5 vs. 8.2% (p<0.004)[2]	(Garcia-Merino et al., 1996)
	TNFa1b5 haplotype frequency patient vs. controls, (p<0.005)[3]	(Monos et al., 1995)
NIDDM	Distribution of TNFa and b alleles different in patients with retinopathy vs. those without (p=0.006)[1]	(Hawrami et al., 1996)
Multiple sclerosis	TNFa11b4c1 haplotype frequency patients vs. controls 30.2 vs. 16.5% (p<0.04))[4]	(Garcia-Merino et al., 1996)
IBD	TNFa2b1c2d4e1 haplotype frequency in Crohn's Disease vs. controls 24 vs. 6.7% (p=0.01)[5]	(PLevy et al., 1996)
Rheumatoid arthritis	TNFa and TNFe [6]	(Field et al., 1997)
	TNFa6 allele frequency in patients vs. controls (p=0.0019)[7]	(Hajeer et al., 1996)
SLE	No association; TNFa microsatellite only tested	(Mascher et al., 199)
Coeliac disease	TNFa2 allele frequency in patients vs. controls 61.2 vs. 26.3% (p<0.001). Also TNFb3 association[8]	(McManus et al., 1996)

Table 4.5 Studies of the TNF microsatellite polymorphisms in immune linked, inflammatory, infective, and malignant disorders (continued...)

Disease	Details of TNF microsatellite allele or haplotype association	Reference
Renal allograft rejection	TNFa9 and TNFd4 alleles increased in rejection vs. rejection free[9]	(Asano et al., 1997)
Colorectal cancer	TNFa2 allele frequency in patients vs. controls (35 vs. 20%,p=0.01)[10]	(Gallagher et al., 1997b)
AIDS	TNFa2 allele associated with faster disease progression vs. Controls (15 vs. 46%, p=0.006)[11]	(Khoo et al., 1997)

Table 4.5 Studies of the TNF microsatellite polymorphisms in immune linked, inflammatory, infective, and malignant disorders

TNF is thought to be important in the pathogenesis of all disorders studied
The study populations are all N European Caucasians unless otherwise specified
details and additional information of studies are given in footnotes, below:

- denotes relationship not tested
IDDM Insulin dependent diabetes mellitus
AIDS Acquired immune deficiency syndrome
NIDDM Non-insulin dependent diabetes mellitus

1. Study in Southern Indian subjects
2. TNFa2b3c2 association not independent of HLA-B8,SCo1,DR3 association. Study in N American Caucasians
3. Only TNFab microsatellites tested. In linkage with HLA-DR3,B18, a haplotype known to be associated with IDDM. This association not tested in patient cohort
4. TNFa11b4c1 association 80% attributable to known HLA-B7,SC31,DR2 association. Study in N American Caucasians
5. TNFa2b1c2d4e1 (and 3 locus TNFa2b1c2) association greater than HLA class I and II haplotype association that is in linkage with TNFa2b1c2d4e1 (HLA-DR1, DQ5). No association with ulcerative colitis found. Study in N American Caucasians
6. TNFa and TNFe only tested. Differences observed consistent with extended MHC haplotypes, therefore no independent contribution of the TNF locus
7. TNFa6 association due to linkage with DRB1*0401. Not an independent marker.

8. TNFb3 also increased in Coeliac patients vs. controls (53.9 vs. 13%, $p<0.001$). This association due to linkage with HLA-DQB1*0201. TNFa2 association was independent of HLA class I or II association
9. TNFa9 and TNFd4 showed strong associations with HLA-B35 and B44, respectively. However the TNF microsatellite loci were more closely related to acute rejection than HLA-B. Japanese population
10. Two independent populations confirmed this association.
11. TNFa,b,c, and d microsatellite characterisation. The 2 subgroup numbers were ≤ 24.

4.2 Interleukin-10 (IL-10) locus

The gene encoding IL-10 has been mapped to chromosome 1 (Kim et al., 1992). It's exact location has yet to be confirmed, but it is probably in the region of 1q31 and 1q32 (Eskdale et al., 1997a). Other genes in this region include genes encoding proteins with functions within the immune system; for example the CD21(lymphocyte cell surface molecule) locus is nearby, as are genes encoding complement system components (decay accelerating factor, complement receptor 1, complement receptor 2).

The intron/exon structure of human IL10 has yet to be determined. The mouse IL-10 gene, which has considerable homology to the human form (Kube et al., 1995), also maps to chromosome 1 and is composed of five exons arrayed over c. 5.5kb of DNA (Kim et al., 1992). The IL-10 region is shown in Fig. 4.4.

The human IL-10 promoter region (5' region) has been investigated in some depth. Kube and co-workers (1995) isolated the promoter region and produced deletion mutants of it by partially digesting the fragment with a selection of restriction enzymes, and then re-ligating. Subsequently a chemoluminescent reporter gene assay was constructed in lymphoma cell lines (Kube et al., 1995). This work showed that areas of the promoter had different effects upon reporter gene activity, identifying elements within the promoter region that could alter the constitutive expression of IL-10. Elements that upregulated gene expression were identified between -1100 and -900, and sequences that reduced gene expression were located between -800 and -300. All positions are relative to the transcription initiation start site of the IL-10 gene.

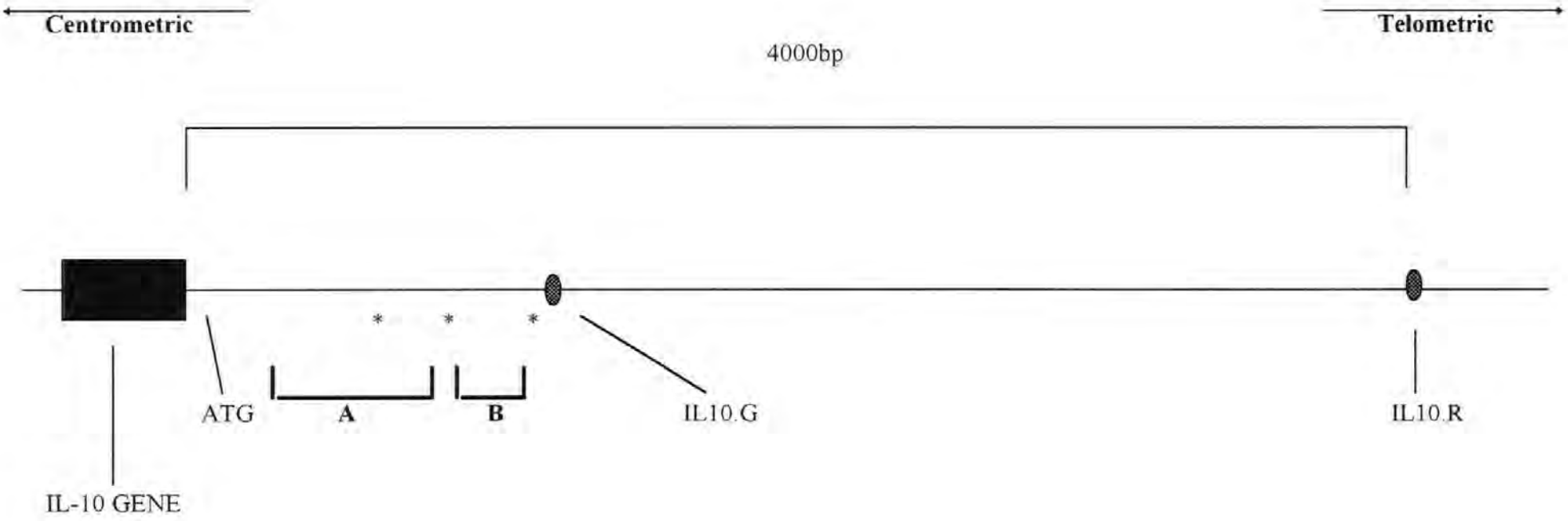

Figure 4.4 IL-10 gene and promoter region on chromosome 1

IL-10 Gene, intron/exon structure unknown
ATG - Transcription start site
A - region of promoter reported to reduce gene expression[1]
B - region of promoter reported to increase gene expression[1]
IL10.G - microsatellite $(CA)_n$ repeat, 16 alleles
IL10.R - microsatellite $(CA)_n$ repeat, 5 alleles
Biallelic (base substitution) polymorphisms at -1117, -854, and -627 from transcription start site

microsatellite

gene locus, intron/exon structure unknown

1 (Kube et al 1995)

(not to scale)

4.2.1 Polymorphisms in the IL10 locus

Polymorphic sites have subsequently been identified within the promoter region. These consist of two polymorphic dinucleotide repeats (microsatellites), named IL10.G and IL10.R, and a number of base substitution polymorphisms (point mutations). These are shown in Figure 4.4.

The first to be described was the IL10.G microsatellite, a $(CA)_n$ repeat located between positions -1193 and -1151 (Eskdale and Gallagher, 1995). IL10.R, another $(CA)_n$ repeat, was subsequently reported to be c. 2.8kb upstream of this (Eskdale et al., 1996). They have sixteen and five alleles respectively.

Three single (biallelic) base substitution polymorphisms have also been characterised. They are named according to position from transcription initiation start site, which causes some confusion, as the two sets of investigators involved seem to have named and identified the polymorphisms using different sequences deposited at the GenEMBL database. In this work, the sequence with accession number X78437 will be used to name the biallelic polymorphisms by position, with their other published nomenclature put in parentheses. Turner et al (1997) characterised three sites at -1117 (they called -1082), -854 (they called -819), and -627 (they called -592). -1117 is a C-A transition, -854 is a C-T transition, and -627 is a C-A transition (Turner et al., 1997). The sites at -854 and -627 have been confirmed by Eskdale et al , who did not analyse the region beyond -1002 (Eskdale et al., 1997a). They found the two sites (-854 and -627) to be in complete linkage disequilibrium in their population, -854.1 was always linked with -627. 2 and *vice versa*.

4.2.2 Nomenclature

Alleles of the two microsatellites (IL10.G and IL10.R) are named according to the common system for this type of polymorphism. The allele with the least number of dinucleotide repeats (n=x) is designated allele 1, and subsequent alleles are numbered according to the number of repeats (n=x+1,allele2; n=x+2, allele 3; etc.).

With regard to the biallelic polymorphisms at positions -1117, -854, -627, as well as having two different names (in this work the sequence with accession number X78437 will be used to name the biallelic polymorphisms by position, with their other published nomenclature put in parentheses), they are in possession of two different systems of allelic nomenclature. Eskdale et al (1997) typed the -854 and -627 loci by PCR-RFLP, and name alleles according to whether the fragment is undigested (allele 1) or digested (allele 2). Turner et al (1997) name alleles according to the nucleotide. This work will follow the convention outlined for the alleles of the TNF locus (see section 4.1.4.2), and will name alleles according to the nucleotide.

e.g. -1117.G, -854.T, -627.A

4.2.2 Effects of polymorphisms upon IL10 production?

As discussed above, comparable with genetic influences upon secretion of TNF, a high proportion (75%) of the variation in IL-10 production between individuals is estimated to be genetically influenced (Westendorp et al., 1996).

It is important to note that (as detailed in fig. 4.4) the three biallelic polymorphisms and the IL10.G microsatellite are all either within or close to the regions identified as having effects upon gene transcription, -1100 to -900, and -800 to -300 (Kube et al., 1995), leading to speculation that the polymorphisms may play a functional role in IL10 gene transcription and subsequent protein expression.

To date, there have been no studies to determine if the IL10.G or IL10.R microsatellites have any functional relevance upon IL-10 production. However, all three of the biallelic polymorphisms have been investigated to see if they have any effect upon IL-10 protein production (Turner et al., 1997). Mean IL-10 production, as measured by *in vitro* stimulation of peripheral blood leucocytes, has been shown to be significantly higher in healthy controls typed for -1117.G (called -1082*G in study) compared to those typed for -1117.A (called -1082*A in study), 1720 pg ml^{1-} vs. 1297 pg ml^{-1} ($p=0.035$, t-test).

Sequencing of the IL10 5'flanking region has also revealed that close to all the polymorphisms so far characterised there are numerous and varied transcription factor binding sites (Eskdale et al., 1997a). These include NKκB/REL sites known to mediate TNF signalling (Baeuerle and Henkel, 1994), STAT.1 sites known to mediate interferon and interleukin signalling, and the NF-IL-6 recognition sequence. A concentration of such sites are situated downstream of the IL10.R polymorphism (and this is described by the authors as being the putative cytokine responsive area), but there also are many located in close proximity to the IL10.G polymorphism and the three biallelic polymorphisms. For example there are two of the nine potential NFκB/REL sites 60 and 80bp immediately upstream of IL10.G (Eskdale et al., 1997a). It can be speculated that the known ability of TNF to influence IL10 transcription (Platzer et al., 1995) is mediated through these sites, and the other transcription factor binding sites may be involved in a similar way in cytokine mediated modulation of IL10 gene expression. Further conjecture could lead one to suppose the polymorphisms in this region may be functional through their associated alteration of an important transcription factor binding site.

4.2.3 IL-10 allele associations with disease

As polymorphisms around the IL-10 locus have been characterised relatively recently, there are a limited number of studies utilising their potential role as tools to determine the conceivable role of IL-10 in genetic susceptibility to inflammatory and (auto)immune disorders.

Systemic lupus erythematosus (SLE) is a disorder in which elevated IL-10 levels are found, particularly in association with the presence of pathogenic autoantibodies. Both the microsatellite polymorphisms and the three biallelic loci have been characterised in several patient populations with SLE.

No differences have been noted in the distribution of IL10.R alleles between SLE patients and controls, however, the two populations differed significantly in the distribution of

IL10.G alleles (p=0.028). In particular allele 9 was decreased whilst allele 13 was increased in frequency (Eskdale et al., 1997b). Differences were confirmed by examining the genotype distribution. The data suggested that those subgroups of patients without renal disease or with anti-DNA antibodies accounted for this, as the other subgroups (with renal disease or with anti-ENA antibodies) had allele 9 and 13 frequencies comparable with the control population. This is a tentative interpretation of the observation, as subgroup numbers and the total were relatively small (total=56).

Two other studies have looked at the biallelic polymorphisms in SLE patients. One also looked at a larger group of UK Caucasian SLE patients and found no significant change in -1117, -854, and -627 allele frequency compared with controls (Lazarus et al., 1997). The authors did note, within the SLE population, a significant difference in the distribution of their (three) putative haplotype frequencies according to positive Ro antibody status (p=0.005). The other published study is in a population of Chinese (n=88). In this population of SLE patients, a significantly different IL-10 allelic distribution was not noted between patients and controls, however an association seen between alleles -854.T and -627.A and the development of lupus nephritis was seen in this small subgroup of patients (Mok et al., 1998).

In another autoimmune associated disease, rheumatoid arthritis (RA), the allelic frequency of -1117, -854, and -627 was found to be the same in Caucasian patients and controls (Hajeer et al., 1998). To assess whether there was any association with disease severity the patients were subdivided on the basis of whether their rheumatoid factor (RF) was IgG, IgA or IgM (IgA associated with worse prognosis). The patients with IgA+/IgG- RF were found to have a higher frequency of the –1117.A allele and the (-1117.A / -854. C / -627.C) haplotype than controls, (28 vs. 50% p=0003). However, as this is a haplotype the authors had previously associated with low IL-10 production (Turner et al., 1997), the significance of this finding is unclear, as IL-10 is known to induce B cells to switch to IgA production

(in combination with TGFβ). The same group of investigators have also characterised the same three polymorphic loci in heart transplant patients, in association with the -308 TNF polymorphism. They found that the patients typed as 'low secretors' of IL-10 (-1117.A homozygotes) and 'high secretors' of TNF (-308.A allele present) were more likely to have high levels of rejection (5-8 episodes). 5 of 19 patients with high levels of rejection had this combination, as compared to 4 of 96 patients with low levels of rejection. (Yates' <0.005). These findings are very interesting and worthy of further study, but the numbers are relatively small.

4.3 Other cytokine genes and inflammatory diseases

Polymorphisms have also been characterised at other cytokine loci within the genome. Three of the most interesting are polymorphisms in the IL-1ra, IL-1, and IL-4 genes.

IL-1ra, the natural antagonist to IL-1, has a well characterised variable number tandem repeat (VNTR) polymorphism in the second intron of it's gene on chromosome 2 (Tarlow et al., 1993). The repeat unit is an 86 bp sequence The polymorphism has 5 alleles, the four-repeat (allele 1) and the two-repeat (allele 2) being the most common, whilst the other alleles have a combined frequency of less than 5%. A relationship has been shown to exist between allele 2 and increased IL-1ra production, and decreased IL-1 production by stimulated monocytes *in vitro* (Danis et al., 1995).

Investigations have suggested that allele 2 of the IL-1ra VNTR is a marker of disease severity in a number of inflammatory diseases and processes. These include Juvenile RA (Mc Dowell et al., 1995), nephropathy in NIDDM and IDDM (Blakemore et al., 1996), and ulcerative colitis (Mansfield et al., 1994). The association between severity of UC and allele 2 has been a disputed finding as other studies with increased (and perhaps more adequate) numbers of patients have partially confirmed (Heresbach et al., 1997; Roussomoustakaki et al., 1997) and refuted the finding (Hacker et al., 1997). Nevertheless, especially as the

VNTR has been shown to have functional relevance, testing for associations between the polymorphism and severity of inflammatory disease is a very attractive hypothesis which warrants testing. There are also identified biallelic (RFLP) polymorphisms in the IL-1 gene itself on the same chromosome. These have also been investigated with the IL-1ra polymorphisms as markers of disease susceptibility and severity in IDDM and IBD (Heresbach et al., 1997).

IL-4 is an interesting cytokine that is produced by and has effects upon mast cells and stimulates increased production of IgE. Therefore it has an important role in atopic responses. The IL-4 gene and IL-4 receptor (IL-4R) genes both have identified biallelic (base substitution) polymorphisms. These have been characterised in groups of patients with asthma and atopy. No association has been found with the IL-4 promoter polymorphism, but an allele, termed R576, of the IL-4R gene (which causes an amino acid change in the cytoplasmic domain of the receptor), has been reported to be associated with atopy. Furthermore, carriers of this allele seem to have enhanced expression of CD23 in response to IL-4, indicating that the mutant allele may have effects upon receptor signalling (Hershey et al., 1997).

4.4 Genetic association studies in alcoholism and alcohol related diseases

Because of the significant number of patients with alcohol-induced pancreatic disease, and in view of the nature of the immunogenetic studies being undertaken in them in this work, consideration will be given to possible HLA associations with alcoholism and alcohol related diseases, and to genetic studies on alcohol-metabolising enzyme genes.

4.4.1 Inherited susceptibility to alcoholism

Alcoholism is well known to run in families, but the important question is whether this is due to the influence of genes or the environment.

Twin studies indicate that alcoholism is more concordant in monozygotic (MZ) than

dizygotic (DZ) twins, and also this does not completely rule out the influence of environment it is suggestive that alcoholism is in part inherited. Adoption studies, which go some way towards separating environmental and genetic influences upon behaviour and disease, confirm this. Animal studies support the concept of a genetic influence on alcoholism; it is possible to selectively breed rats for high and low voluntary alcohol intake (Lumeng and Crabb, 1994).

A recent American study, while confirming that concordance rates for alcoholism are higher in MZ compared to DZ twins, claims that there exists little additional genetic liability to the cirrhotic or psychotic complications of alcoholism, beyond that already seen in relation to alcoholism. Studies that have used different variance modelling methodology are, however, in direct contradiction to this finding (Reed et al., 1996).

4.4.2 HLA associations with alcoholism and alcohol related liver disease

There have been a large number of studies looking at the association between HLA antigens, encoded by the MHC, and alcoholism and alcohol-related liver disease in a number of different races. All published studies used serological methods to perform HLA typing and used healthy control subjects from the same geographical area.

In Caucasian populations there is no consistent HLA type which is associated with alcoholism or alcohol-related liver disease. However, HLA-B15, B40, DR2, and DR4 have all been reported of an increased frequency in groups of such patients in Northern European and Australian Caucasians populations, but a meta-analysis of all the studies done in this area concludes that there is no HLA association (List and Gluuid, 1994).

4.4.3 HLA associations with alcohol related pancreatitis

Studies have also been undertaken regarding serological HLA types and association with alcohol-induced pancreatic disease. Although many of the studies are poorly controlled certain HLA types have been found to have a significant association with the development of alcoholic chronic pancreatitis in certain Caucasian populations:

French: B40 (Fauchet et al., 1979)

UK: C5 (Forbes et al., 1987)

Italian: B13 (Gullo et al., 1982)

Australian: B39 (Wilson et al., 1984)

Again, the HLA type in question differed between studies, and only one used a control group of alcoholics without pancreatic disease (Wilson et al., 1984). Other studies in similar Caucasian populations have found no associations (Haber et al., 1995).

4.4.4 Alcohol metabolising enzyme genes, alcoholism, and alcohol-related liver disease

As previously discussed there are three main families of alcohol metabolising enzymes; alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH), and the cytochrome P450 2E1 enzyme (CYP450 2E1).

Although hepatic ADH is the major pathway for ethanol oxidation in the liver, there are over 20 different ADH isoenzymes grouped in five classes. They have diverse tissue distribution, including stomach mucosa. The five classes (I to V) are encoded by seven gene loci, which encode for subunits of ADH isozymes. The genes ADH1, ADH2, and ADH3 encode for the subunits which make up the isoenzyme class found predominantly and extensively in the liver. ADH2 and ADH3 are polymorphic, biallelic base substitution polymorphisms result in amino acid residue changes in the subunits. The ADH2 residue changes are in the coenzyme binding site, so directly affect the kinetic properties of the enzyme. The same is postulated for ADH3 (reviewed by (Arnon et al., 1995)).

The ALDH enzyme has two classes, but it seems that only the ALDH2 isozyme, encoded by the ALDH2 gene, is a major participant in ethanol metabolism (reviewed by (Arnon et al., 1995)). It too exhibits biallelic polymorphism.

The CYP450 2E1 gene has four polymorphic RFLP sites, indicating biallelic base substitution changes (Savolainen et al., 1997).

In Caucasian populations the ADH2 and ALDH2 allelic variations do not occur or are very

rare , however, they exist in Far Eastern populations (Suzuki et al., 1994). Associations with ADH2*1 and ALDH2*1 alleles are claimed in Japanese alcoholic subjects, but conflicting results have been obtained as to whether any allele is associated with the development of alcoholic liver disease (Tanaka et al., 1996; Yamaguchi et al., 1995). Interestingly possession of the ALDH2*1 allele protects Far Eastern individuals from the severe facial flushing commonly seen with alcohol in these populations.

In Caucasian alcoholics with liver disease the ADH3*1 allele is reported to be increased in frequency compared to controls, but the (weak, and non-significant by standard tests) association seen by the authors could be due to an association with alcoholism as a non-alcoholic control group was used (Day et al., 1991).

CYP450 2E1 polymorphisms have been characterised in subjects with heavy alcohol intakes and in patents with alcoholic liver disease. Again, markedly different allele frequencies are seen in different racial groups, the Japanese population (Tsutsumi et al., 1994) exhibiting more variability than Caucasians (Savolainen et al., 1997). Unsurprisingly therefore, only in the Japanese has an association between alcoholic liver disease and polymorphism of CYP450 2E1 been claimed. It does not seem to be associated with alcoholism *per se.*

4.4.5 Alcohol metabolising enzyme genes and alcohol-related pancreatic disease

There have only been a few studies that have characterised alcohol metabolising gene polymorphisms in pancreatitis patients. These have been in subjects with alcohol-induced chronic pancreatitis.

Studies in Caucasians have been of the ADH2, ADH3, and ALDH2 loci. Unsurprisingly, little or no allelic variation of the ADH2 and ALDH2 loci was detected. An increased frequency of the ADH3*1 allele is reported to be found in Caucasian alcoholics with chronic pancreatitis compared to normal healthy controls (Day et al., 1991), however the association is very weak and does not achieve significance with standard statistical methodology. Furthermore, only 13 patients were studied. A similarly sized study reports

no ADH3 association (Couzigou et al., 1990).

Larger studies, also characterising and the CYP450 2E1 locus, have been carried out in Japanese patients with chronic pancreatitis, comparing them with alcoholic patients with no chronic pancreatitis (Matsumoto et al., 1996). No differences in allele distribution between patients and controls were seen for the ADH3, ALDH2, or CYP450 2E1 loci. The authors noted that the frequency of the ADH2*2/*2 genotype was increased in the group with pancreatic disease (42 vs. 30%), and in fact the genotype distribution is significantly different ($p<0.05$).

All in all, this is scanty evidence that polymorphisms of the alcohol metabolising enzyme genes are associated with susceptibility to alcoholic chronic pancreatitis or alcohol-induced liver disease, especially in Caucasian populations. To do more than suggest an association, more studies are needed.

4.5 Aims of this study

The clinical course of acute pancreatitis is often mild with only minimal associated organ dysfunction, but a significant proportion of patients develop severe pancreatitis which is associated with organ failure, systemic complications, local inflammatory and infective manifestations and, in up to 10% of cases, death. The aetiology of acute pancreatitis is also diverse, gallstones and heavy consumption of alcohol accounting for the majority of cases. The reason for the development of alcoholic acute pancreatitis is unclear, but does not appear to be related to the amount or type of alcohol consumed, implying that factors relating to the host account for why only a proportion of patients with heavy alcohol consumption develop the disease.

Pro-inflammatory and regulatory cytokines play a fundamental role in the local and systemic inflammatory response in the initial stages of disease and in the development of severe acute pancreatitis. As has been discussed, polymorphisms of cytokine genes may influence protein production, so influencing regulation of the inflammatory response.

This study aimed to characterise TNF and IL-10 polymorphisms in a population of normal Europids, and patients with acute pancreatitis. This enabled analysis to determine if any of the polymorphisms studied were determinants of disease severity or susceptibility.

5 Materials and Methods

5.1 Study Subjects

5.1.1 Patients

One hundred and thirty five Europid patients admitted with a diagnosis of acute pancreatitis were identified shortly after admission to Derriford Hospital, Plymouth. Ethical committee approval had been obtained. The criteria for diagnosis of acute pancreatitis were:

i. a clinical history and examination consistent with the disease (Table 5.1)

ii. a serum amylase level greater than 660units/l (Hitachi 911, Hitachi Corporation, Japan; normal level <220 units).

iii. appropriate radiological evidence (Table 5.1)

Patients who had clinical or radiological evidence suggestive of a diagnosis of chronic pancreatitis were excluded (Table 5.2) as were patients who subsequently proven to have radiological or ERCP evidence of chronic pancreatitis. CT or ERCP was performed if there was a clinical suspicion of chronic pancreatitis in those patients with alcohol induced disease. 71% (15 of 21) patients classified as having acute alcohol-induced acute pancreatitis underwent CT or ERCP. The other 19% (6 of 21) had an adequate ultrasound scan (pancreas clearly visualised), with no evidence of chronic pancreatitis in this imaging modality.

Venupuncture was performed using a *Vacutainer*® blood collection system (Becton Dickinson, Meylan, Cedex-France). Blood was collected into 5% disodium ethylene diamine tetra-acetic acid (Na_2EDTA), and stored at -20°C in sterile polypropylene containers prior to subsequent DNA extraction.

Patients who had received a blood transfusion within the previous three months were not included in the study as donor white cells still may have been present in peripheral blood, so contaminating subsequent DNA preparation.

5.1.2 Determination of aetiology of disease

Aetiology of acute pancreatitis was classified as being due to gallstones in the presence of appropriate radiological or ERCP findings, due to alcohol if subjects admitted on questioning to being consistent consumers of greater than 80g of alcohol per day (Wilson et al., 1985), or idiopathic if no other identifiable cause could be discovered.

Patients were classified as having idiopathic pancreatitis following an optimal ultrasound or CT scan reporting the absence of gallstones in the biliary tree and gallbladder. Patients who had an inadequate initial examination underwent repeat ultrasound scan.

Consistent consumption of alcohol was defined as daily consumption of greater than 80g of alcohol per day over a time period exceeding six months. No subject who had acute pancreatitis secondary to excessive 'binge' consumption of alcohol was encountered during recruitment for this study. Any history of alcohol consumption recorded in the medical notes was confirmed by interviews with individual patients.

5.1.3 Severity of disease episode

The progress of individuals with regard to development of complications was monitored during their disease episode. The recording of physiological, biochemical, and haematological parameters enabled patients to be classified as having mild disease or severe local or systemic disease according to criteria defined by the Atlanta convention (Bradley, 1993). This is summarised in Table 5.3.

The occurrence of a local pancreatic complication was defined as described in the Atlanta classification, except an acute fluid collection was not included in this category for the purposes of this study.

Maximal organ failure scores according to the method of Bernard (Kingsnorth et al., 1995) were also calculated to provide another means of patient stratification (Table 5.4).

Clinical history and examination	Radiological evidence
Abdominal pain	Plain X-rays:
Referred pain to back	Intestinal ileus
Anorexia	Gallstones
Nausea	Absence of free sub-diaphragmatic air[1]
Vomiting	Absence of pancreatic calcification[2]
Epigastric tenderness	Ultrasound Scan: (may be normal)
Dehydration	Pancreatic oedema
Hypovolaemia	Free intraperitoneal fluid
Confusion secondary to hypoxia	Fluid collection (pseudocyst, abscess)
Jaundice (10-20%)	CT Scan: (may be normal)
Grey-Turner/Cullen sign (<5%)	Pancreatic oedema
	Free intraperitoneal fluid
	Fluid collection (pseudocyst, abscess)
	Area of necrosis

Table 5.1 Clinical and radiological criteria consistent with diagnosis of acute pancreatitis

CT = computerised tomography

[1] Indicates perforated viscus

[2] Indicates probability of chronic pancreatic inflammation

Clinical	Radiological
History:	Plain Radiographs, USS, CT Scan:
Chronic epigastric pain	Calcification
Steatorrhoea	Ductal stones
Weight loss	Pseudocysts
Diabetes	ERCP:
	Calcification
Pancreatic function test:	Ductal stones
Evidence of pancreatic insufficiency	Pseudocysts
	Ductal stricture

Table 5.2 Clinical and Radiological evidence suggestive of Chronic Pancreatitis

CT = computerised tomography

USS = ultrasound scan

ERCP = endoscopic retrograde cholangiopancreatography

CATEGORY	DEFINING CRITERIA
Mild acute pancreatitis	**Minimal organ dysfunction**
Severe acute pancreatitis:	**Organ failure and / or local complications**
Predicted severe disease	Apache II Score ≥ 8
Organ failure:	
i. Cardiovascular insufficiency	Systolic blood pressure < 90 mmHg
ii. Respiratory insufficiency	PaO_2 < 60 mmHg (8 KPa)
iii. Renal failure	Serum Creatinine > 177μmol l^{-1} (after rehydration)
iv. Gastrointestinal bleeding	> 500ml of blood loss in 24 hours
v. DIC	Platelets ≤ 100,000 per mm^3, Fibrinogen < 1g l^{-1}
vi. Metabolic failure	Serum calcium ≤ 1.87 mmol l^{-1}
Local complications	
i. Acute fluid collection	Located in or near the pancreas, lack a wall of granulation or fibrous tissue
ii. Pseudocyst	Collection of pancreatic secretion bounded by fibrous or granulation tissue wall
iii. Abscess	Circumscribed collection of pus
iv. Necrosis	Diffuse or focal nonviable pancreatic parenchyma

Table 5.3 Atlanta Convention Classification of acute pancreatitis[1]

Apache II	Acute Physiology and Chronic Health Evaluation II (Larvin and McMahon, 1989)
DIC	Disseminated Intravascular Coagulation
mmHg	millimetres of mercury
Kpa	Kilopascals

[1] (Bradley, 1993)

OFS	CVS SBP (mmHg) Lactate (mmol l^{-1})	RS PaO_2/FiO_2 (mmHg)	CNS Glasgow Coma Score	COAG. Platelets ($x10^9$ l^{-1})	RENAL Creatinine (μl l^{-1})
0	≤ 90	≥ 400	15	≥ 120	≤ 133
1	<90 responsive to fluid	≥300 - <400	13-14	≥80 - <120	>133 - ≤169
2	<90 unresponsive to fluid	≥200 - <300	10-12	≥50 - <80	>169 - ≤310
3	2-5	≥100 - <200	6-9	≥20 - <50	>310 - ≤440
4	>5	<100	≤ 5	<20	>440

Table 5.4 Organ failure score system used in this study

OFS Organ failure score

CVS Cardiovascular system

RS Respiratory system

CNS Central nervous system

COAG. Coagulation system

RENAL Renal system

SBP Systolic blood pressure

$Pa0_2$ Arterial partial pressure of oxygen

$Fi0_2$ Fraction of inspired oxygen

Organ failure score was the sum of the score obtained in each system

Modified from (Kingsnorth et al., 1995)

5.1.4 Control subjects

Two sets of control subjects were used for this study:

5.1.4.1 Normal healthy controls Cord blood samples from 134 Europids following a normal healthy obstetric delivery at Derriford Hospital, Plymouth were used to obtain control allele and genotype frequencies.

5.1.4.2 Alcohol related disease 'controls' Due to methodological difficulties in recruiting alcoholics who are definitely free of organ specific alcohol related disease, a group of 33 Europids with alcohol induced liver disease was used as an extra 'control' group for the group of alcoholic pancreatitis patients.

49 alcoholics admitted to Derriford Hospital, Plymouth with acute alcoholic hepatitis or alcohol-induced cirrhosis were screened for inclusion in the 'control' group. Diagnosis was by clinical history and examination, liver enzyme estimation, and ultrasonography in all, but further confirmed by post mortem in 1, and liver biopsy in 13 patients. 4 patients (8.2%) with a history of pancreatic disease were excluded. Of the remainder, DNA was available for analysis in 33 subjects.

All subjects admitted to the consumption of >80g alcohol day^{-1} for a minimum period of 2 years.

5.2 Materials

5.2.1 Water

Double distilled tap water was used to make up all stock, general purpose and specialist solutions. Sterile water (Baxter Healthcare, Thetford, UK) was used to dilute amplimer and probe solutions, and used for PCR.

5.2.2 Reagents

All reagents used were analytical or molecular biology grade or equivalent.

Acetic acid, Disodium ethylene diamine tetra-acetic acid, Glycerol, Hydrochloric acid,

Magnesium chloride, Maleic acid, Orthoboric acid, Sodium chloride, Sodium citrate, Sodium dodecyl sulphate, Sodium hydroxide, Sucrose, Tris (hydroxymethyl) aminomethane and Tween 20 were purchased from BDH Laboratory Supplies-Merck Limited (Poole,UK). Chloroform, Ethanol, and Methanol were purchased from Rathburns Limited (Walkerburn, UK). Ammonium persulphate, Ethidium bromide, N-Lauroylsarcosine, Tetra-methyl-ammonium-chloride, Triton-X-100 and Xylene cyanol were purchased from Sigma Chemicals (Poole, UK) and Orange G from Fisher Scientific (Loughborough, UK).

5.2.3 Specialised reagents, enzymes and materials

Agarose and the DIG luminescent detection system was purchased from Boehringer Mannheim GmbH, (Germany). Taq polymerase and PCR buffer was purchased from HT Biotechnology, (Cambridge, UK). Deoxynucleoside 5'-triphosphates (dNTP's) and T4 Polynucleotide kinase (T4 PNK) were purchased from Pharmacia Biotech (Sweden). 3MM Whatman paper (Whatman International, Maidstone, UK) was used in the Southern blotting station and 50 x 30cm filter paper from Heto Laboratory Equipment (Surrey, UK) was used for polyacrylamide gel support and drying.

5.2.4 Stock solutions

i. Sodium chloride/sodium citrate (SSC)

20x solution: 3 M NaCl, 0.3M trisodium citrate, pH 7.0

ii. Tris/borate electrophoresis buffer (TBE)

10x solution: 0.89mM Tris base, 0.89M Boric acid, 2mM EDTA(pH 8.0)

iii. Ethidium bromide

10 mg ml^{-1} in H_2O

iv. Xylene cyanol track dye / loading buffer

0.25% w/v Xylene cyanol, 10% v/v Glycerol in 10 x TBE

v. Orange G track dye / loading buffer

0.25% Orange G, 10% v/v Glycerol in 10 x TBE

5.2.5 Autoclaving

All solutions, and all glassware and plasticware used in the techniques of DNA analysis were autoclaved at a temperature of 121°C , and pressure of 15 p.s.i for 30 minutes in a steam autoclave (Rodwell Instruments, UK).

5.3 Preparation of high molecular weight DNA

10 to 15 ml of peripheral venous blood was collected into 5% disodium ethylene diamine tetra-acetic acid (Na_2EDTA), and stored at -20°C in sterile polypropylene containers prior to subsequent DNA extraction.

The method utilised for DNA extraction used a Nucleon® BACC (blood and cell culture) genomic DNA extraction kit (Scotlab Ltd, Coatbridge, Lanarkshire, UK). The manufacturer's protocol was followed with only slight modification, described below. The kit reagents supplied (reagents A & B, sodium perchlorate, Nucleon® resin) were used, other and additional reagents were supplied and made up in house as described in section 5.2.

5.3.1 Cell preparation

Stored blood was thawed at room temperature, and an aliquot of 7.5 mls transferred to a 50 ml Falcon tube (Becton Dickinson, New Jersey, USA). To the sample a 4x volume of Reagent A (10mM Tris-HCl, 320mM Sucrose, 5mM $MgCl_2$, 1% Triton X-100, pH 8.0) was added and mixed on a Luckham R100/TW Rotatest shaker for 4 minutes. After centrifugation at 1300 g for 4 minutes on a MSE Mistral 1000, supernatant containing lysed red cells was separated from the pellet and discarded.

5.3.2 Cell lysis and deproteinisation of solution

The pellet was resuspended in 2mls Reagent B (400mM Tris-HCl pH 8.0, 60mM EDTA, 150mM NaCl, 1% SDS) and incubated at 37°C for 10-15 minutes, enabling nuclear membrane disruption to occur. Then the suspension was transferred to a 15 ml Falcon tube

and 500µl of sodium perchlorate added and mixed by inversion.

5.3.3 DNA extraction and precipitation

2mls of chloroform (-20°C) was added and mixed to emulsify the 2 phases, which were the separated by centrifugation at 1300g for 3 minutes. 200µl of Nucleon® silica resin was added prior to another centrifugation at 1300g for 3 minutes. The aqueous phase containing the DNA was carefully transferred to a fresh tube, ensuring the interphase with silica resin and the underlying organic phase were not disturbed. The solution was re-spun at 1300g for 1 minute to pellet any residual silica and transferred to another fresh tube. Precipitation of DNA was by inverting gently with 2x volume 100% ethanol (-20°C). A sterilised glass pipette with a sealed tip was used to hook out the precipitated DNA which was washed in 70% ethanol prior to dilution in 500µl of sterile water over 24 hours.

5.3.4 Quantification and dilution

The quantity of DNA was estimated by eye after precipitation, and this information used to guide dilution. Generally a 1 in 5 dilution of concentrated DNA was used which gave 100-500ng of DNA per µl of dilute solution. This was confirmed on a sample of specimens by estimation of concentration using a Cecil spectrophotometer.

5.4 Polymorphism detection

All assays used for the detection of polymorphisms at gene loci were PCR based. Specific assays for the different polymorphisms studied will be described in following sections.

5.4.1 Polymerase Chain Reaction (PCR)

The principle of PCR is shown in Fig 5.1. Thermostable DNA polymerase copies DNA, using a template and priming DNA molecules (amplimers) complementary to sequences on either side of the sequence targeted for amplification.

100-500ng of genomic DNA was used in each reaction with 0.5µM of each amplimer pair 10mM Tris-HCL(pH 9.0), 50mM KCl, 2-2.5mM $MgCl_2$, 0.1% Triton X-100, 300µM of

each dNTP, and 0.8 units of Taq DNA polymerase. All PCR reactions were performed in 20µl volumes in 0.2ml thin walled microtubes (Advanced Biotechnology, Epsom, UK) in a PTC-200 Thermal Cycler (MJ Research, Essex, UK) or a Cyclogene thermocycler (Techne, Cambridge, UK) under the following conditions.

Denaturation	95°C for 3 minutes
then 30 cycles of	
Denaturation	95°C for 30 seconds
Annealing	55-62°C for 1 minute
Extension	72°C for 1 minute
then	
Extension	72°C for 3 minutes

Samples were cooled at 4°C until further use.

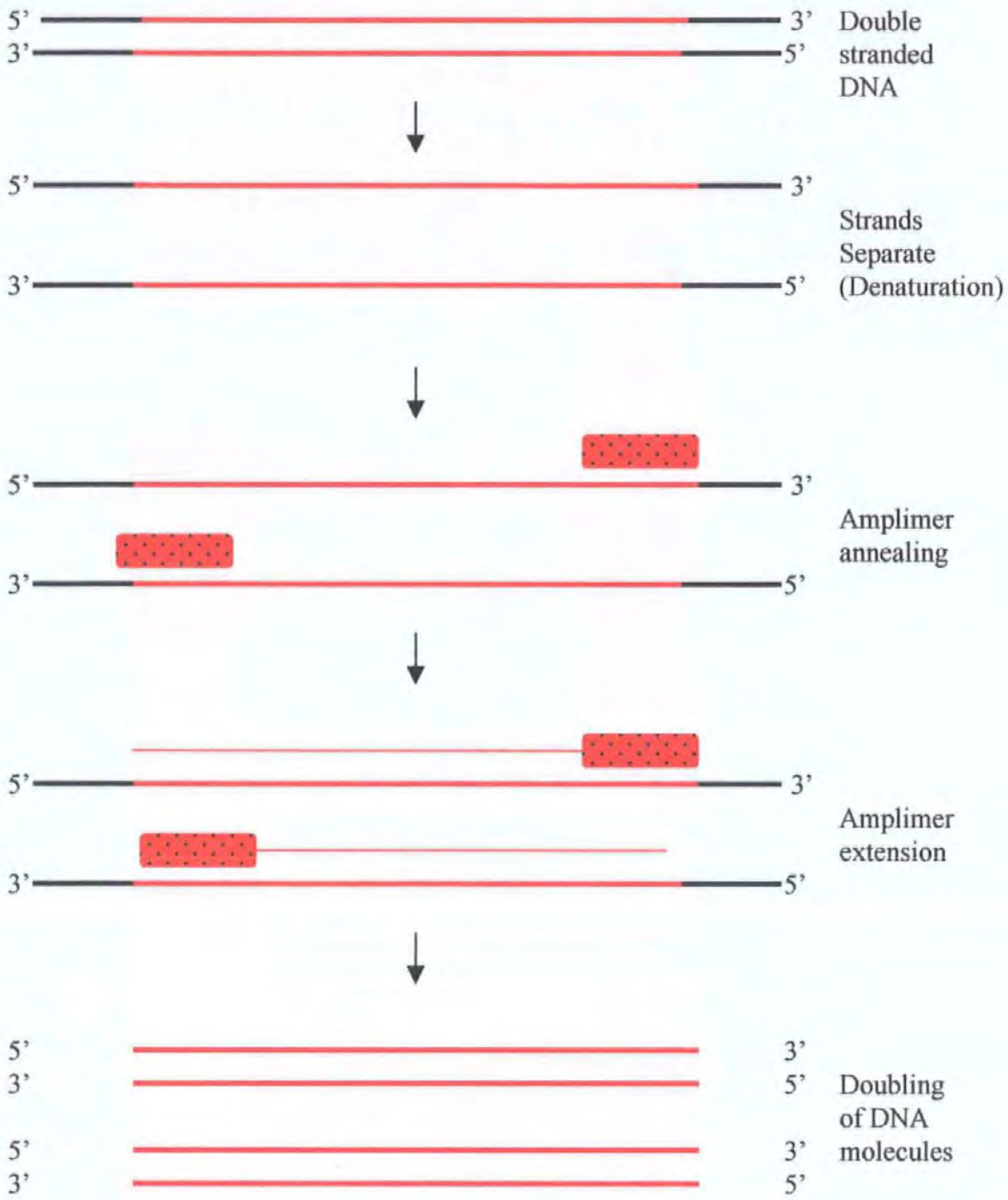

Figure 5.1 The polymerase chain reaction (PCR)

DNA amplification. Denaturation of the DNA separates the strands, allowing sequence specific amplimers to anneal. A new strand of DNA is made from dNTP's using the template DNA, catalysed by *Taq* DNA polymerase. Subsequent cycles of amplification give rise to exponential increase of product.

5.4.2 Agarose gel electrophoresis

Amplification efficiency and product size was checked by electrophoresis of an aliquot of the product mixed with track dye on a 1% agarose gel (1% w/v agarose in 0.5 x TBE buffer) containing 0.01% v/v ethidium bromide. The tank contained 0.5x TBE. A 123 bp ladder (Life Technologies, Paisley, UK) was used as a size marker.

Gels were electrophoresed for 30 minutes at 180V. The gels were then photographed under UV light (320nm).

5.4.3 Amplimer design and production

Amplimers were designed with reference to sequences deposited in Genbank. All amplimers were 18-21 bases in length with an approximate GC content of 50%. Amplimers used were commercially produced on an oligonucleotide synthesiser (Pharmacia Biotech, Sweden), and diluted for use to concentrations of 10pmol μl^{-1}, so 1µl of diluted amplimer in a 20 µl volume was at 0.5M concentration.

5.5 Microsatellite markers

Three microsatellite markers of the TNF gene (TNFa, TNFb, and TNFc) and one marker associated with the IL-10 gene (IL-10G) were studied. These have been described in sections 4.1 and 4.2. Alleles were detected by separation on denaturing polyacrylamide gels.

5.5.1 Amplimers for microsatellite amplification

Amplimers used for the assays are listed in Table 5.5 The forward amplimer in each reaction was 5'-end labelled with [γ ^{32}P] ATP (Amersham International, Amersham, UK) using T4 Polynucleotide kinase (T4 PNK).

Microsatellite		
TNFa	Forward	5'-GCACTCCAGCCTAGGCCACAGA-3'
	Reverse	5'-GCCTCTAGATTTCATCCAGCCACAG-3'
TNFb	Forward	5'-CCTCTCTCCCCTGCAACACACA-3'
	Reverse	5'-TGTGTGTTGCAGGGGAGAGAGG-3'
TNFc	Forward	5'-GGGAGGTCTGTCTTCCGCCG-3'
	Reverse	5'-CGTTCAGGTGGTGTCATGGG-3'
IL10.G	Forward	5'-TCCTTCCCCAGGTAGAGCAACACTCC-3'
	Reverse	5'-TCCCAAAGAAGCCTTAGTAGTGTTG-3'

Table 5.5 Amplimer pairs used to amplify microsatellite polymorphisms

The TNF-a microsatellite is a $(CA)_n$ dinucleotide repeat

The TNF-b & TNF-c microsatellites are $(CT)_n$ dinucleotide repeats

The IL-10.G microsatellite is a $(CA)_n$ dinucleotide repeat

Their position and significance is described in section 4.1 and 4.2.

5.5.2 5'-end labelling of amplimers

T4 PNK was bought in kit form (Pharmacia Biotech, Sweden). 25µl of sterile H_2O was added to the tube containing the T4 PNK, incubated at room T° for 5 minutes, then mixed. 50 pmol of amplimer, 1µl of [γ ^{32}P] ATP (10µCi/µl), and sterile H_2O to make the volume up to 50 µl were added prior to brief centrifugation then incubation at 37°C for 45 minutes. The reaction was stopped by placing on ice.

5.5.3 Precipitation of labelled amplimer

0.1 volumes of 5M NaCl, 2µl of Quick-Precip® (Advanced Genetic Technologies Corp. USA), and 3 volumes of Ethanol (-20°C) were added to the reaction tube to precipitate the DNA. Centrifugation at 1300 rpm in Biofuge 13 microcentrifuge (Heraeus Sepatech, Germany) pelleted the DNA, from which supernatant was pipetted off. A wash of the pellet in 70% Ethanol was performed prior to dissolution in 50µl of sterile H_2O.

5.5.4 Assessment of efficacy of amplimer labelling

1µl of the labelled amplimer was counted in a Beckman scintillation counter. This gave a count per minute (cpm) reading. Knowing the cpm of the whole sample allowed calculation of the volume of labelled amplimer for each reaction when the desired amount of radioactivity is 25,000 cpm.

5.5.5 PCR of microsatellite markers

PCR was carried out as previously described. 25,000cpm of the labelled amplimer was added to the reaction mixture in addition.

Annealing temperatures for each assay were:

TNFa & TNFb	62°C
TNFc	58°C
IL-10.G	65°C

An extension temperature of 68°C was employed for all microsatellite amplifications.

5.5.6 Polyacrylamide gel electrophoresis

A 6% polyacrylamide gel with 8M Urea was cast in a 30 x 50 cm *Sequi-Gen GT* Electrophoresis cell (Biorad Laboratories, Hemel Hempstead, UK). Sequagel™ sequencing system gel reagents were used (National Diagnostics, Atlanta, USA) and made up to the manufacturers instructions. The gel was equilibrated in 1x TBE buffer.

Amplification products (6µl) mixed with 3µL of Stop solution containing Formamide (Amersham Life Science, Buckinghamshire, UK) were then separated on a 6% polyacrylamide gel with 8M urea at 1700V for 2.5 hours (TNFa and TNFc, IL-10R), or 3 hours (TNFb, IL-10G).

The gel was fixed in 10% Methanol and Acetic Acid and removed from the cell plate onto filter paper.

5.5.7 Exposure of gel

After drying the gels were exposed to Kodak XLS5 X-ray film (Scientific Imaging Systems, Cambridge, UK) with intensifying screens at -80°C for 18 hours. Film was developed in Kodak developing and fixing agents.

5.5.8 Scoring of microsatellite alleles

An example of microsatellite alleles revealed by this method is shown in Fig 6.1.

Allele scoring was by two independent observers. Description of how alleles were assigned for each microsatellite is given in the results section.

5.6 PCR-Sequence specific primer reaction (PCR-SSP)

This method was used to type the bi-allelic -308 polymorphism of the TNF promoter region previously described in section 4.1.4.1. Amplification of an 836 base pair (bp) fragment of the TNF promoter region was performed in two PCR-SSP reactions. PCR-SSP is commonly used for HLA typing (Bunce et al., 1995). It was described as the 'Amplification refractory mutation system' (ARMS) (Newton et al., 1989), and is

dependent upon the fact that *Taq* polymerase lacks 3' exonuclease activity. Therefore amplimers (primers) employing allele specific nucleotides at their 3' end will amplify only those alleles if PCR conditions are exacting and stringently applied.

5.6.1 PCR-SSP Amplimers

Allele specific amplimers employed are detailed in Table 5.6. A common forward amplimer and one of two reverse amplimers with a 3' mismatch corresponding to a G or an A at position -308 were used. Amplimers for a 256 bp control amplicon from exon 15 of the adenomatous polyposis coli gene was included in the same reaction (Table 5.6). The assay had an annealing T° of 55°C.

5.6.2 PCR and electrophoresis

As described in sections 5.4.1 and 5.4.2. Loading dye used was Orange-G as this migrates with the amplimer enabling simplified reading of gels.

5.6.3 Scoring of alleles detected by PCR-SSP

Gels were scored for the presence or absence of an allele specific band providing a PCR control band was present.

Amplimer		
Common	Forward	5'-CTGCATCCCCGTCTTTCTCC-3'
Allele specific for 'G'	Reverse	5'-ATAGGTTTTGAGGGGCATCG-3'
Allele specific for 'A'	Reverse	5'-ATAGGTTTTGAGGGGCATCA-3'
Control amplicon	Forward	5'-ATGATGTTGACCTTTCCAGGG-3'
	Reverse	5'-TTCTGTAACTTTTCATCAGTTGC-3'

Table 5.6 Amplimers used in PCR-SSP assay for TNF promoter (-308) polymorphism

5.7 PCR-Sequence-specific oligonucleotide probing (PCR-SSOP)

This method was employed to type the three bi-allelic polymorphisms of the IL-10 promoter region as described in section 4.2. The method relies on the hybridisation of sequence-specific oligonucleotide probes to an amplified fragment of the gene locus. Detection of bound probe was by non-radioactive detection of 5'-digoxygenin labelled probe.

5.7.1 Amplification of DNA

PCR to amplify a 626 bp fragment of the IL-10 promoter was carried out as described in section 5.4.1 using the amplimers:

Forward 5'-ATCCAAGCAAC ACTACTAA-3'

Reverse 5'-TAAATATCCTCAAAGTCCC-3'

An annealing temperature of 56°C was employed

5.7.2 Southern Blotting

Following electrophoresis on a 1% agarose gel DNA was transferred to *Hybond*-N+ nucleic acid transfer membrane (Amersham Life Science, Buckinghamshire, UK), using the capillary transfer method described by Southern (1975) using 0.4M NaOH as the solvent. Transfer took place overnight following which the membranes were washed thoroughly in 3x SSC. Storage of membranes wrapped in cling film was at 4°C.

5.7.3 Oligonucleotide probes

Hybridisation was initially performed using oligonucleotide probes previously described (Turner et al., 1997). However, non-specific binding of probes occurred in spite of variation of wash stringency. Therefore probes for 2 of the loci (-1117 and -627) were redesigned by shortening the length of the probe or placing the allele-specific oligonucleotide at the 3' end. It was determined that the probes which bound with specificity to these two loci were those with the allele-specific oligonucleotide at the 3' end, and these were subsequently used. Sequences of oligonucleotide probes are given in Table 5.7. Probes were purchased from the usual commercial supplier labelled at the 5'-end with digoxygenin (DIG).

5.7.4 Pre-hybridisation and hybridisation

This was performed in glass roller tubes in a HB-1D oven (Techne, Cambridge, UK). The membranes were pre-hybridised for 1 hour in 20ml of hybridisation solution per 100cm^2 of membrane (5x SSC, 1% Blocking agent, 1% N-Lauroylsarcosine, 0.02% SDS). Subsequent hybridisation was at 42°C in 6 ml of hybridisation buffer per 100cm^2 membrane for 1 hour. DIG-labelled probes were used at a concentration of 5pmol ml^{-1}.

Allele	Nucleotide	Probe sequence	Wash T^O
1117*G	G	TAAGGCTTCTTTGGGAGG	60°C
1117*A	A	TAAGGCTTCTTTGGGAAG	60°C
854*C	C	CAGGTGATGTAACATCTCTGTCG	70°C
854*T	T	GCACAGAGATATTACATCACCTGT	70°C
627*C	C	CTGTGACCCCGCCTGCC	62°C
627*A	A	CTGTGACCCCGCCTGAC	62°C

Table 5.7 Oligonucleotide probes used for PCR-SSOP typing of the three polymorphisms in the IL-10 promoter

Nucleotide differences in the probes for the two alleles at each polymorphism are underlined

5.7.5 Post hybridisation washing

Initial washing was performed in the hybridisation oven and roller tubes. Washing was with 3.2M Tetra-methyl-ammonium chloride (TMAC) solution (3.2M TMAC, 0.02M EDTA, 0.1% SDS, and 0.05M Tris-HCl , pH 8.0). TMAC is a reagent which binds AT-rich DNA polymers, and abolishes the preferential melting of AT compared to GC base pairs. This means hybridisation of probes is only a function of the length of the probe.

The washing protocol was:

5x SSC at 42°C for 10 minutes, then 5x SSC at specific probe temperature* for 10 minutes, then 3.2M TMAC solution at specific probe temperature* for 20 minutes.

Filters were then transferred to 'sandwich boxes' and washed twice at room T° for 10 minutes.

* Specific probe washing T° in Table 5.7.

5.7.6 Detection of bound probe

A commercially available DIG luminescent detection system was used (Boehringer Mannheim GmbH, Germany) and the manufacturers protocol was followed with slight modifications.

Buffers used:

Buffer 1: 0.15M NaCl, 0.1M Maleic acid, pH 7.5

Blocking reagent: 10% w/v *Marvel* ™ skimmed milk in Buffer 1

Buffer 2: Blocking reagent 1:10 in Buffer 1

Buffer 3: 0.1M Tris-HCl (pH 9.5), 0.1M NaCl

Wash Buffer: 0.3% *Tween 20®* in Buffer 1

Filters placed in 'sandwich boxes' were washed briefly in Wash buffer, then blocked for 30 minutes in Buffer 2. After transfer to roller tubes in the hybridisation oven, filters were incubated at room T° in 10mls per 100cm^2 of anti-DIG ab solution (1:10,000 in Buffer 2). Excess ab was washed off with Wash buffer x2 for 15 minutes. The filters were then

equilibrated in Buffer 3 for 5 minutes, prior to exposure in the roller tube to 2ml per 100cm^2 of a 1:100 solution (in Buffer 3) of the chemoluminescence substrate, CSPD®. Filters were then briefly blotted on filter paper (DNA side up) prior to being wrapped in cling-film. The filters were then incubated at 37°C for 15 minutes prior to exposure against Kodak XLS5 X-ray film (Scientific Imaging Systems, Cambridge, UK) in a cassette with intensifying screens for 2hours.

Films were then developed and scored by 2 independent observers (Fig. 6.7)

5.7.7 Stripping bound probe from filters

To enable re-use of filters, they were first rinsed briefly in H_2O. This was followed by two 15 minute washes at 37°C in 0.2 M Na OH, SDS 0.1% to remove the DIG-labelled probe, and a thorough wash in 2x SSC.

5.8 Analysis of data

All data was entered into a computerised spreadsheet (Microsoft ® Excel 7.0) registered with and subject to the Data Protection Act 1988. This enabled statistical analysis to be performed within the same application, or in applications that could import Excel ® spreadsheets.

5.8.1 Patient and control subject demographics

Comparison was made between control subjects and patient groups with regard to sex distribution using the χ^2 test.

Distribution of age in patient groups was tested for normality using *Statgraphics software* (Statistical Graphics Corp. USA. 1996). Distribution in subject groups was tested for normality using the Chi-square goodness-of-fit statistic, the Shapiro-Wilkes W statistic, the Z score for skewness, and the Z score for kurtosis.

If distribution was normal, variances of each group were compared by the F-test, with subsequent comparison of means using the t-test. If the distribution in any group being

compared was not normal a non-parametric test (Kolmogorov-Smirnov) was used to compare age distribution.

P values of ≤ 0.05 were considered significant.

5.8.2 Allele and gene frequencies

Allele frequencies, expressed as decimals, were calculated from the number of copies of an allele out of the total number of chromosomes in the population, counting homozygotes as two copies. Gene frequencies in control subject and patient groups were calculated as the percentage of subjects positive for each gene.

5.8.3 Hardy-Weinberg equilibrium

This was used to determine whether a bi-allelic polymorphism is conforming to normal distribution within a population. Expected gene frequencies are calculated by the formula: $1 = p^2 \times q^2 \times 2(pq)$. p and q are the frequencies of the two alleles within the population. The observed gene frequencies are the compared using 2 x n tables and the Chi-square test. Hardy-Weinberg equilibrium is established if the expected frequencies do not differ significantly ($p \leq 0.05$) from the observed.

5.8.4 Haplotype analysis

In the absence of family studies haplotypes were assigned dependant upon observed possibilities. As long as the subject was not heterozygous at more than one locus used to create the haplotype, the possible haplotypes can be determined and the frequency calculated. If the subject was heterozygous at more than one locus, the two different haplotypes were possible on each chromosome, so the information was disregarded. Using this method a bias may be introduced if there is a large number of double heterozygotes. Where haplotypes and their frequency are shown, the number excluded from the analysis is shown. As such bias is likely to be systematic it should be the same for the two populations being compared.

Linkage across the TNFabc microsatellite loci, producing distinct three locus haplotypes,

has been characterised by maximum likelihood estimates in previous population based and family studies (Jongeneel et al., 1991; Nedaspasov et al., 1991; Crouau-Roy et al., 1993) and their existence has been confirmed in homozygous cell lines(Weissensteiner and Lanchbury, 1997; Udalova et al., 1993). The eleven putative haplotypes previously described in Caucasian populations were used for haplotype analysis.

5.8.5 Linkage analysis

Linkage of alleles at two loci occurs when they combine more frequently than could be expected by random association. Expected frequency of an allelic association between two loci was calculated by multiplying the frequency of one of the alleles with the other. Expected and observed frequencies were compared using the Chi squared statistic [$(O-E)^2$ / E]. Linkage was considered to occur if the associated p value was ≤ 0.05.

5.8.6 Comparison of allelic, gene, and haplotype frequencies

Comparisons of allelic, gene, and haplotype frequencies between the control group and patient groups were made in 2 x 2 contingency tables using the Chi-squared test. If any cell value was <5 Fisher's exact test was used. Both were performed on the *Statcalc* program of the computer software package *Epi Info 6* (World Health Organisation, Geneva, Switzerland). P values were corrected (p_c) for multiple comparisons made using the Bonferroni inequality method(Tiwari and Terasaki, 1985). This involves multiplying the p value by the number of comparisons made.

The Bonferroni correction is considered a conservative procedure as it assumes that in making multiple comparisons one is testing multiple independent hypotheses. In testing n independent hypotheses, Bonferroni assumes a significance level of $0.5 \div n$.

Statistical significance was achieved when the corrected p value (p_c) was ≤ 0.05.

6 Results

6.1 Study subjects

6.1.1 Normal control subjects

The normal control group was taken from a bank of cord bloods collected at the time of normal healthy deliveries at Derriford Hospital, Plymouth. 107 controls were used for the TNF locus typing, and 136 were used for the IL-10 typing. The sex distribution within the normal control group is shown in Table 6.1.

6.1.2 Alcoholic 'control' subjects

Of the 49 patients with alcoholic liver disease (Cirrhosis or alcoholic hepatitis), 4 (8.2%) had a history of pancreatic disease (episodes of acute/chronic pancreatitis), so were excluded. Of the remainder, DNA was available from 33 patients for DNA typing. Basic demographic characteristics are shown in Table 6.3.

6.1.3 Acute pancreatitis patients

135 patients were included in this study. A total of 144 patients were initially thought to fit inclusion criteria during the recruitment period. However, 8 were subsequently excluded. 3 of these were alcoholic patients who were subsequently proven over a time period of 3-6 months from screening to have chronic pancreatitis on ERCP. 4 patients were excluded as insufficient data was available, and in 2 subjects DNA preparation was inadequate. Basic demographic characteristics of the patient group are shown in Table 6.1.

Of the 135 patients in this study, 97 (71.9%) had mild disease as defined by the Atlanta criteria. Of the 38 patients (28.1%) with severe disease, 30 (22.2%) had systemic complications defined by at least one organ dysfunction, 21 (15.5%) had a local pancreatic collection (fluid, necrosis, or pseudocyst), of whom only 8 did not have concurrent systemic complications. 35 (25.9%) patients had maximal organ failure scores of 2 or more during their disease episode and 18 (13.3%) patients had organ failure scores of 3 or more, the respiratory system always accounting for at least 3 of the score. 12 (8.9%) patients had

organ failure scores of 4 or more, and in only 5 patients (3.7%) did death occur. The reason for the difference in the numbers having systemic complications as defined by the Atlanta classification and those having organ dysfunction as evidenced by OFS of 2 or more is due to the different criteria in the two classification systems. Basic demographic characteristics of the patient groups stratified according to disease severity are shown in Table 6.1 and 6.2. In 21 patients (15.6%) the episode of acute pancreatitis was caused by alcohol (≥80g per day), in 77 (57.0%) the cause was cholelithiasis, and in 37 patients (27.4%) no cause could be identified following adequate radiological (USS or CT) visualisation of the gallbladder and biliary tree (classified as idiopathic).

Basic demographic characteristics of the patient groups stratified according to aetiology of disease are shown in Table 6.3.

6.1.4 Age and sex distribution in subjects

Age is not applicable to the normal controls as they were from a bank of cord bloods. Although mean age and standard deviation are shown in Tables 6.1-6.3 to allow approximate comparison between groups, derivation of these statistics is not strictly applicable as the study population or study groups do not fit normal distribution for age. Distribution in subject groups was tested for normality using the Chi-square goodness-of-fit statistic, the Shapiro-Wilkes W statistic, the Z score for skewness, and the Z score for kurtosis.

As no group fitted normal distribution this tends to invalidate comparison of means and standard deviations using the F-test and t-test. Therefore, a non-parametric test (Kolmogorov-Smirnov) was used to compare whether there is a statistically significant difference in distribution of age between study groups. Distribution of age in the group of patients with severe disease was significantly different compared to the group with mild disease ($p=0.007$), the patients with mild disease having a younger age distribution. The same was true for those with a local complication vs. mild disease ($p=0.001$), and those

with an OFS ≥ 2 (p=0.008). However, using the Kolmogorov-Smirnov test, there was not a significant difference in distribution of age between those with mild disease vs. OFS ≥ 3 (p=0.11) or vs. OFS ≥ 4 (p=0.27).

With patients stratified according to aetiology, there was a significant difference in the distribution of age between:

Alcoholic pancreatitis and idiopathic pancreatitis groups (p=0.003), alcoholic pancreatitis and gallstone pancreatitis groups (p=0.0002), and alcoholic pancreatitis and idiopathic and gallstone groups combined (p=0.0001). As indicated by the age range shown in table 6.3, the alcoholic pancreatitis group were younger. Also the alcoholic pancreatitis group were younger than the alcoholic 'controls', as distribution of age was again significantly different (p=0.006). However, as indicated by their age ranges, using the Kolmogorov-Smirnov test, there was not a significant difference in distribution of age between gallstone and idiopathic groups (p=0.68).

Male to female ratio was comparable between the normal controls and acute pancreatitis patients as a whole (57 vs. 51% female, χ^2=0.84, p=0.4), and between patient groups stratified according to disease severity. However, only 1 of 21 patients (4.8%) in the alcoholic pancreatitis group was female, meaning that there was a significant difference in sex distribution between alcoholic pancreatitis patients and the normal control group and the other aetiological groups.

Normal controls and alcoholic pancreatitis (χ^2 =19.2, p<0.0001), idiopathic pancreatitis (female=47.4%) and alcoholic pancreatitis (χ^2 =11.7, p<0.001), gallstone pancreatitis (female=66.2%) and alcoholic pancreatitis (χ^2 =25.0, p<10^{-6}).

The alcoholic 'control' group were 33.3% female, meaning that there was also a significant difference in sex distribution between the alcoholic 'controls' and the normal controls (33.3 vs. 57%, χ^2=5.7, p=0.017), and the alcoholic 'controls' and the alcoholic pancreatitis group (Fisher's exact test, p=0.013).

In spite of there being almost twice as many females than males with gallstone pancreatitis, no statistically significant difference in sex distribution was seen between gallstone and idiopathic pancreatitis groups, or between the gallstone pancreatitis group and the normal controls.

	Controls [1]	Study patients	Mild	Severe	Local complication
	n=107	n=135	n=97	n=38	n=21
mean age (years)	n/a [2]	56.1 [3]	53.5 [3]	62.6 [3]	64.5 [3]
age range (years)	n/a	21-86	21-86	26-78	35-78
S.D	n/a	15.4	16.0	11.6	10.7
M:F	46:61	66:69 [4]	49:48	17:21	10:11

Table 6.1 Demographic characteristics of normal control and study subjects, and patient subgroups according to (local and systemic) disease severity

Age and sex characteristics of normal control and study subjects, and patient subgroups of those with mild or severe pancreatitis, and those with a local (pancreatic) complication.

n represents the number of subjects in each group
S.D standard deviation
M Male
F Female

Mild and **severe** disease severity groups are according to the Atlanta convention classification (Bradley, 1993), described in section 5.1.4.

Local complication is defined as occurrence of pancreatic necrosis, abscess or pseudocyst.

1. For the studies involving the IL-10 polymorphisms, a larger group of controls (up to 136) was used. The male: female ratio is comparable for this group (55:71).
2. Age not applicable to controls as taken from a bank of cord bloods
3. Although mean age and standard deviation are shown to allow approximate comparison, derivation of these statistics is not strictly applicable as the study population or study groups shown do not fit normal distribution for age. Distribution in subject groups was tested for normality using the Chi-square goodness-of-fit statistic, the Shapiro-Wilkes W statistic, the Z score for skewness, and the Z score for kurtosis.
   As no group fitted normal distribution this tends to invalidate comparison of means and standard deviations using the F-test and t-test. Therefore, a non-parametric test (Kolmogorov-Smirnov) was used to compare whether there is a statistically significant difference in distribution of age between study groups.
   As indicated by the means in the table there were significant differences between distribution of age in:
   Mild vs. Severe, p=0.007
   Mild vs. Local complication, p=0.001
3. Comparison of sex distribution using χ^2 test, no significant difference in sex distribution between normal control and study patient groups (χ^2 =0.84, p=0.4)

	Controls	Mild	OFS ≥ 2	OFS ≥ 3	OFS ≥ 4
	n=107	n=97	n=35	n=18	n=12
mean age (years)	n/a [1]	53.5 [2]	63.4 [2]	62.1 [2]	61.3 [2]
age range (years)	n/a	21-86	26-84	26-78	26-76
S.D	n/a	15.4	11.4	13.7	14.9
sex M:F	46:61 [3]	49:48	17:18	10:8	7:5

Table 6.2 Demographic characteristics of normal control and study subjects, and patient subgroups according to (systemic) disease severity as assessed by organ failure score

Age and sex characteristics of normal control and study subjects, and patient subgroups as defined by organ failure score

n represents the number of subjects in each group
S.D standard deviation
M Male
F Female
OFS Organ failure score

OFS calculated according to method described in section 5.1.4 (Kingsnorth et al, 1995).

1. Age not applicable to controls as taken from a bank of cord bloods
2. Although mean age and standard deviation are shown to allow approximate comparison, derivation of these statistics is not strictly applicable as the study population or study groups shown do not fit normal distribution for age. Distribution in subject groups was tested for normality using the Chi-square goodness-of-fit statistic, the Shapiro-Wilkes W statistic, the Z score for skewness, and the Z score for kurtosis.
   As no group fitted normal distribution this tends to invalidate comparison of means and standard deviations using the F-test and t-test. Therefore, a non-parametric test (Kolmogorov-Smirnov) was used to compare whether there is a statistically significant difference in distribution of age between study groups.
   As indicated by the means in the table there was a significant difference between distribution of age in:
   Mild vs. OFS ≥ 2, p=0.008
   However, using the Kolmogorov-Smirnov test, there was not a significant difference in distribution of age between:
   Mild vs. OFS ≥ 3, p=0.11
   Mild vs. OFS ≥ 4, p=0.27
3. Comparison of sex distribution using χ^2 test, no significant difference in sex distribution between normal control and study patient groups.

	Controls	Alcohol	Idiopathic	Gallstones	'Alcoholic' controls
	n=107	n=21	n=37	n=77	n=33
mean age (years)	n/a [1]	43.8 [2]	57.4 [2]	58.5 [2]	51.0
age range (years)	n/a	28-64	23-84	21-86	37-71
S.D	n/a	11.6	17.2	14.3	9.0
sex M:F	46:61 [3]	20:1 [3]	19:18 [3]	26:51 [3]	22:11 [3]

Table 6.3 Demographic characteristics of normal and 'alcoholic' control and study subjects grouped according to aetiology of acute pancreatitis

Age and sex characteristics of normal and 'alcoholic' control and study subjects grouped according to aetiology of disease

n represents the number of subjects in each group

S.D standard deviation

M Male

F Female

Alcoholic pancreatitis if daily consumption of ≥ 80g alcohol day^{-1}

Gallstones if radiological or ERCP evidence of cholelithiasis

Idiopathic if no identifiable aetiology

1. Age not applicable to controls as taken from a bank of cord bloods
2. Although mean age and standard deviation are shown to allow approximate comparison, derivation of these statistics is not strictly applicable as the study population or study groups shown do not fit normal distribution for age. Distribution in subject groups was tested for normality using the Chi-square goodness-of-fit statistic, the Shapiro-Wilkes W statistic, the Z score for skewness, and the Z score for kurtosis.
   As no group fitted normal distribution this tends to invalidate comparison of means and standard deviations using the F-test and t-test. Therefore, a non-parametric test (Kolmogorov-Smirnov) was used to compare whether there is a statistically significant difference in distribution of age between study groups.
   As indicated by the means in the table there was a significant difference between distribution of age in:
   Alcoholic pancreatitis vs. Idiopathic, p=0.003
   Alcoholic pancreatitis vs. Gallstones, p=0.0002
   Alcoholic pancreatitis vs. Idiopathic & Gallstone combined, p=0.0001
   Alcoholic pancreatitis vs. alcoholic 'controls', p=0.0056
   Alcoholic 'controls' vs. Idiopathic & Gallstone combined, p=0.00005
   Alcoholic 'controls' vs. all acute pancreatitis patients, p=0.0006
   However, as indicated by their means, using the Kolmogorov-Smirnov test, there was not a significant difference in distribution of age between:
   Gallstone and Idiopathic groups, p=0.68
3. Comparison of sex distribution using χ^2 test or Fisher's exact test. Significant differences

in sex distribution between:
Normal controls and alcoholic pancreatitis, χ^2 =19.2, p<0.0001
Idiopathic and alcoholic pancreatitis, χ^2 =11.7, p<0.001
Gallstone and alcoholic pancreatitis, χ^2 =25.0, $p<10^{-6}$
'Alcoholic controls' and normal controls, χ^2=5.7, p=0.017
'Alcoholic controls' and alcoholic pancreatitis, Fisher's exact test, p=0.013
'Alcoholic controls' and gallstone pancreatitis, χ^2=10.2, p=0.0014
In spite of there being almost twice as many females than males with gallstone pancreatitis, no statistically significant difference in sex distribution was seen between gallstone and idiopathic pancreatitis groups, or between the gallstone pancreatitis group and the normal controls:

Normal controls and gallstone pancreatitis, χ^2 =1.6, p=0.2
Idiopathic and gallstone pancreatitis, χ^2 =3.2, p=0.07

6.2 Severity of disease according to aetiology

Severe disease, according to the Atlanta classification, occurred in 38 of 135 patients (28.1%). 17 of those had idiopathic disease, a group which accounts for only 27.4% of patients. This apparent excess of those with severe disease (Atlanta) in the idiopathic group is non significant however when compared to the expected number (χ^2=4.2, p=0.12, 2 d.f). Likewise, there is an apparent excess of patients (11 of 18) with an OFS of 3 or more in the group classified as having idiopathic disease. When compared to the expected number this approaches significance (χ^2=7.2, p=0.027, 2 d.f). However, as the expected number is less than 5 (4.9), the χ^2 test is not valid. Testing with Fisher's exact test gives a p value of 0.074. There is no variation from expected numbers in patients with a pancreatic collection in the aetiological groups.

6.3 TNF microsatellite alleles

Allelic frequencies of the 3-locus TNFabc microsatellite system were measured in 100% of patients. 13 of 14 known alleles of TNFa (Fig. 6.1) were observed in the population studied. Six of the seven known alleles of TNFb (Fig. 6.2) were found in the patient and control groups. TNFc (Fig. 6.3) is biallelic. Tables 6.4 and 6.5 show the allelic frequencies at the three loci in normal control and acute pancreatitis subjects. Tables 6.6 and 6.7 show allelic frequencies at the 3 loci in patient groups stratified according to disease severity classified according to the Atlanta classification and the occurrence of a local complication. Tables 6.8 and 6.9 show patient groups stratified according to organ failure score.

There are no statistically significant differences in the allelic frequencies of the TNF-a, TNF-b, or TNF-c loci when comparing all acute pancreatitis patients with controls or when patients grouped according to disease course (mild vs. severe, mild vs. OFS≥2, mild vs. OFS≥3, mild vs. OFS≥4) were compared. The distribution of all TNFabc alleles between controls and patients was also not different (TNFa, χ^2 =14.99, p_c=0.31, 13 d.f; TNFb, χ^2 =6.21, p_c=0.4, 6 d.f; TNFc, χ^2 =0.02).

When patients were grouped according to the aetiology of their disease there was a decrease in the frequency of the TNFa2 allele (Tables 6.10 and 6.11) in those patients with alcoholic acute pancreatitis compared to normal controls (χ^2 =7.24, p=0.007, p_c=0.098), those with pancreatitis of other aetiology (χ^2 =4.51, p= 0.034, p_c=0.4), and alcoholic 'controls' (χ^2=8.57, p=0.0034, p_c=0.048). However this was only significant in comparison with the group of alcoholic 'controls' after correction of p-values.

The reduction in the a2 allele in the alcoholic group was not accounted for by an increase in any one allele, but both a6 and a11 were increased in frequency, although again this was not statistically significant. For allele a6, alcoholic pancreatitis vs. normal controls (28.6 vs. 15.4%, χ^2=1.5, p=0.2), alcoholic pancreatitis vs. non-alcoholic pancreatitis group (28.6 vs. 16.2%, χ^2 =3.64, p= 0.056, p_c>0.5), alcoholic pancreatitis vs. alcohol 'controls' (28.6 vs. 12.1%, χ^2=4.6, p=0.032, p_c=0.44). For allele a11, alcoholic pancreatitis vs. normal controls (26.2 vs. 16.8%, χ^2=2.1, p=0.15), alcoholic pancreatitis vs. non-alcoholic pancreatitis (26.2 vs. 16.7%, χ^2 =2.17, p= 0.14), alcoholic pancreatitis vs. alcohol 'controls' (26.2 vs. 13.6%, χ^2=2.7, p=0.1).

It is unlikely that the difference in TNFa allelic frequencies could be accounted for by gender (20 of the 21 alcoholic patients were male) as the control population had an equal distribution of all alleles in the males and females.

There was a trend towards reduced frequency of the TNFb1 allele in the alcoholic pancreatitis group, although this did not achieve statistical significance (Table 6.12). Alcoholic pancreatitis vs. controls (4.8 vs. 14.0%, χ^2 =2.75, p=0.097, p_c>0.5), alcoholic pancreatitis vs. non-alcoholic pancreatitis (4.8 vs. 15.8%, χ^2 =3.57, p= 0.059, p_c>0.5).

In the group of 'alcoholic controls' the b1 allele was very slightly reduced in frequency compared to the normal controls, so the difference in b1 frequencies between the alcoholic pancreatitis group and alcoholic 'controls' was much reduced. Alcoholic pancreatitis vs. alcohol 'controls' (4.8 vs. 10.6%, χ^2=1.2, p=0.28), normal controls vs. alcohol 'controls'

(14.0 vs 10.6%, χ^2=0.5, p=0.5).

TNFc allelic frequencies were comparable between control groups and aetiological patient groups (Table 6.13).

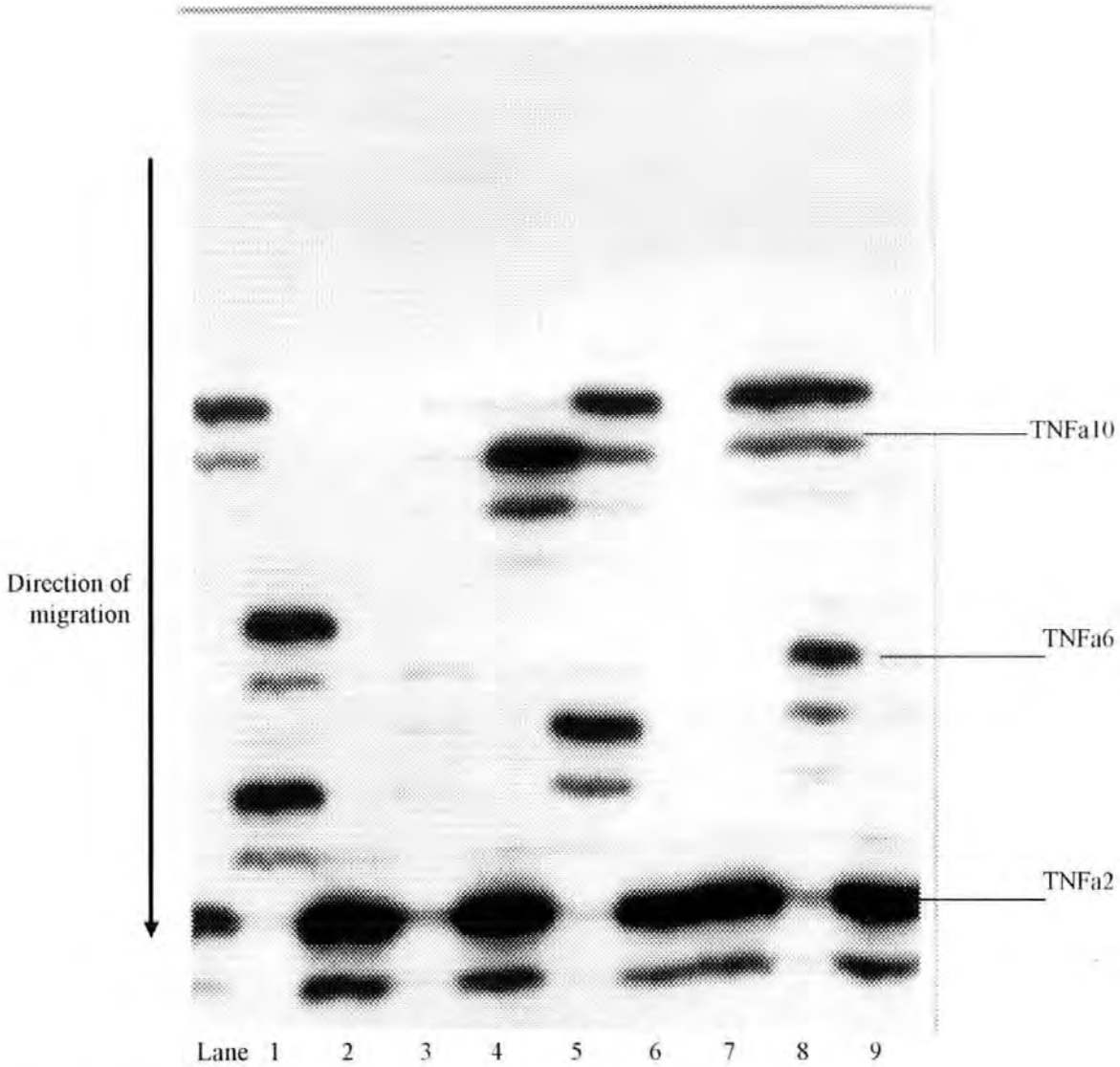

Figure 6.1 TNFa microsatellite autoradiograph

Amplification of a region containing the microsatellite was performed as described in section 5.5. Amplification products (6µl) mixed with 3µl of Stop solution containing Formamide (Amersham Life Science, Buckinghamshire, UK) are separated on a 6% polyacrylamide gel with 8M urea at 1700V for 2.5 hours, and alleles revealed with autoradiography.

TNFa has 14 known alleles. Alleles were assigned as proposed by Nedaspasov et al (1991). Allele1denotes the allele with the least number of repeats. For TNFa, this is $(AC/GT)_6$. Allele 2 (TNFa2) is the most common allele in Europids. Each subsequent allele differs by one base pair repeat.

Alleles TNFa2, TNFa6, and TNFa10 are labelled in this figure. Genotypes for each of the samples shown in lanes 1-9 are as follows:

Lane1: a4,7
Lane2: a2,2
Lane3: null
Lane4: a2, 10
Lane5: a5,11
Lane6: a2,2
Lane7: a2,11
Lane8: a6,11
Lane9: a2,2

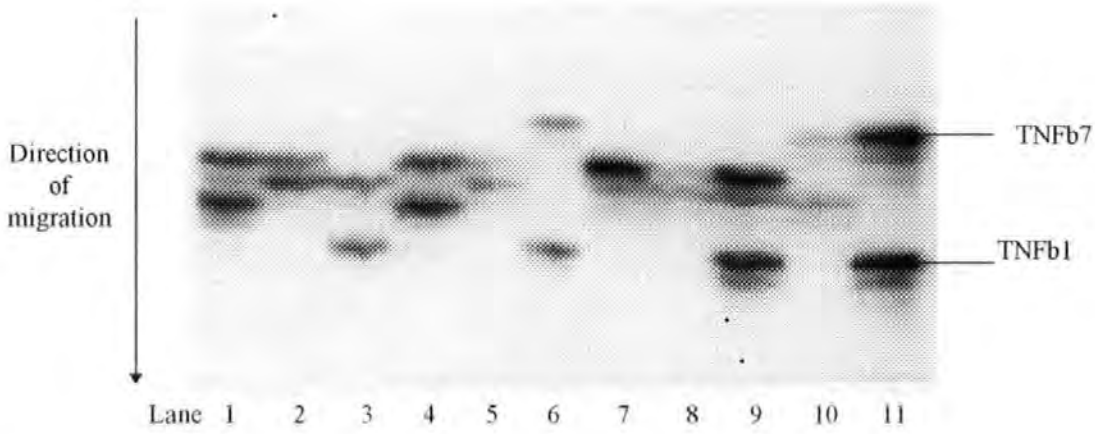

Figure 6.2 TNFb microsatellite autoradiograph

Amplification of a region containing the microsatellite was performed as described in section 5.5. Amplification products (6μl) mixed with 3μl of Stop solution containing Formamide (Amersham Life Science, Buckinghamshire, UK) are separated on a 6% polyacrylamide gel with 8M urea at 1700V for 3.5 hours, and alleles revealed with autoradiography.

TNFb has 7 known alleles. Alleles were assigned as proposed by Nedaspasov et al (1991). Allele1denotes the allele with the least number of repeats. For TNFb, this is $(TC/GA)_8$. Each subsequent allele differs by one base pair repeat. Allele 4 (TNFb4) is the most common allele in Europids.

Alleles TNFb1and TNFb7 are labelled in this figure. Genotypes for each of the samples shown in lanes 1-9 are as follows:

Lane1: b3,5
Lane2: b4,5
Lane3: b1,4
Lane4: b3,5
Lane5: b4,5
Lane6: b1,7
Lane7: b5,5
Lane8: b4,5
Lane9: b1,4
Lane10: b4,7
Lane11: b1,7

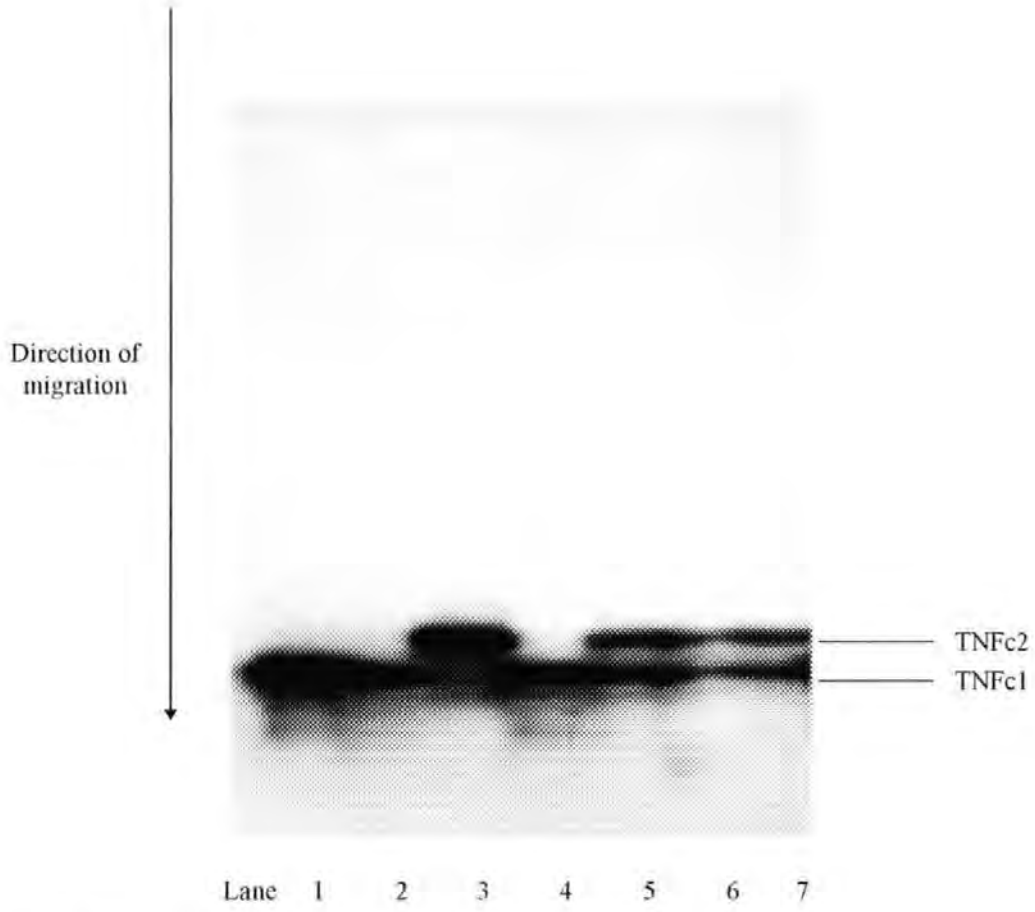

Figure 6.3 TNFc microsatellite autoradiograph

Amplification of a region containing the microsatellite was performed as described in section 5.5. Amplification products (6μl) mixed with 3μl of Stop solution containing Formamide (Amersham Life Science, Buckinghamshire, UK) are separated on a 6% polyacrylamide gel with 8M urea at 1700V for 2.5 hours, and alleles revealed with autoradiography.
TNFc is biallelic. Alleles were assigned as proposed by Nedaspasov et al (1991). Allele1denotes the allele with the least number of repeats. For TNFc, this is $(TC/GA)_9$. Allele 2 is $(TC/GA)_{10}$. Genotypes for each of the samples shown in lanes 1-7 are as follows:
Lane1: c1,1
Lane2: c1,1
Lane3: c2,2
Lane4: c1,1
Lane5: c1,2
Lane6: c1,2
Lane7: c1,2

TNFa allele	Controls n=107 %	Study patients n=135 %
a1	**0.5** (1)	**0.7** (2)
a2	**35.5** (76)	**27.4** [1] (74)
a3	**0.9** (2)	**0.0** (0)
a4	**8.4** (18)	**9.3** (25)
a5	**1.9** (4)	**4.8** [2] (13)
a6	**15.4** (43)	**18.1** (49)
a7	**7.5** (16)	**7.4** (20)
a8	**1.4** (3)	**2.6** (7)
a9	**0.9** (2)	**0.7** (2)
a10	**9.3** (20)	**9.6** (26)
a11	**16.8** (36)	**18.1** (49)
a12	**0.0** (0)	**0.7** (2)
a13	**0.9** (2)	**0.0** (0)
a14	**0.5** (1)	**0.4** (1)
Total alleles(n)	214	270

Table 6.4 **TNFa microsatellite allele frequencies in normal controls and acute pancreatitis patients**

This table displays TNFa microsatellite allele frequencies in control and patient subjects. For each allele the **% frequency** and the actual number of alleles (in brackets) is given.

n represents the number of subjects in each group

(n) represents the number of alleles in each group.

Comparisons were made for each allele between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of

comparisons made.
1 Control vs. patient χ^2 =3.67, p=0.055, p_c=0.77.
2 Control vs. patient χ^2 =3.06, p=0.08, p_c>0.5.
No statistically different differences in individual TNFa alleles are seen between the control and patient subjects.
The distribution of all TNFa alleles between controls and patients was also not different
(χ^2 =14.99, p_c=0.31, 13 d.f.).

TNFb allele	Controls n=107 %	Study patients n=135 %
b1	**14.0** (30)	**14.4** (39)
b2	**0.0** (0)	**0.4** (1)
b3	**18.2** (39)	**12.2**[1] (33)
b4	**35.5** (76)	**36.3** (98)
b5	**30.4** (65)	**32.6** (88)
b6	**0.0** (0)	**0.7** (2)
b7	**1.9** (4)	**3.3** (4)
Total alleles (n)	214	270
TNFc allele		
c1	**73.4** (157)	**73.0** (197)
c2	**26.6** (57)	**27.0** (73)
Total alleles (n)	214	270

Table 6.5 TNFb and TNFc microsatellite allele frequencies in normal controls and acute pancreatitis patients

This table displays TNFb and TNFc microsatellite allele frequencies in control and patient subjects. For each allele the **% frequency** and the actual number of alleles (in brackets) is given.

n represents the number of subjects in each group

(n) represents the number of alleles in each group.

Comparisons were made for each allele between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.

1 Control vs. patient χ^2 =3.4, p=0.065, p_c>0.5.

No statistically different differences in individual TNFb or TNFc alleles are seen between the control and patient subjects.

Distribution of TNFb alleles (χ^2 =6.21, p_c=0.4, 6 d.f.), and TNFc alleles (χ^2 =0.02) between controls and patients was also not different

TNFa allele	Controls	Mild	Severe	Local complication
	n=107	n=97	n=38	n=21
	%	%	%	%
a1	**0.5** (1)	**0.5** (1)	**1.3** (1)	**2.4** (1)
a2	**35.5** (76)	**25.8** (50)	**31.6** (24)	**28.6** (12)
a3	**0.9** (2)	**0.0** (0)	**0.0** (0)	**0.0** (0)
a4	**8.4** (18)	**9.3** (18)	**9.2** (7)	**9.5** (4)
a5	**1.9** (4)	**4.6** (9)	**5.3** (4)	**7.1** (3)
a6	**15.4** (43)	**18.6** (36)	**17.1** (13)	**21.4** (9)
a7	**7.5** (16)	**7.7** (15)	**6.6** (5)	**4.8** (2)
a8	**1.4** (3)	**2.1** (4)	**3.9** (3)	**4.8** (2)
a9	**0.9** (2)	**1.0** (2)	**0.0** (0)	**0.0** (0)
a10	**9.3** (20)	**8.2** (16)	**13.2** (10)	**7.1** (3)
a11	**16.8** (36)	**20.6** (40)	**11.8** (9)	**14.3** (6)
a12	**0.0** (0)	**1.0** (2)	**0.0** (0)	**0.0** (0)
a13	**0.9** (2)	**0.0** (0)	**0.0** (0)	**0.0** (0)
a14	**0.5** (1)	**0.5** (1)	**0.0** (0)	**0.0** (0)
Total alleles (n)	214	194	76	42

Table 6.6 TNFa microsatellite allele frequencies in normal controls and acute pancreatitis patients grouped according to (local and systemic) disease severity

This table displays TNFa microsatellite allele frequencies in control and patient group subjects. For each allele the **% frequency** and the actual number of alleles (in brackets) is given.

n represents the number of subjects in each group

(n) represents the number of alleles in each group.

(continued.....)

Mild and severe disease severity groups are according to the Atlanta convention classification (Bradley, 1993), described in section 5.1.4.
Local complication is defined as occurrence of pancreatic necrosis, abscess or pseudocyst.
Comparisons were made for each allele between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.
No differences are seen in the frequency of individual alleles between patient groups or between patient groups and controls.

TNFb allele	Controls	Mild	Severe	Local complication
	n=107	n=97	n=38	n=21
	%	%	%	%
b1	**14.0** (30)	**13.9** (27)	**15.8** (12)	**11.9** (5)
b2	**0.0** (0)	**0.5** (1)	**0.0** (0)	**0.0** (0)
b3	**18.2** (39)	**12.4** (24)	**11.8** (9)	**9.5** (4)
b4	**35.5** (76)	**37.6** (73)	**32.9** (25)	**33.3** (14)
b5	**30.4** (65)	**32.0** (62)	**34.2** (26)	**42.9** (18)
b6	**0.0** (0)	**1.0** (2)	**0.0** (0)	**0.0** (0)
b7	**1.9** (4)	**2.6** (5)	**5.3** (4)	**2.4** (1)
Total alleles (n)	214	194	76	42
TNFc allele				
c1	**73.4** (157)	**75.3** (146)	**67.1** (51)	**69.0** (29)
c2	**26.6** (57)	**24.7** (48)	**32.9** (25)	**31.0** (13)
Total alleles (n)	214	194	76	42

Table 6.7 TNFb and TNFc microsatellite allele frequencies in normal controls and acute pancreatitis patients grouped according to (local and systemic) disease severity

This table displays TNFb and TNFc microsatellite allele frequencies in control and patient group subjects. For each allele the **% frequency** and the actual number of alleles (in brackets) is given.

n represents the number of subjects in each group

(n) represents the number of alleles in each group.

Mild and severe disease severity groups are according to the Atlanta convention classification (Bradley, 1993), described in section 5.1.4.

Local complication is defined as occurrence of pancreatic necrosis, abscess or pseudocyst.

Comparisons were made for each allele between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.

No differences are seen in the frequency of individual alleles between patient groups or between patient groups and controls.

TNFa allele	Controls n=107 %	OFS ≥ 2 n=35 %	OFS ≥ 3 n=18 %	OFS ≥ 4 n=12 %
a1	**0.5** (1)	**0.0** (0)	**0.0** (0)	**0.0** (0)
a2	**35.5** (76)	**32.9** (23)	**36.1** (13)	**37.5** (9)
a3	**0.9** (2)	**0.0** (0)	**0.0** (0)	**0.0** (0)
a4	**8.4** (18)	**8.6** (6)	**8.3** (3)	**8.3** (2)
a5	**1.9** (4)	**7.1** (5)	**5.6** (2)	**8.3** (2)
a6	**15.4** (43)	**15.7** (11)	**16.7** (6)	**20.8** (5)
a7	**7.5** (16)	**5.7** (4)	**11.1** (4)	**4.2** (1)
a8	**1.4** (3)	**4.3** (3)	**0.0** (0)	**0.0** (0)
a9	**0.9** (2)	**0.0** (0)	**0.0** (0)	**0.0** (0)
a10	**9.3** (20)	**12.9** (9)	**16.7** (6)	**12.5** (3)
a11	**16.8** (36)	**12.9** (9)	**5.6** (2)	**8.3** (2)
a12	**0.0** (0)	**0.0** (0)	**0.0** (0)	**0.0** (0)
a13	**0.9** (2)	**0.0** (0)	**0.0** (0)	**0.0** (0)
a14	**0.5** (1)	**0.0** (0)	**0.0** (0)	**0.0** (0)
Total alleles (n)	214	70	36	24

Table 6.8 TNFa microsatellite allele frequencies in normal controls and acute pancreatitis patients grouped according to (systemic) disease severity as assessed by organ failure score

This table displays TNFa microsatellite allele frequencies in control and patient group subjects. For each allele the **% frequency** and the actual number of alleles (in brackets) is given.

n represents the number of subjects in each group
(n) represents the number of alleles in each group.
OFS Organ failure score

(continued.....)

OFS calculated according to method described in section 5.1.4 (Kingsnorth et al, 1995). Comparisons were made for each allele between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.

No differences are seen in the frequency of individual alleles between patient groups or between patient groups and controls.

TNFb allele	Controls n=107 %	OFS ≥ 2 n=35 %	OFS ≥ 3 n=18 %	OFS ≥ 4 n=12 %
b1	**14.0** (30)	**15.7** (11)	**19.4** (7)	**20.8** (5)
b2	**0.0** (0)	**0.0** (0)	**0.0** (0)	**0.0** (0)
b3	**18.2** (39)	**12.9** (9)	**13.9** (5)	**12.5** (3)
b4	**35.5** (76)	**34.3** (24)	**33.3** (12)	**25.0** (6)
b5	**30.4** (65)	**32.9** (23)	**33.3** (12)	**41.7** (10)
b6	**0.0** (0)	**0.0** (0)	**0.0** (0)	**0.0** (0)
b7	**1.9** (4)	**4.3** (3)	**0.0** (0)	**0.0** (0)
Total alleles (n)	214	70	36	24
TNFc allele				
c1	**73.4** (157)	**67.1** (47)	**66.7** (24)	**58.3** (14)
c2	**26.6** (57)	**32.9** (23)	**33.3** (12)	**41.7** (10)
Total alleles (n)	214	70	36	24

Table 6.9 TNFab and TNFc microsatellite allele frequencies in normal controls and acute pancreatitis patients grouped according to (systemic) disease severity as assessed by organ failure score

This table displays TNFb and TNFc microsatellite allele frequencies in control and patient group subjects. For each allele the **% frequency** and the actual number of alleles (in brackets) is given.

n represents the number of subjects in each group

(n) represents the number of alleles in each group.

OFS Organ failure score

OFS calculated according to method described in section 5.1.4 (Kingsnorth et al, 1995). Comparisons were made for each allele between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.

No differences are seen in the frequency of individual alleles between patient groups or between patient groups and controls.

TNFa allele	Controls n=107 %	Alcohol n=21 %	Idiopathic n=37 %	Gallstones n=77 %
a1	**0.5** (1)	**2.4** (1)	**1.4** (1)	**0.0** (0)
a2	**35.5** (76)	**14.3** [1] (6)	**27.0** (20)	**31.8** (49)
a3	**0.9** (2)	**0.0** (0)	**0.0** (0)	**0.0** (0)
a4	**8.4** (18)	**0.0** (0)	**12.2** (9)	**10.4** (16)
a5	**1.9** (4)	**2.4** (1)	**4.1** (3)	**5.8** (9)
a6	**15.4** (43)	**28.6** [2] (12)	**16.2** (12)	**16.2** (25)
a7	**7.5** (16)	**9.5** (4)	**10.8** (8)	**4.5** (7)
a8	**1.4** (3)	**4.8** (2)	**1.4** (1)	**2.6** (4)
a9	**0.9** (2)	**2.4** (1)	**1.4** (1)	**0.0** (0)
a10	**9.3** (20)	**7.1** (3)	**9.5** (7)	**10.4** (16)
a11	**16.8** (36)	**26.2** [3] (11)	**14.9** (11)	**17.5** (27)
a12	**0.0** (0)	**2.4** (1)	**0.0** (0)	**0.6** (1)
a13	**0.9** (2)	**0.0** (0)	**0.0** (0)	**0.0** (0)
a14	**0.5** (1)	**0.0** (0)	**1.4** (1)	**0.0** (0)
Total alleles (n)	214	42	74	154

Table 6.10 TNFa microsatellite allele frequencies in normal controls and acute pancreatitis patients grouped according to aetiology of acute pancreatitis

This table displays TNFa microsatellite allele frequencies in control and patient group subjects. For each allele the **% frequency** and the actual number of alleles (in brackets) is given.

n represents the number of subjects in each group

(n) represents the number of alleles in each group.

Alcoholic pancreatitis if daily consumption of ≥ 80g alcohol day^{-1}

Gallstones if radiological or ERCP evidence of cholelithiasis
Idiopathic if no identifiable aetiology
Comparisons were made for each allele between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.

1. There was a trend towards reduced frequency of the TNFa2 allele in patients with alcohol induced acute pancreatitis, although this did not achieve statistical significance.
   Controls vs. alcohol group, χ^2 =7.24, p=0.007, p_c=0.098
   Alcohol vs. idiopathic and gallstone groups combined, χ^2 =4.51, p= 0.034, p_c=0.4
   The reduction in the a2 allele in the alcoholic group was not accounted for by an increase in any one allele, but both a6 and a11 were increased in frequency, especially when compared to patients with non-alcoholic aetiology, although again this was not statistically significant.
2. Alcohol vs. idiopathic and gallstone groups combined, χ^2 =3.64, p= 0.056, p_c=>0.5.
   Alcohol vs. normal controls, χ^2=1.5, p=0.2.
3. Alcohol vs. idiopathic and gallstone groups combined, χ^2 =2.17, p= 0.14.
   Alcohol vs. normal controls, χ^2=2.1, p=0.15.

TNFb allele	Controls n=107 %	Alcohol n=21 %	Idiopathic n=37 %	Gallstones n=77 %
b1	**14.0** (30)	**4.8** [1] (2)	**20.3** (15)	**13.6** (21)
b2	**0.0** (0)	**0.0** (0)	**0.0** (0)	**0.0** (0)
b3	**18.2** (39)	**16.7** (7)	**12.2** (9)	**13.0** (20)
b4	**35.5** (76)	**38.1** (16)	**36.5** (27)	**33.8** (52)
b5	**30.4** (65)	**38.1** (16)	**29.7** (22)	**33.8** (52)
b6	**0.0** (0)	**2.4** (1)	**0.0** (0)	**0.6** (1)
b7	**1.9** (4)	**0.0** (0)	**1.4** (1)	**5.2** (8)
Total alleles (n)	214	42	72	154
TNFc allele				
c1	**73.4** (157)	**88.1** (37)	**68.9** (51)	**70.8** (109)
c2	**26.6** (57)	**11.9** (5)	**31.1** (23)	**29.2** (45)
Total alleles (n)	214	42	74	154

Table 6.11 TNFb and TNFc microsatellite allele frequencies in normal controls and acute pancreatitis patients grouped according to aetiology of acute pancreatitis

This table displays TNFa microsatellite allele frequencies in control and patient group subjects. For each allele the **% frequency** and the actual number of alleles (in brackets) is given.

n represents the number of subjects in each group

(n) represents the number of alleles in each group.

Alcoholic pancreatitis if daily consumption of ≥ 80g alcohol day^{-1}

Gallstones if radiological or ERCP evidence of cholelithiasis

Idiopathic if no identifiable aetiology

Comparisons were made for each allele between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for number of comparisons.

1. There was a slight trend towards reduced frequency of the TNFb1 allele in patients with alcohol induced acute pancreatitis, although this did not achieve statistical significance. Controls vs. alcohol group, χ^2 =2.75, p=0.097, p_c>0.5.
   Alcohol vs. idiopathic and gallstone groups combined, χ^2 =3.57, p= 0.059, p_c>0.5.

TNFa allele	Controls n=107 %	Alcohol 'controls' n=33 %	Alcohol n=21 %	Not alcohol n=114 %
a1	**0.5** (1)	**0.0** (0)	**2.4** (1)	**0.4** (1)
a2	**35.5** (76)	**40.9** (27)	**14.3** [1] (6)	**30.3** (69)
a3	**0.9** (2)	**1.5** (1)	**0.0** (0)	**0.0** (0)
a4	**8.4** (18)	**7.6** (5)	**0.0** (0)	**11.0** (25)
a5	**1.9** (4)	**3.0** (2)	**2.4** (1)	**5.3** (12)
a6	**15.4** (43)	**12.1** (8)	**28.6** [2] (12)	**16.2** (37)
a7	**7.5** (16)	**7.6** (5)	**9.5** (4)	**6.6** (15)
a8	**1.4** (3)	**1.5** (1)	**4.8** (2)	**2.2** (5)
a9	**0.9** (2)	**0.0** (0)	**2.4** (1)	**0.4** (1)
a10	**9.3** (20)	**12.1** (8)	**7.1** (3)	**10.1** (23)
a11	**16.8** (36)	**13.6** (9)	**26.2** [3] (11)	**16.7** (38)
a12	**0.0** (0)	**0.0** (0)	**2.4** (1)	**0.4** (1)
a13	**0.9** (2)	**0.0** (0)	**0.0** (0)	**0.0** (0)
a14	**0.5** (1)	**0.0** (0)	**0.0** (0)	**0.4** (1)
Total alleles (n)	214	66	42	228

Table 6.12 TNFa microsatellite allele frequencies in normal controls, alcohol-induced and non alcohol-induced acute pancreatitis patients, and alcoholic 'controls'

This table displays TNFa microsatellite allele frequencies in both control and patient group subjects. For each allele the **% frequency** and the actual number of alleles (in brackets) is given.

n represents the number of subjects in each group

(n) represents the number of alleles in each group.

Alcoholic pancreatitis if daily consumption of ≥ 80g alcohol day^{-1}
Non-alcohol induced pancreatitis includes:

- Gallstones if radiological or ERCP evidence of cholelithiasis
- Idiopathic if no identifiable aetiology

Comparisons were made for each allele between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.

1. There was a trend towards reduced frequency of the TNFa2 allele in patients with alcohol induced acute pancreatitis, although this achieved statistical significance only when comparing with the group of 'alcoholic controls'.
   Controls vs. alcoholic pancreatitis group, χ^2 =7.24, p=0.007, p_c=0.098
   Alcoholic pancreatitis vs. non-alcoholic group, χ^2 =4.51, p= 0.034, p_c=0.4
   Alcoholic pancreatitis vs. alcohol 'controls', χ^2=8.6, p=0.0034, p_c=0.048
   The reduction in the a2 allele in the alcoholic group was not accounted for by an increase in any one allele, but both a6 and a11 were increased in frequency, although again this was not statistically significant.
2. Alcohol vs. non-alcoholic group, χ^2 =3.64, p= 0.056, p_c>0.5.
   Alcohol vs. alcohol 'controls', χ^2=4.6, p=0.032, p_c=0.44.
3. Alcohol vs. non-alcoholic group, χ^2 =2.17, p= 0.14.
   Alcohol vs. alcohol 'controls', χ^2=2.7, p=0.1.

TNFb allele	Controls n=107 %	Alcohol 'controls' n=33 %	Alcohol n=21 %	Not alcohol n=114 %
b1	**14.0** (30)	**10.6** (7)	**4.8** [1] (2)	**15.8** (36)
b2	**0.0** (0)	**0.0** (0)	**0.0** (0)	**0.0** (0)
b3	**18.2** (39)	**19.7** (13)	**16.7** (7)	**12.7** (29)
b4	**35.5** (76)	**31.8** (21)	**38.1** (16)	**34.6** (79)
b5	**30.4** (65)	**36.4** (24)	**38.1** (16)	**32.5** (74)
b6	**0.0** (0)	**0.0** (0)	**2.4** (1)	**0.4** (1)
b7	**1.9** (4)	**1.5** (1)	**0.0** (0)	**3.9** (9)
Total alleles (n)	214	66	70	228
TNFc allele				
c1	**73.4** (157)	**66.7** (44)	**88.1** (37)	**70.2** (160)
c2	**26.6** (57)	**33.3** (22)	**11.9** (5)	**29.8** (68)
Total alleles (n)	214	66	42	228

Table 6.13 TNFb and c microsatellite allele frequencies in normal controls, alcohol-induced and non alcohol-induced acute pancreatitis patients, and alcoholic 'controls'

This table displays TNFb and c microsatellite allele frequencies in both control and patient group subjects. For each allele the **% frequency** and the actual number of alleles (in brackets) is given.

n represents the number of subjects in each group

(n) represents the number of alleles in each group.

Alcoholic pancreatitis if daily consumption of ≥ 80g alcohol day^{-1}

Non-alcohol induced pancreatitis includes:

- Gallstones if radiological or ERCP evidence of cholelithiasis
- Idiopathic if no identifiable aetiology

Comparisons were made for each allele between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.

1. There was a trend towards reduced frequency of the TNFb1 allele in patients with

alcohol induced acute pancreatitis, although this did not achieve statistical significance.
Controls vs. alcoholic pancreatitis group, χ^2 =2.75, p=0.097, p_c>0.5.
Alcoholic pancreatitis vs. non-alcohol group, χ^2 =3.57, p= 0.059, p_c>0.5.
In the group of 'alcoholic controls' the b1 allele was very slightly reduced in frequency compared to the normal controls.
Alcoholic pancreatitis vs. alcohol 'controls', χ^2=1.2, p=0.28.
Controls vs. alcohol 'controls', χ^2=0.5, p=0.5.

6.4 TNF microsatellite genotypes

Due to the number of alleles, there are many possible TNFa and TNFb microsatellite genotypes. This limits analysis of genotype distribution because of very small numbers. The TNFc locus has only 2 alleles, so only 3 genotypes are possible. TNFa, b, and c genotypes in normal control and patients are displayed in Tables 6.14, 6.15, and 6.16. Genotypes in patient groups stratified according to disease severity classified according to the Atlanta classification and the occurrence of a local complication are shown in Tables 6.17, 6.18, and 6.19. No significant differences were seen between controls and patient groups or between patient groups.

Tables 6.20, 6.21 and 6.22 show genotypes in patient groups stratified according to organ failure score. No significant differences were seen between controls and patient groups or between patient groups. Tables 6.23, 6.24, 6.25, 6.26, 6.27, and 6.28 show genotypes in patient groups stratified according aetiology of acute pancreatitis, and alcoholic 'controls'. No significant differences were seen between controls and patient groups or between patient groups.

TNFa genotype	Controls n=107 %	Study patients n=135 %
1,2	0.9 (1)	0.0 (0)
1,7	0.0 (0)	0.7 (1)
1,11	0.0 (0)	0.7 (1)
2,2	13.1 (14)	5.9 (8)
2,3	0.9 (1)	0.0 (0)
2,4	5.6 (6)	6.7 (9)
2,5	0.9 (1)	3.7 (5)
2,6	12.1 (13)	11.1 (15)
2,7	7.5 (8)	5.2 (7)
2,8	0.9 (1)	0.0 (0)
2,9	0.0 (0)	0.7 (1)
2,10	6.5 (7)	8.1 (11)
2,11	9.3 (10)	7.4 (10)
3,6	0.9 (1)	0.0 (0)
4,4	0.9 (1)	0.7 (1)
4,5	0.0 (0)	2.2 (3)
4,6	0.9 (1)	3.0 (4)
4,7	1.9 (2)	1.5 (2)
4,8	0.0 (0)	0.7 (1)
4,9	0.0 (0)	0.7 (1)
4,10	0.9 (1)	0.7 (1)
4,11	4.7 (5)	1.5 (2)

Table 6.14 **Genotypes at the TNFa microsatellite locus in normal control and patient groups** (continued.....)

TNFa genotype	Controls n=107 %	Study patients n=135 %
4,13	**0.9**	**0.0**
	(1)	(0)
5,6	**0.0**	**1.5**
	(0)	(2)
5,10	**0.9**	**0.7**
	(1)	(1)
5,11	**1.9**	**1.5**
	(2)	(2)
6,6	**1.9**	**3.0**
	(2)	(4)
6,7	**1.9**	**0.7**
	(2)	(1)
6,10	**1.9**	**6.7**
	(2)	(9)
6,11	**9.3**	**7.4**
	(10)	(10)
7,7	**0.9**	**1.5**
	(1)	(2)
7,8	**0.9**	**0.0**
	(1)	(0)
7,11	**0.9**	**3.0**
	(1)	(4)
7,13	**0.9**	**0.0**
	(1)	(0)
7,14	**0.0**	**0.7**
	(0)	(1)
8,10	**0.9**	**0.7**
	(1)	(1)
8,11	**0.0**	**3.7**
	(0)	(5)
9,10	**0.9**	**0.0**
	(1)	(0)
9,11	**0.9**	**0.0**
	(1)	(0)
10,10	**0.9**	**0.0**
	(1)	(0)
10,11	**4.7**	**2.2**
	(5)	(3)
11,11	**0.9**	**3.7**
	(1)	(5)
11,12	**0.0**	**1.5**
	(0)	(2)
Total, n	**107**	**135**

Table 6.14 Genotypes at the TNFa microsatellite locus in normal control and patient groups

This table displays TNFa microsatellite genotype frequencies in control and patient subjects. For each genotype the **% frequency** and the actual number of genotypes (in brackets) is

given. Comparisons were made for each genotype between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made. No significant differences were seen in genotype frequencies between patient and control groups.

TNFb genotype	Controls n=107 %	Study patients n=135 %
1,1	1.9 (2)	1.5 (2)
1,2	0.0 (0)	0.7 (1)
1,3	4.7 (5)	4.4 (6)
1,4	17.8 (19)	6.7 [1] (9)
1,5	7.5 (8)	10.4 (14)
1,6	0.0 (0)	0.7 (1)
1,7	0.0 (0)	2.2 (3)
3,3	2.8 (3)	0.0 (0)
3,4	12.1 (13)	8.1 (11)
3,5	11.2 (12)	11.1 (15)
3,7	0.9 (1)	0.0 (0)
4,4	7.5 (8)	15.6 (21)
4,5	25.2 (27)	24.4 (33)
4,6	0.0 (0)	0.7 (1)
4,7	2.8 (3)	3.0 (4)
5,5	5.6 (6)	8.9 (12)
5,7	0.0 (0)	1.5 (2)
Total	107	135

Table 6.15 Genotypes at the TNFb microsatellite locus in normal control and patient groups

This table displays TNFb microsatellite genotype frequencies in control and patient subjects. For each genotype the **% frequency** and the actual number of genotypes (in brackets) is given.

Comparisons were made for each genotype between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.

No significant differences were seen in genotype frequencies between patient and control groups.

1. Control vs. patient groups, χ^2 =7.18, p=0.007, p_c=0.12.

TNFc genotype	Controls n=107 %	Study patients n=135 %
1,1	**52.3** (56)	**53.3** (72)
1,2	**42.1** (45)	**39.3** (53)
2,2	**5.6** (6)	**7.4** (10)
Total	107	135

Table 6.16 Genotypes at the TNFc microsatellite locus in normal control and patient groups

This table displays TNFc microsatellite genotype frequencies in control and patient subjects. For each genotype the **% frequency** and the actual number of genotypes (in brackets) is given.
Comparisons were made for each genotype between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.
No significant differences were seen in genotype frequencies between patient and control groups.

TNFa genotype	Controls n=107 %	Mild n=97 %	Severe n=38 %	Local complication n=21 %
1,2	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
1,7	**0.0**	**1.0**	**0.0**	**0.0**
	(0)	(1)	(0)	(0)
1,11	**0.0**	**0.0**	**2.6**	**4.8**
	(0)	(0)	(1)	(1)
2,2	**13.1**	**5.2**	**7.9**	**9.5**
	(14)	(5)	(3)	(2)
2,3	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
2,4	**5.6**	**6.2**	**7.9**	**4.8**
	(6)	(6)	(3)	(1)
2,5	**0.9**	**4.1**	**2.6**	**0.0**
	(1)	(4)	(1)	(0)
2,6	**12.1**	**10.3**	**13.2**	**14.3**
	(13)	(10)	(5)	(3)
2,7	**7.5**	**5.2**	**5.3**	**4.8**
	(8)	(5)	(2)	(1)
2,8	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	**(0)**	(0)
2,9	**0.0**	**1.0**	**0.0**	**0.0**
	(0)	(1)	(0)	(0)
2,10	**6.5**	**6.2**	**13.2**	**4.8**
	(7)	(6)	(5)	(1)
2,11	**9.3**	**8.2**	**5.3**	**9.5**
	(10)	(8)	(2)	(2)
3,6	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
4,4	**0.9**	**1.0**	**0.0**	**0.0**
	(1)	(1)	(0)	(0)
4,5	**0.0**	**1.0**	**5.3**	**9.5**
	(0)	(1)	(2)	(2)
4,6	**0.9**	**4.1**	**0.0**	**0.0**
	(1)	(4)	(0)	(0)
4,7	**1.9**	**1.0**	**2.6**	**4.8**
	(2)	(1)	(1)	(1)
4,8	**0.0**	**1.0**	**0.0**	**0.0**
	(0)	(1)	(0)	(0)
4,9	**0.0**	**1.0**	**0.0**	**0.0**
	(0)	(1)	(0)	(0)
4,10	**0.9**	**1.0**	**0.0**	**0.0**
	(1)	(1)	(0)	(0)
4,11	**4.7**	**1.0**	**2.6**	**0.0**
	(5)	(1)	(1)	(0)

Table 6.17 **Genotypes at the TNFa microsatellite locus in normal control and acute pancreatitis patients grouped according to (local and systemic) disease severity** (cont.....)

TNFa genotype	Controls n=107 %	Mild n=97 %	Severe n=38 %	Local complication n=21 %
4,13	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
5,6	**0.0**	**1.0**	**2.6**	**4.8**
	(0)	(1)	(1)	(1)
5,10	**0.9**	**1.0**	**0.0**	**0.0**
	(1)	(1)	(0)	(0)
5,11	**1.9**	**2.1**	**0.0**	**0.0**
	(2)	(2)	(0)	(0)
6,6	**1.9**	**3.1**	**2.6**	**4.8**
	(2)	(3)	(1)	(1)
6,7	**1.9**	**1.0**	**0.0**	**0.0**
	(2)	(1)	(0)	(0)
6,10	**1.9**	**5.2**	**10.5**	**9.5**
	(2)	(5)	(4)	(2)
6,11	**9.3**	**9.3**	**2.6**	**4.8**
	(10)	(9)	(1)	(1)
7,7	**0.9**	**1.0**	**2.6**	**0.0**
	(1)	(1)	(1)	(0)
7,8	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
7,11	**0.9**	**4.1**	**0.0**	**0.0**
	(1)	(4)	(0)	(0)
7,13	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
7,14	**0.0**	**1.0**	**0.0**	**0.0**
	(0)	(1)	(0)	(0)
8,10	**0.9**	**1.0**	**0.0**	**0.0**
	(1)	(1)	(0)	(0)
8,11	**0.0**	**2.1**	**7.9**	**9.5**
	(0)	(2)	(3)	(2)
9,10	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
9,11	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
10,10	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
10,11	**4.7**	**2.1**	**2.6**	**0.0**
	(5)	(2)	(1)	(0)
11,11	**0.9**	**5.2**	**0.0**	**0.0**
	(1)	(5)	(0)	(0)
11,12	**0.0**	**2.1**	**0.0**	**0.0**
	(0)	(2)	(0)	(0)
Total	107	97	38	21

Table 6.17 **Genotypes at the TNFa microsatellite locus in normal control and acute pancreatitis patients grouped according to (local and systemic) disease severity**

Table 6.17 Genotypes at the TNFa microsatellite locus in normal control and acute pancreatitis patients grouped according to (local and systemic) disease severity

This table displays TNFa microsatellite genotype frequencies in control and patient groups. For each genotype the **% frequency** and the actual number of genotypes (in brackets) is given.
Mild and severe disease severity groups are according to the Atlanta convention classification (Bradley, 1993), described in section 5.1.4.
Local complication is defined as occurrence of pancreatic necrosis, abscess or pseudocyst.
Comparisons were made for each genotype between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.

TNFb genotype	Controls n=107 %	Mild n=97 %	Severe n=38 %	Local complication n=21 %
1,1	**1.9**	**2.1**	**0.0**	**0.0**
	(2)	(2)	(0)	(0)
1,2	**0.0**	**1**	**0.0**	**0.0**
	(0)	(1)	(0)	(0)
1,3	**4.7**	**4.1**	**5.3**	**0.0**
	(5)	(4)	(2)	(0)
1,4	**17.8**	**5.2**	**10.5**	**9.5**
	(19)	(5)	(4)	(2)
1,5	**7.5**	**11.3**	**7.9**	**14.3**
	(8)	(11)	(3)	(3)
1,6	**0.0**	**1.0**	**0.0**	**0.0**
	(0)	(1)	(0)	(0)
1,7	**0.0**	**1.0**	**5.3**	**0.0**
	(0)	(1)	(2)	(0)
3,3	**2.8**	**0.0**	**0.0**	**0.0**
	(3)	(0)	(0)	(0)
3,4	**12.1**	**9.3**	**5.3**	**9.5**
	(13)	(9)	(2)	(2)
3,5	**11.2**	**11.3**	**10.5**	**9.5**
	(12)	(11)	(4)	(2)
3,7	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
4,4	**7.5**	**15.5**	**15.8**	**9.5**
	(8)	(15)	(6)	(2)
4,5	**25.2**	**25.8**	**21.1**	**23.8**
	(27)	(25)	(8)	(5)
4,6	**0.0**	**1.0**	**0.0**	**0.0**
	(0)	(1)	(0)	(0)
4,7	**2.8**	**3.1**	**2.6**	**4.8**
	(3)	(3)	(1)	(1)
5,5	**5.6**	**7.2**	**13.2**	**19.0**
	(6)	(7)	(5)	(4)
5,7	**0.0**	**1.0**	**2.6**	**0.0**
	(0)	(1)	(1)	(0)
Total	107	97	38	135

Table 6.18 Genotypes at the TNFb microsatellite locus in normal control and acute pancreatitis patients grouped according to (local and systemic) disease severity

This table displays TNFb microsatellite genotype frequencies in control and patient groups. For each genotype the **% frequency** and the number of genotypes (in brackets) is given. Mild and severe disease severity groups are according to the Atlanta convention classification (Bradley, 1993), described in section 5.1.4.
Local complication is defined as occurrence of pancreatic necrosis, abscess or pseudocyst. Comparisons were made for each genotype between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made. No significant differences were seen.

TNFc genotype	Controls n=107 %	Mild n=97 %	Severe n=38 %	Local complication n=21 %
1,1	**52.3** (56)	**57.7** (56)	**42.1** (16)	**42.9** (9)
1,2	**42.1** (45)	**35.1** (34)	**50.0** (19)	**52.4** (11)
2,2	**5.6** (6)	**7.2** (7)	**7.9** (3)	**4.8** (1)
Total	107	97	38	21

Table 6.19 Genotypes at the TNFc microsatellite locus in normal control and acute pancreatitis patients grouped according to (local and systemic) disease severity

This table displays TNFc microsatellite genotype frequencies in control and patient groups. For each genotype the **% frequency** and the number of genotypes (in brackets) is given. Mild and severe disease severity groups are according to the Atlanta convention classification (Bradley, 1993), described in section 5.1.4.
Local complication is defined as occurrence of pancreatic necrosis, abscess or pseudocyst. Comparisons were made for each genotype between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made. No significant differences were seen.

TNFa genotype	Controls n=107 %	OFS ≥ 2 n=35 %	OFS ≥ 3 n=18 %	OFS ≥ 4 n=12 %
1,2	0.9	0.0	0.0	0.0
	(1)	(0)	(0)	(0)
2,2	13.1	8.6	16.7	25.0
	(14)	(3)	(3)	(3)
2,3	0.9	0.0	0.0	0.0
	(1)	(0)	(0)	(0)
2,4	5.6	5.7	5.6	0.0
	(6)	(2)	(1)	(0)
2,5	0.9	2.9	0.0	0.0
	(1)	(1)	(0)	(0)
2,6	12.1	17.1	11.1	16.7
	(13)	(6)	(2)	(2)
2,7	7.5	5.7	11.1	8.3
	(8)	(2)	(2)	(1)
2,8	0.9	0.0	0.0	0.0
	(1)	(0)	(0)	(1)
2,9	0.0	0.0	0.0	0.0
	(0)	(0)	(0)	(0)
2,10	6.5	14.3	11.1	0.0
	(7)	(5)	(0)	(0)
2,11	9.3	2.9	0.0	0.0
	(10)	(1)	(0)	(0)
3,6	0.9	0.0	0.0	0.0
	(1)	(0)	(0)	(0)
4,4	0.9	0.0	0.0	0.0
	(1)	(0)	(0)	(0)
4,5	0.0	5.7	5.6	8.3
	(0)	(2)	(1)	(1)
4,6	0.9	2.9	0.0	0.0
	(1)	(1)	(0)	(0)
4,7	1.9	0.0	0.0	0.0
	(2)	(0)	(0)	(0)
4,8	0.0	0.0	0.0	0.0
	(0)	(0)	(0)	(0)
4,9	0.0	0.0	0.0	0.0
	(0)	(0)	(0)	(0)
4,10	0.9	0.0	0.0	0.0
	(1)	(0)	(0)	(0)
4,11	4.7	2.9	5.6	8.3
	(5)	(1)	(1)	(1)
4,13	0.9	0.0	0.0	0.0
	(1)	(0)	(1)	(0)

Table 6.20 **TNFa genotype frequencies in normal controls and acute pancreatitis patients grouped according to (systemic) disease severity as assessed by organ failure score** (continued....)

TNFa genotype	Controls	OFS ≥ 2	OFS ≥ 3	OFS ≥ 4
	n=107	n=35	n=18	n=12
	%	%	%	%
5,6	**0.0**	**2.9**	**5.6**	**8.3**
	(0)	(1)	(1)	(1)
5,10	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
5,11	**1.9**	**2.9**	**0.0**	**0.0**
	(2)	(1)	(0)	(0)
6,6	**1.9**	**0.0**	**0.0**	**0.0**
	(2)	(0)	(0)	(0)
6,7	**1.9**	**0.0**	**0.0**	**0.0**
	(2)	(0)	(0)	(0)
6,10	**1.9**	**8.6**	**16.7**	**16.7**
	(2)	(3)	(3)	(2)
6,11	**9.3**	**0.0**	**0.0**	**0.0**
	(10)	(0)	(0)	(0)
7,7	**0.9**	**2.9**	**5.6**	**0.0**
	(1)	(1)	(1)	(0)
7,8	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
7,11	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
7,13	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
7,14	**0.0**	**0.0**	**0.0**	**0.0**
	(0)	(0)	(0)	(0)
8,10	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
8,11	**0.0**	**8.6**	**0.0**	**0.0**
	(0)	(3)	(0)	(0)
9,10	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
9,11	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
10,10	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
10,11	**4.7**	**2.9**	**5.6**	**8.3**
	(5)	(1)	(1)	(1)
11,11	**0.9**	**2.9**	**0.0**	**0.0**
	(1)	(1)	(0)	(0)
Total	107	35	18	12

Table 6.20 TNFa genotype frequencies in normal controls and acute pancreatitis patients grouped according to (systemic) disease severity as assessed by organ failure score

This table displays TNFa microsatellite genotype frequencies in control and patient groups. For each genotype the **% frequency** and the number of genotypes (in brackets) is given.
OFS Organ failure score
OFS calculated according to method described in section 5.1.4 (Kingsnorth et al, 1995).

TNFb genotype	Controls	OFS ≥ 2	OFS ≥ 3	OFS ≥ 4
	n=107	n=35	n=18	n=12
	%	%	%	%
1,1	**1.9**	**0.0**	**0.0**	**0.0**
	(2)	(0)	(0)	(0)
1,2	**0.0**	**0.0**	**0.0**	**0.0**
	(0)	(0)	(0)	(0)
1,3	**4.7**	**5.7**	**5.6**	**8.3**
	(5)	(2)	(1)	(1)
1,4	**17.8**	**8.6**	**16.7**	**8.3**
	(19)	(3)	(3)	(1)
1,5	**7.5**	**11.4**	**16.7**	**25.0**
	(8)	(4)	(3)	(3)
1,6	**0.0**	**0.0**	**0.0**	**0.0**
	(0)	(0)	(0)	(0)
1,7	**0.0**	**5.7**	**0.0**	**0.0**
	(0)	(2)	(0)	(0)
3,3	**2.8**	**0.0**	**0.0**	**0.0**
	(3)	(0)	(0)	(0)
3,4	**12.1**	**5.7**	**11.1**	**8.3**
	(13)	(2)	(2)	(1)
3,5	**11.2**	**11.4**	**11.1**	**8.3**
	(12)	(4)	(2)	(1)
3,7	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
4,4	**7.5**	**20.0**	**5.6**	**0.0**
	(8)	(7)	(1)	(0)
4,5	**25.2**	**17.1**	**27.8**	**33.3**
	(27)	(6)	(5)	(4)
4,6	**0.0**	**0.0**	**0.0**	**0.0**
	(0)	(0)	(0)	(0)
4,7	**2.8**	**0.0**	**0.0**	**0.0**
	(3)	(0)	(0)	(0)
5,5	**5.6**	**11.4**	**5.6**	**8.3**
	(6)	(4)	(1)	(1)
5,7	**0.0**	**2.9**	**0.0**	**0.0**
	(0)	(1)	(0)	(0)
Total	107	35	18	12

Table 6.21 TNFb genotype frequencies in normal controls and acute pancreatitis patients grouped according to (systemic) disease severity as assessed by organ failure score

This table displays TNFb microsatellite genotype frequencies in control and patient groups. For each genotype the **% frequency** and the number of genotypes (in brackets) is given.

OFS Organ failure score

OFS calculated according to method described in section 5.1.4 (Kingsnorth et al, 1995). Comparisons were made for each genotype between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made. No significant differences were seen

TNFc genotype	Controls	OFS ≥ 2	OFS ≥ 3	OFS ≥ 4
	n=107	n=35	n=18	n=12
	%	%	%	%
1,1	**52.3**	**42.9**	**44.4**	**25.0**
	(56)	(15)	(8)	(3)
1,2	**42.1**	**48.6**	**50.0**	**66.7**
	(45)	(17)	(9)	(8)
2,2	**5.6**	**8.6**	**5.6**	**8.3**
	(6)	(3)	(1)	(1)
Total	107	35	18	12

Table 6.22 TNFc genotype frequencies in normal controls and acute pancreatitis patients grouped according to (systemic) disease severity as assessed by organ failure score

This table displays TNFc microsatellite genotype frequencies in control and patient groups. For each genotype the **% frequency** and the number of genotypes (in brackets) is given.
OFS Organ failure score
OFS calculated according to method described in section 5.1.4 (Kingsnorth et al, 1995).
Comparisons were made for each genotype between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made. No significant differences were seen

TNFa genotype	Controls n=107 %	Alcohol n=21 %	Idiopathic n=37 %	Gallstones n=77 %
1,2	0.9 (1)	0.0 (0)	0.0 (0)	0.0 (0)
1,7	0.0 (0)	4.8 (1)	0.0 (0)	0.0 (0)
1,11	0.0 (0)	0.0 (0)	2.7 (1)	0.0 (0)
2,2	13.1 (14)	0.0 (0)	5.4 (2)	7.8 (6)
2,3	0.9 (1)	0.0 (0)	0.0 (0)	0.0 (0)
2,4	5.6 (6)	0.0 (0)	8.1 (3)	7.8 (6)
2,5	0.9 (1)	4.8 (1)	0.0 (0)	5.2 (4)
2,6	12.1 (13)	14.3 (3)	13.5 (5)	9.1 (7)
2,7	7.5 (8)	0.0 (0)	8.1 (3)	5.2 (4)
2,8	0.9 (1)	0.0 (0)	0.0 (0)	0.0 (0)
2,9	0.0 (0)	4.8 (1)	0.0 (0)	0.0 (0)
2,10	6.5 (7)	4.8 (1)	10.8 (4)	7.8 (6)
2,11	9.3 (10)	0.0 (0)	2.7 (1)	11.7 (9)
3,6	0.9 (1)	0.0 (0)	0.0 (0)	0.0 (0)
4,4	0.9 (1)	0.0 (0)	0.0 (0)	1.3 (1)
4,5	0.0 (0)	0.0 (0)	2.7 (1)	2.6 (2)
4,6	0.9 (1)	0.0 (0)	5.4 (2)	2.6 (2)
4,7	1.9 (2)	0.0 (0)	2.7 (1)	1.3 (1)
4,8	0.0 (0)	0.0 (0)	0.0 (0)	1.3 (1)
4,9	0.0 (0)	0.0 (0)	2.7 (1)	0.0 (0)
4,10	0.9 (1)	0.0 (0)	0.0 (0)	1.3 (1)
4,11	4.7 (5)	0.0 (0)	2.7 (1)	1.3 (1)
4,13	0.9 (1)	0.0 (0)	0.0 (0)	0.0 (0)

Table 6.23 **Genotypes at the TNFa microsatellite locus in normal control and acute pancreatitis patients grouped according to aetiology** (continued...)

TNFa genotype	Controls	Alcohol	Idiopathic	Gallstones
	n=107	n=21	n=37	n=77
	%	%	%	%
5,6	**0.0**	**0.0**	**2.7**	**1.3**
	(0)	(0)	(1)	(1)
5,10	**0.9**	**0.0**	**0.0**	**1.3**
	(1)	(0)	(0)	(1)
5,11	**1.9**	**0.0**	**2.7**	**1.3**
	(2)	(0)	(1)	(1)
6,6	**1.9**	**4.8**	**0.0**	**3.9**
	(2)	(1)	(0)	(3)
6,7	**1.9**	**4.8**	**0.0**	**0.0**
	(2)	(1)	(0)	(0)
6,10	**1.9**	**9.5**	**5.4**	**6.5**
	(2)	(2)	(2)	(5)
6,11	**9.3**	**19.0**	**5.4**	**5.2**
	(10)	(4)	(2)	(4)
7,7	**0.9**	**0.0**	**2.7**	**1.3**
	(1)	(0)	(1)	(1)
7,8	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
7,11	**0.9**	**9.5**	**2.7**	**1.3**
	(1)	(2)	(1)	(1)
7,13	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
7,14	**0.0**	**0.0**	**2.7**	**0.0**
	(0)	(0)	(1)	(0)
8,10	**0.9**	**0.0**	**0.0**	**1.3**
	(1)	(0)	(0)	(1)
8,11	**0.0**	**9.5**	**2.7**	**2.6**
	(0)	(2)	(1)	(2)
9,10	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
9,11	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
10,10	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
10,11	**4.7**	**0.0**	**2.7**	**2.6**
	(5)	(0)	(1)	(2)
11,11	**0.9**	**4.8**	**2.7**	**3.9**
	(1)	(1)	(1)	(3)
11,12	**0.0**	**4.8**	**0.0**	**1.3**
	(0)	(1)	(0)	(1)
Total	107	21	37	77

Table 6.23 Genotypes at the TNFa microsatellite locus in normal control and acute pancreatitis patients grouped according to aetiology

This table displays TNFa microsatellite genotype frequencies in control and patient groups. For each genotype the **% frequency** and the number of genotypes (in brackets) is given.

Alcoholic pancreatitis if daily consumption of ≥ 80g alcohol day^{-1}
Gallstones if radiological or ERCP evidence of cholelithiasis
Idiopathic if no identifiable aetiology
Comparisons were made for each genotype between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made. No significant differences were seen

TNFb genotype	Controls n=107 %	Alcohol n=21 %	Idiopathic n=37 %	Gallstones n=77 %
1,1	**1.9**	**0.0**	**2.7**	**1.3**
	(2)	(0)	(1)	(1)
1,2	**0.0**	**0.0**	**0.0**	**0.0**
	(0)	(0)	(0)	(0)
1,3	**4.7**	**0.0**	**5.4**	**5.2**
	(5)	(0)	(2)	(4)
1,4	**17.8**	**0.0**	**16.2**	**5.2**
	(19)	(0)	(6)	(4)
1,5	**7.5**	**9.5**	**13.5**	**9.1**
	(8)	(2)	(5)	(7)
1,6	**0.0**	**0.0**	**0.0**	**1.3**
	(0)	(0)	(0)	(1)
1,7	**0.0**	**0.0**	**0.0**	**3.9**
	(0)	(0)	(0)	(3)
3,3	**2.8**	**0.0**	**0.0**	**0.0**
	(3)	(0)	(0)	(0)
3,4	**12.1**	**9.5**	**8.1**	**9.1**
	(13)	(2)	(3)	(7)
3,5	**11.2**	**14.3**	**8.1**	**11.7**
	(12)	(3)	(3)	(9)
3,7	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
4,4	**7.5**	**19.0**	**10.8**	**15.6**
	(8)	(4)	(4)	(12)
4,5	**25.2**	**38.1**	**27.0**	**18.2**
	(27)	(8)	(10)	(14)
4,6	**0.0**	**4.8**	**0.0**	**0.0**
	(0)	(1)	(0)	(0)
4,7	**2.8**	**0.0**	**2.7**	**3.9**
	(3)	(0)	(1)	(3)
5,5	**5.6**	**4.8**	**5.4**	**13.0**
	(6)	(1)	(2)	(10)
5,7	**0.0**	**0.0**	**0.0**	**2.6**
	(0)	(0)	(0)	(2)
Total	107	21	35	77

Table 6.24 Genotypes at the TNFb microsatellite locus in normal control and acute pancreatitis patients grouped according to aetiology

This table displays TNFb microsatellite genotype frequencies in control and patient groups. For each genotype the **% frequency** and the number of genotypes (in brackets) is given.
Alcoholic pancreatitis if daily consumption of ≥ 80g alcohol day^{-1}
Gallstones if radiological or ERCP evidence of cholelithiasis
Idiopathic if no identifiable aetiology
Comparisons were made for each genotype between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made. No significant differences were seen

TNFc genotype	Controls	Alcohol	Idiopathic	Gallstones
	n=107	n=21	n=37	n=77
	%	%	%	%
1,1	**52.3**	**76.2**	**43.2**	**51.9**
	(56)	(16)	(16)	(40)
1,2	**42.1**	**23.8**	**51.4**	**37.7**
	(45)	(5)	(19)	(29)
2,2	**5.6**	**0.0**	**5.4**	**10.4**
	(6)	(0)	(2)	(8)
Total	107	21	37	77

Table 6.25 Genotypes at the TNFc microsatellite locus in normal control and acute pancreatitis patients grouped according to aetiology

This table displays TNFc microsatellite genotype frequencies in control and patient groups. For each genotype the **% frequency** and the number of genotypes (in brackets) is given.
Alcoholic pancreatitis if daily consumption of ≥ 80g alcohol day^{-1}
Gallstones if radiological or ERCP evidence of cholelithiasis
Idiopathic if no identifiable aetiology
Comparisons were made for each genotype between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made. No significant differences were seen.

TNFa genotype	Controls n=107 %	Alcohol 'controls' n=33 %	Alcohol n=21 %	Not alcohol n=114 %
1,2	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
1,7	**0.0**	**0.0**	**4.8**	**0.0**
	(0)	(0)	(1)	(0)
1,11	**0.0**	**0.0**	**0.0**	**0.9**
	(0)	(0)	(0)	(1)
2,2	**13.1**	**18.2**	**0.0**	**7.0**
	(14)	(6)	(0)	(8)
2,3	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
2,4	**5.6**	**6.1**	**0.0**	**7.9**
	(6)	(2)	(0)	(9)
2,5	**0.9**	**0.0**	**4.8**	**3.5**
	(1)	(0)	(1)	(4)
2,6	**12.1**	**9.1**	**14.3**	**10.5**
	(13)	(3)	(3)	(12)
2,7	**7.5**	**6.1**	**0.0**	**6.1**
	(8)	(2)	(0)	(7)
2,8	**0.9**	**3.0**	**0.0**	**0.0**
	(1)	(1)	(0)	(0)
2,9	**0.0**	**0.0**	**4.8**	**0.0**
	(0)	(0)	(1)	(0)
2,10	**6.5**	**15.2**	**4.8**	**8.8**
	(7)	(5)	(1)	(10)
2,11	**9.3**	**6.1**	**0.0**	**8.8**
	(10)	(2)	(0)	(10)
3,6	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
3,7	**0.0**	**3.0**	**0.0**	**0.0**
	(0)	(1)	(0)	(0)
4,4	**0.9**	**0.0**	**0.0**	**0.9**
	(1)	(0)	(0)	(1)
4,5	**0.0**	**3.0**	**0.0**	**2.6**
	(0)	(1)	(0)	(3)
4,6	**0.9**	**3.0**	**0.0**	**3.5**
	(1)	(1)	(0)	(4)
4,7	**1.9**	**0.0**	**0.0**	**1.8**
	(2)	(0)	(0)	(2)
4,8	**0.0**	**0.0**	**0.0**	**0.9**
	(0)	(0)	(0)	(1)
4,9	**0.0**	**0.0**	**0.0**	**0.9**
	(0)	(0)	(0)	(1)
4,10	**0.9**	**0.0**	**0.0**	**0.9**
	(1)	(0)	(0)	(1)

Table 6.26 **Genotypes at the TNFa microsatellite locus in normal controls, alcohol-induced and non alcohol-induced acute pancreatitis patients, and alcoholic 'controls'** (continued...)

TNFa genotype	Controls n=107 %	Alcohol 'controls' n=33 %	Alcohol n=21 %	Not alcohol n=114 %
4,11	**4.7**	**3.0**	**0.0**	**1.8**
	(5)	(1)	(0)	(2)
4,13	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
5,6	**0.0**	**0.0**	**0.0**	**1.8**
	(0)	(0)	(0)	(2)
5,10	**0.9**	**0.0**	**0.0**	**0.9**
	(1)	(0)	(0)	(1)
5,11	**1.9**	**3.0**	**0.0**	**1.8**
	(2)	(1)	(0)	(2)
6,6	**1.9**	**0.0**	**4.8**	**2.6**
	(2)	(0)	(1)	(3)
6,7	**1.9**	**0.0**	**4.8**	**0.0**
	(2)	(0)	(1)	(0)
6,10	**1.9**	**6.1**	**9.5**	**6.1**
	(2)	(2)	(2)	(7)
6,11	**9.3**	**6.1**	**19.0**	**5.3**
	(10)	(2)	(4)	(6)
7,7	**0.9**	**0.0**	**0.0**	**1.8**
	(1)	(0)	(0)	(2)
7,8	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
7,10	**0.0**	**3.0**	**0.0**	**0.0**
	(0)	(1)	(0)	(0)
7,11	**0.9**	**3.0**	**9.5**	**1.8**
	(1)	(1)	(2)	(2)
7,13	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
7,14	**0.0**	**0.0**	**0.0**	**0.9**
	(0)	(0)	(0)	(1)
8,10	**0.9**	**0.0**	**0.0**	**0.9**
	(1)	(0)	(0)	(1)
8,11	**0.0**	**0.0**	**9.5**	**2.6**
	(0)	(0)	(2)	(3)
9,10	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
9,11	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
10,10	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
10,11	**4.7**	**0.0**	**0.0**	**2.6**
	(5)	(0)	(0)	(3)
11,11	**0.9**	**3.0**	**4.8**	**3.5**
	(1)	(1)	(1)	(4)
11,12	**0.0**	**0.0**	**4.8**	**0.9**
	(0)	(0)	(1)	(1)
Total	107	33	21	114

Table 6.26 **Genotypes at the TNFa microsatellite locus in normal controls, alcohol-induced and non alcohol-induced acute pancreatitis patients, and alcoholic 'controls'** (footnotes next page...)

Table 6.26 Genotypes at the TNFa microsatellite locus in normal controls, alcohol-induced and non alcohol-induced acute pancreatitis patients, and alcoholic 'controls'

This table displays TNFa microsatellite genotype frequencies in control and patient groups. For each genotype the **% frequency** and the number of genotypes (in brackets) is given.
n represents the number of subjects in each group
(n) represents the number of alleles in each group.
Alcoholic pancreatitis if daily consumption of ≥ 80g alcohol day^{-1}
Non-alcohol induced pancreatitis includes:

- Gallstones if radiological or ERCP evidence of cholelithiasis
- Idiopathic if no identifiable aetiology

Comparisons were made for each genotype between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made. No significant differences were seen.

TNFb genotype	Controls n=107 %	Alcohol 'controls' n=33 %	Alcohol n=21 %	Not alcohol n=114 %
1,1	**1.9**	**0.0**	**0.0**	**1.8**
	(2)	(0)	(0)	(2)
1,3	**4.7**	**6.1**	**0.0**	**5.3**
	(5)	(2)	(0)	(6)
1,4	**17.8**	**15.2**	**0.0**	**8.8**
	(19)	(5)	(0)	(10)
1,5	**7.5**	**0.0**	**9.5**	**10.5**
	(8)	(0)	(2)	(12)
1,6	**0.0**	**0.0**	**0.0**	**0.9**
	(0)	(0)	(0)	(1)
1,7	**0.0**	**0.0**	**0.0**	**2.6**
	(0)	(0)	(0)	(3)
3,3	**2.8**	**9.1**	**0.0**	**0.0**
	(3)	(3)	(0)	(0)
3,4	**12.1**	**9.1**	**9.5**	**8.8**
	(13)	(3)	(2)	(10)
3,5	**11.2**	**6.1**	**14.3**	**10.5**
	(12)	(2)	(3)	(12)
3,7	**0.9**	**0.0**	**0.0**	**0.0**
	(1)	(0)	(0)	(0)
4,4	**7.5**	**9.1**	**19.0**	**14.0**
	(8)	(3)	(4)	(16)
4,5	**25.2**	**21.2**	**38.1**	**21.1**
	(27)	(7)	(8)	(24)
4,6	**0.0**	**0.0**	**4.8**	**0.0**
	(0)	(0)	(1)	(0)
4,7	**2.8**	**0.0**	**0.0**	**3.5**
	(3)	(0)	(0)	(4)
5,5	**5.6**	**21.2**	**4.8**	**10.5**
	(6)	(7)	(1)	(12)
5,7	**0.0**	**3.0**	**0.0**	**1.8**
	(0)	(1)	(0)	(2)
Total	107	33	21	114

Table 6.27 Genotypes at the TNFb microsatellite locus in normal controls, alcohol-induced and non alcohol-induced acute pancreatitis patients, and alcoholic 'controls'

This table displays TNFb microsatellite genotype frequencies in control and patient groups. For each genotype the **% frequency** and the number of genotypes (in brackets) is given.
Alcoholic pancreatitis if daily consumption of ≥ 80g alcohol day^{-1}
Non-alcohol induced pancreatitis includes:

- Gallstones if radiological or ERCP evidence of cholelithiasis
- Idiopathic if no identifiable aetiology

Comparisons were made for each genotype between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made. No significant differences were seen.

TNFc genotype	Controls n=107 %	Alcohol 'controls' n=33 %	Alcohol n=21 %	Not alcohol n=114 %
1,1	**52.3** (56)	**45.5** (15)	**76.2** (16)	**49.1** (56)
1,2	**42.1** (45)	**42.4** (14)	**23.8** (5)	**42.1** (48)
2,2	**5.6** (6)	**12.1** (4)	**0.0** (0)	**8.8** (10)
Total	107	33	21	114

Table 6.28 Genotypes at the TNFc microsatellite locus in normal controls, alcohol-induced and non alcohol-induced acute pancreatitis patients, and alcoholic 'controls'

This table displays TNFc microsatellite genotype frequencies in control and patient groups. For each genotype the **% frequency** and the number of genotypes (in brackets) is given.
Alcoholic pancreatitis if daily consumption of ≥ 80g alcohol day^{-1}
Non-alcohol induced pancreatitis includes:

- Gallstones if radiological or ERCP evidence of cholelithiasis
- Idiopathic if no identifiable aetiology

Comparisons were made for each genotype between patient and control groups using 2x2 contingency tables and the $\chi 2$ test. P values were corrected for the number of comparisons made. No significant differences were seen.

6.5 TNF microsatellite 3-locus haplotypes

Haplotypes, alleles combining in non-random association across the TNF 3 locus microsatellite system, have been characterised by maximum likelihood estimates in previous population based and family studies (Jongeneel et al., 1991; Nedaspasov et al., 1991; Crouau-Roy et al., 1993) and their existence has been confirmed in homozygous cell lines (Weissensteiner and Lanchbury, 1997; Udalova et al., 1993). TNF haplotype analysis was performed using all 11 haplotypes described in European populations (Crouau-Roy et al., 1993; Weissensteiner and Lanchbury, 1997).

There were no significant differences in haplotype frequencies between patients and healthy controls (Table 6.29), or between any of the patient groups stratified according to disease severity classified according to the Atlanta classification and the occurrence of a local complication (Table 6.30).

Table 6.31 shows haplotype frequencies in patient groups stratified according to organ failure score. No significant differences were seen between controls and patient groups or between patient groups.

Table 6.32 shows TNFabc 3-locus haplotype frequency in normal controls and subjects grouped according to disease aetiology, and Table 6.33 also shows haplotype frequency in the alcoholic 'controls'. The TNF a2b1c2 haplotype shows a strong trend toward reduced frequency in the alcohol induced acute pancreatitis group compared to the two other aetiologies. This is not statistically significant after correction for multiple comparisons, however. Alcoholic pancreatitis group vs. non-alcoholic pancreatitis group (2.4 vs. 15.8%,χ^2 =5.39, p=0.02, p_c=0.22), alcoholic pancreatitis group vs. normal controls (2.4 vs. 13.1%, χ^2 =4.59,p=0.032, p_c=0.38).

When compared with the alcohol 'control' group however, the difference in frequency is even less marked, alcoholic pancreatitis group vs. alcoholic 'controls', (2.4 vs. 9.1%, Fisher's exact test, p=0.16).

The TNF a2b5c2 haplotype is present at higher frequency in the alcoholic 'control' group compared to all other groups. This is not statistically significant after correction for multiple comparisons, however. Alcoholic pancreatitis group vs. 'alcoholic controls' (0.0 vs. 13.6%, Fisher's exact test, $p=0.0095$, $p_c=0.105$), non-alcoholic pancreatitis group vs. alcoholic 'controls' (4.4 vs. 13.6%, Fisher's exact test, $p=0.012$, $p_c=0.132$), normal control group vs. 'alcoholic controls' (4.7 vs. 13.6%, Fisher's exact test, $p=0.016$, $p_c=0.176$).

TNFabc haplotype	Controls n=107 %	Study patients n=135 %
a2b1c2	**13.1** (28)	**13.7** (37)
a2b3c1	**15.4** (33)	**9.6** (26)
a2b5c2	**4.7** (10)	**3.7** (10)
a5b5c2	**1.4** (3)	**3.3** (9)
a1b5c2	**0.5** (1)	**0.7** (2)
a4b7c2	**1.4** (3)	**2.2** (6)
a6b5c1	**14.5** (31)	**17.4** (47)
a7b4c1	**6.5** (14)	**6.3** (17)
a11b4c1	**16.4** (35)	**18.1** (49)
a10b4c1	**8.4** (18)	**7.8** (21)
a4b5bc1	**6.1** (13)	**7.4** (20)
X	**11.7** (25)	**9.6** (26)
Total chromosomes (n)	214	270

Table 6.29 TNFabc haplotype frequency in control and patient groups

Table shows TNFabc 3-locus haplotype frequency in normal controls and patient subjects. Haplotype analysis using 11 putative haplotypes previously characterised in Caucasian populations and confirmed in homozygous cell lines.
For each haplotype the **% frequency** and the actual number of haplotypes (in brackets) is given.
n represents the number of subjects in each group
(n) represents the number of chromosomes in each group.
X denotes unable to determine haplotype
Comparisons were made for each haplotype between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made. No significant differences were seen.

TNFabc haplotype	Controls n=107 %	Mild n=97 %	Severe n=38 %	Local complication n=21 %
a2b1c2	**13.1**	**13.4**	**14.5**	**11.9**
	(28)	(26)	(11)	(5)
a2b3c1	**15.4**	**9.8**	**9.2**	**9.5**
	(33)	(19)	(7)	(4)
a2b5c2	**4.7**	**3.1**	**5.3**	**7.1**
	(10)	(6)	(4)	(3)
a5b5c2	**1.4**	**2.6**	**5.3**	**7.1**
	(3)	(5)	(4)	(3)
a1b5c2	**0.5**	**0.5**	**1.3**	**2.4**
	(1)	(1)	(1)	(1)
a4b7c2	**1.4**	**2.1**	**2.6**	**2.4**
	(3)	(4)	(2)	(1)
a6b5c1	**14.5**	**18.0**	**15.8**	**21.4**
	(31)	(35)	(12)	(9)
a7b4c1	**6.5**	**6.2**	**6.6**	**4.8**
	(14)	(12)	(5)	(2)
a11b4c1	**16.4**	**20.6**	**11.8**	**14.3**
	(35)	(40)	(9)	(6)
a10b4c1	**8.4**	**6.7**	**10.5**	**7.1**
	(18)	(13)	(8)	(3)
a4b5bc1	**6.1**	7.7	**6.6**	**7.1**
	(13)	(15)	(5)	(3)
X	**11.7**	**9.3**	**10.5**	**4.8**
	(25)	(18)	(8)	(2)
Total chromosomes (n)	214	194	76	42

Table 6.30 TNFabc haplotype frequency in control and acute pancreatitis patients grouped according to (local and systemic) disease severity

Table shows TNFabc 3-locus haplotype frequency in normal controls and patient subjects grouped according to disease severity.
Mild and severe disease severity groups are according to the Atlanta convention classification (Bradley, 1993), described in section 5.1.4.
Local complication is defined as occurrence of pancreatic necrosis, abscess or pseudocyst
Haplotype analysis using 11 putative haplotypes previously characterised in Caucasian populations and confirmed in homozygous cell lines.
For each haplotype the **% frequency** and the actual number of haplotypes (in brackets) is given.

n represents the number of subjects in each group
(n) represents the number of chromosomes in each group.
X denotes unable to determine haplotype

Comparisons were made for each genotype between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made. No significant differences were seen.

TNFabc haplotype	Controls n=107 %	OFS ≥ 2 n=35 %	OFS ≥ 3 n=18 %	OFS ≥ 4 n=12 %
a2b1c2	**13.1** (28)	**15.7** (11)	**19.4** (7)	**20.8** (5)
a2b3c1	**15.4** (33)	**10.0** (7)	**11.1** (4)	**8.3** (2)
a2b5c2	**4.7** (10)	**4.3** (3)	**5.6** (2)	**8.3** (2)
a5b5c2	**1.4** (3)	**7.1** (5)	**5.6** (2)	**8.3** (2)
a1b5c2	**0.5** (1)	**0.0** (0)	**0.0** (0)	**0.0** (0)
a4b7c2	**1.4** (3)	**1.4** (1)	**0.0** (0)	**0.0** (0)
a6b5c1	**14.5** (31)	**14.3** (10)	**16.7** (6)	**20.8** (5)
a7b4c1	**6.5** (14)	**5.7** (4)	**11.1** (4)	**4.2** (1)
a11b4c1	**16.4** (35)	**12.9** (9)	**5.6** (2)	**8.3** (2)
a10b4c1	**8.4** (18)	**10.0** (7)	**16.7** (6)	**12.5** (3)
a4b5bc1	**6.1** (13)	**7.1** (5)	**8.3** (3)	**8.3** (2)
X	**11.7** (25)	**11.4** (8)	**0.0** (0)	**0.0** (0)
Total chromosomes (n)	214	70	36	24

Table 6.31 TNFabc 3-locus haplotype frequency in control and acute pancreatitis patients grouped according to (systemic) disease severity as assessed by organ failure score

Table shows TNFabc 3-locus haplotype frequency in normal controls and subjects grouped according to maximal organ failure score for the disease episode.
Haplotype analysis using 11 putative haplotypes previously characterised in Caucasian populations and confirmed in homozygous cell lines.
For each haplotype the **% frequency** and the actual number of haplotypes (in brackets) is given.

n represents the number of subjects in each group
(n) represents the number of chromosomes in each group.
X denotes unable to determine haplotype
OFS Organ failure score

OFS calculated according to method described in section 5.1.4 (Kingsnorth et al, 1995).
Comparisons were made for each genotype between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made. No significant differences were seen.

TNFabc haplotype	Controls n=107 %	Alcohol n=21 %	Idiopathic n=37 %	Gallstones n=77 %
a2b1c2	**13.1** (28)	**2.4** [1] (1)	**20.3** (15)	**13.6** (21)
a2b3c1	**15.4** (33)	**11.9** (5)	**6.8** (5)	**10.4** (16)
a2b5c2	**4.7** (10)	**0.0** (0)	**0.0** (0)	**6.5** (10)
a5b5c2	**1.4** (3)	**2.4** (1)	**4.1** (3)	**3.2** (5)
a1b5c2	**0.5** (1)	**2.4** (1)	**1.4** (1)	**0.0** (0)
a4b7c2	**1.4** (3)	**0.0** (0)	**1.4** (1)	**3.2** (5)
a6b5c1	**14.5** (31)	**28.6** (12)	**16.2** (12)	**14.9** (23)
a7b4c1	**6.5** (14)	**7.1** (3)	**10.8** (8)	**3.9** (6)
a11b4c1	**16.4** (35)	**26.2** (11)	**14.9** (11)	**17.5** (27)
a10b4c1	**8.4** (18)	**7.1** (3)	**8.1** (6)	**7.8** (12)
a4b5bc1	**6.1** (13)	**0.0** (0)	**10.8** (8)	**7.8** (12)
X	**11.7** (25)	**11.9** (5)	**5.4** (4)	**11.0** (17)
Total chromosomes (n)	214	42	74	154

Table 6.32 TNFabc 3-locus haplotype frequency in control and acute pancreatitis patients grouped according to aetiology of acute pancreatitis

Table shows TNFabc 3-locus haplotype frequency in normal controls and subjects grouped according to disease aetiology.

Haplotype analysis using 11 putative haplotypes previously characterised in Caucasian populations and confirmed in homozygous cell lines.

For each haplotype the **% frequency** and actual number of haplotypes (brackets) is given.

n represents the number of subjects in each group

(n) represents the number of chromosomes in each group.

X denotes unable to determine haplotype

Alcoholic pancreatitis if daily consumption of ≥ 80g alcohol day^{-1}

Gallstones if radiological or ERCP evidence of cholelithiasis

Idiopathic if no identifiable aetiology

1. The TNF a2b1c2 haplotype shows a strong trend toward reduced frequency in the alcohol induced acute pancreatitis group compared to the two other aetiologies. This is not statistically significant after correction for multiple comparisons, however.
   Alcohol group vs. other aetiology, χ^2 =6.41, p=0.011, p_c=0.13
   Alcohol group vs. normal controls, χ^2 =4.59,p=0.032, p_c=0.38

TNFabc haplotype	Controls n=107 %	Alcohol 'controls' n=33 %	Alcohol n=21 %	Not alcohol n=114 %
a2b1c2	**13.1**	**9.1**	**2.4** [1]	**15.8**
	(28)	(6)	(1)	(36)
a2b3c1	**15.4**	**16.7**	**11.9**	**9.2**
	(33)	(1)	(5)	(21)
a2b5c2	**4.7**	**13.6** [2]	**0.0**	**4.4**
	(10)	(9)	(0)	(10)
a5b5c2	**1.4**	**4.5**	**2.4**	**3.5**
	(3)	(3)	(1)	(8)
a1b5c2	**0.5**	**0.0**	**2.4**	**0.4**
	(1)	(0)	(1)	(1)
a4b7c2	**1.4**	**1.5**	**0.0**	**2.6**
	(3)	(1)	(0)	(6)
a6b5c1	**14.5**	**12.1**	**28.6**	**15.4**
	(31)	(8)	(12)	(35)
a7b4c1	**6.5**	**7.6**	**7.1**	**6.1**
	(14)	(5)	(3)	(14)
a11b4c1	**16.4**	**13.6**	**26.2**	**16.7**
	(35)	(9)	(11)	(38)
a10b4c1	**8.4**	**9.1**	**7.1**	**7.9**
	(18)	(6)	(3)	(18)
a4b5bc1	**6.1**	**6.1**	**0.0**	**8.8**
	(13)	(4)	(0)	(20)
X	**11.7**	**6.1**	**11.9**	**9.2**
	(25)	(4)	(5)	(21)
Total chromosomes (n)	214	33	42	228

Table 6.33 TNFabc 3-locus haplotype frequency in normal controls, alcohol-induced and non alcohol-induced acute pancreatitis patients, and alcoholic 'controls'

Table shows TNFabc 3-locus haplotype frequency in normal controls and subjects groups. Haplotype analysis using 11 putative haplotypes previously characterised in Caucasian populations and confirmed in homozygous cell lines.

For each haplotype the **% frequency** and the actual number of haplotypes (in brackets) is given.

n represents the number of subjects in each group

(n) represents the number of chromosomes in each group.

X denotes unable to determine haplotype.

Alcoholic pancreatitis if daily consumption of ≥ 80g alcohol day^{-1}

Non-alcohol induced pancreatitis includes:

- Gallstones if radiological or ERCP evidence of cholelithiasis
- Idiopathic if no identifiable aetiology

Comparisons were made for each genotype between patient and control groups using 2x2 contingency tables and the χ^2 test, or Fisher's exact test if expected cell values were less than 5. P values were corrected for the number of comparisons made.

1. The TNF a2b1c2 haplotype shows a strong trend toward reduced frequency in the

alcohol induced acute pancreatitis group compared to the two other aetiologies. This is not statistically significant after correction for multiple comparisons, however.
Alcohol group vs. other non-alcoholic pancreatitis group, χ^2 =5.39, p=0.02, p_c=0.22.
Alcohol group vs. normal controls, χ^2 =4.59,p=0.032, p_c=0.38.
When compared with the alcohol 'control' group however, the difference in frequency is much less marked.
Alcohol group vs. 'alcoholic controls', Fisher's exact test, p=0.16.
2. The TNF a2b5c2 haplotype is present at higher frequency in the alcoholic 'control' group compared to all other groups. This is not statistically significant after correction for multiple comparisons, however.
Alcohol group vs. 'alcoholic controls', Fisher's exact test, p=0.0095, p_c=0.105.
Non-alcoholic pancreatitis group vs. 'alcoholic controls', Fisher's exact test, p=0.012, p_c=0.132.
Normal control group vs. 'alcoholic controls', Fisher's exact test, p=0.016, p_c=0.176.

6.5 TNF microsatellite 3-locus haplotypes (continued)

When haplotype analysis is performed according to the frequency of patients with at least one copy of a particular haplotype, a similar pattern is seen. Table 6.34 shows the frequency (number) of patients with at least one copy of putative TNFabc 3-locus haplotype in normal controls, alcoholic 'controls', study patients, patients grouped according to severity of disease as assessed by the Atlanta classification, severity of disease as assessed by organ failure score, occurrence of a local complication, and aetiology of acute pancreatitis.

Again, the TNF a2b1c2 haplotype shows a strong trend toward reduced frequency in the alcohol induced acute pancreatitis group compared to the two other aetiologies, and normal control groups. This is not statistically significant after correction for multiple comparisons, however.

Alcoholic pancreatitis group vs. other aetiology (4.8 vs. 29.8%, χ^2 =6.76, p=0.009, p_c=0.11), alcoholic pancreatitis group vs. normal controls (4.8 vs. 24.3%, χ^2 =4.03, p=0.045, p_c>0.5). When compared with the alcohol 'control' group however, the difference in frequency is much less marked. Alcoholic pancreatitis group vs. 'alcoholic controls' (4.8 vs 18.2%, Fisher's exact test, p=0.155). This is graphically represented in Fig. 6.1.

These differences in the a2 allele containing haplotype frequencies discussed above reflect the reduced carriage of the a2 allele in the alcoholic pancreatitis group.

No significant differences were seen between controls and patient groups or between patient groups for any other haplotype.

TNFabc haplotype	Controls n=107 %	Study patients n=135 %	Mild n=97 %	Severe n=38 %	Local complic[n] n=21 %	OFS ≥ 2 n=35 %	OFS ≥ 3 n=18 %	OFS ≥ 4 n=12 %	Alcohol n=21 %	Idiopath. n=37 %	G\stones n=77 %	Alcoholic controls n=33 %
a2b1c2	**24.3**	**25.9**	**24.7**	**28.9**	**23.8**	**31.4**	**38.9**	**41.7**	**4.8** [1]	**37.8**	**26.0**	**18.2**
	(26)	(35)	(24)	(11)	(5)	(11)	(7)	(5)	(1)	(14)	(20)	(6)
a2b3c1	**29.0**	**20.0**	**20.6**	**18.4**	**19.0**	**20.0**	**22.2**	**16.7**	**23.8**	**13.5**	**22.1**	**24.2**
	(31)	(27)	(20)	(7)	(4)	(7)	(4)	(2)	(5)	(5)	(17)	(8)
a2b5c2	**9.3**	**7.4**	**6.2**	**10.5**	**14.3**	**8.6**	**11.1**	**16.7**	**0.0**	**0.0**	**13.0**	**21.2**
	(10)	(10)	(6)	(4)	(3)	(3)	(2)	(2)	(0)	(0)	(10)	(7)
a5b5c2	**2.8**	**6.7**	**5.2**	**10.5**	**14.3**	**14.3**	**11.1**	**16.7**	**4.8**	**8.1**	**6.5**	**9.1**
	(3)	(9)	(5)	(4)	(3)	(5)	(2)	(2)	(1)	(3)	(5)	(3)
a1b5c2	**0.9**	**1.5**	**1.0**	**2.6**	**4.8**	**0.0**	**0.0**	**0.0**	**4.8**	**2.7**	**0.0**	**0.0**
	(1)	(2)	(1)	(1)	(1)	(0)	(0)	()0	(1)	(1)	(0)	(0)
a4b7c2	**1.9**	**4.4**	**4.1**	**5.3**	**4.8**	**2.9**	**0.0**	**0.0**	**0.0**	**2.7**	**6.5**	**3.0**
	(2)	(6)	(4)	(2)	(1)	(1)	(0)	(0)	(0)	(1)	(5)	(1)
a6b5c1	**27.1**	**32.6**	**34.0**	**28.9**	**38.1**	**28.6**	**33.3**	**41.7**	**52.4**	**32.4**	**27.3**	**24.2**
	(29)	(44)	(33)	(11)	(8)	(10)	(6)	(5)	(11)	(12)	(21)	(8)
a7b4c1	**13.1**	**11.1**	**11.3**	**10.5**	**9.5**	**8.6**	**16.7**	**8.3**	**14.3**	**18.9**	**6.5**	**15.2**
	(14)	(15)	(11)	(4)	(2)	(3)	(3)	(1)	(3)	(7)	(5)	(5)
a11b4c1	**31.8**	**32.6**	**36.1**	**23.7**	**28.6**	**22.9**	**11.1**	**16.7**	**47.6**	**27.0**	**31.2**	**24.2**
	(34)	(44)	(35)	(9)	(6)	(8)	(2)	(2)	(10)	(10)	(24)	(8)
a10b4c1	**15.9**	**15.6**	**13.4**	**21.1**	**14.3**	**20.0**	**33.3**	**25.0**	**14.3**	**16.2**	**15.6**	**18.2**
	(17)	(21)	(13)	(8)	(3)	(7)	(6)	(3)	(3)	(6)	(12)	(6)
a4b5bc1	**11.2**	**14.8**	**15.5**	**13.2**	**14.3**	**14.3**	**16.7**	**16.7**	**4.8**	**21.6**	**14.3**	**12.1**
	(12)	(20)	(15)	(5)	(3)	(5)	(3)	(2)	(1)	(8)	(11)	(4)
X	**20.6**	**19.3**	**18.6**	**21.1**	**9.5**	**22.9**	**0.0**	**0.0**	**23.8**	**10.8**	**22.1**	**12.1**
	(22)	(26)	(18)	(8)	(2)	(8)	(0)	(0)	(5)	(4)	(17)	(4)

Table 6.34 **Frequency (number) of patients with at least one copy of putative TNFabc 3-locus haplotype** (footnotes continued....)

Table 6.34 Frequency (number) of patients with at least one copy of putative TNFabc 3-locus haplotype

Shows the frequency of possession of at least one copy of specific (putative) TNFabc 3-locus haplotype in

- normal controls
- study patients
- patients grouped according to severity of disease as assessed by the Atlanta classification
- severity of disease as assessed by organ failure score
- occurrence of a local complication
- aetiology of acute pancreatitis.

Local complic[n] Local complication
G/stones Gallstones
OFS Organ failure score, calculated according to method described in section 5.1.4 (Kingsnorth et al, 1995).
Mild and severe disease severity groups are according to the Atlanta convention classification (Bradley, 1993), described in section 5.1.4.
Local complication is defined as occurrence of pancreatic necrosis, abscess or pseudocyst
n represents the number of subjects in each group
X denotes unable to determine haplotype
Alcoholic pancreatitis if daily consumption of ≥ 80g alcohol day^{-1}
Gallstones if radiological or ERCP evidence of cholelithiasis
Idiopathic if no identifiable aetiology
Haplotype analysis using 11 putative haplotypes previously characterised in Caucasian populations and confirmed in homozygous cell lines.
For each haplotype the **% frequency** and the actual number of haplotypes (in brackets) is given.

1. Again, the TNF a2b1c2 haplotype shows a strong trend toward reduced frequency in the alcohol induced acute pancreatitis group compared to the two other aetiologies, and both control groups. This is not statistically significant after correction for multiple comparisons, however.

Alcoholic pancreatitis group vs. other aetiology, $\chi^2 = 6.76$, $p=0.009$, $p_c=0.11$
Alcoholic pancreatitis group vs. normal controls, $\chi^2 = 4.03$, $p=0.045$, $p_c>0.5$
When compared with the alcohol 'control' group however, the difference in frequency is much less marked.
Alcoholic pancreatitis group vs. 'alcoholic controls', Fisher's exact test, $p=0.155$

This is graphically represented in Fig. 6.4.

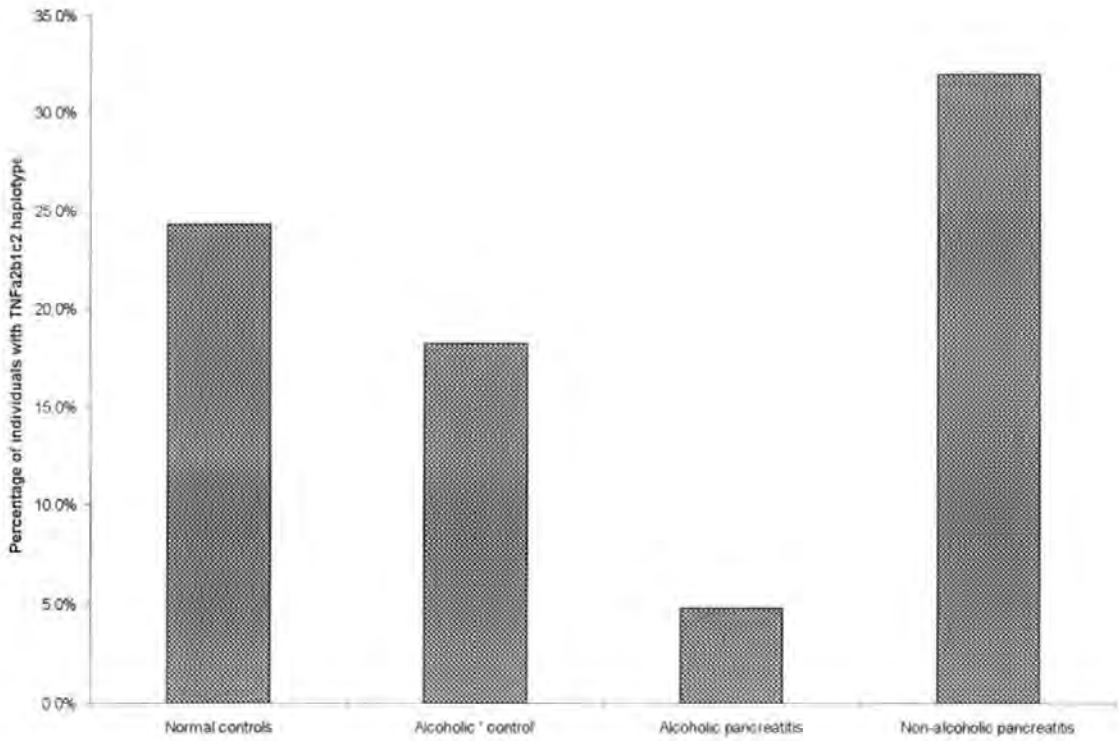

Fig 6.4 Frequency (%) of individuals with the 3-locus TNFa2b1c2 haplotype

The TNF a2b1c2 haplotype shows a strong trend toward reduced frequency in the alcohol induced acute pancreatitis group compared to the two other aetiologies, and normal control groups. This is not statistically significant after correction for multiple comparisons, however.

Alcoholic pancreatitis group vs. other aetiology, χ^2 =6.76, p=0.009, p_c=0.11

Alcoholic pancreatitis group vs. normal controls, χ^2 =4.03, p=0.045, p_c>0.5

When compared with the alcohol 'control' group however, the difference in frequency is much less marked.

Alcoholic pancreatitis group vs. 'alcoholic controls', Fisher's exact test, p=0.155

6.6 TNF microsatellite 3-locus genotypes

Haplotypes, like individual alleles, combine in individuals to form haplotype 'genotypes. Table 6.35 shows TNFabc 3-locus genotypes in normal control and patient groups. Due to the number of possible different combinations, analysis of 3-locus genotypes is limited because of the small numbers of each combination present. No differences in 3-locus genotype numbers are seen between patient and normal control groups. Data showing 3-locus genotypes for all the different patient subgroups is displayed in appendix 1.

TNFabc haplotype combinations	Controls n=107 n	Study patients n=135 n
a2b1c2- a2b1c2	2	2
a2b1c2- a11b4c1	4	3
a2b1c2- a6b5c1	4	7
a2b1c2- a10b4c1	4	3
a2b1c2- a2b3c1	5	3
a2b1c2- a7b4c1	1	4
a2b1c2- a2b5c2	2	3
a2b1c2- a5b5c2	0	1
a2b1c2- a1b5c2	1	0
a2b1c2- a4b5c1	2	1
a2b1c2- a4b7c2	0	2
a2b1c2- X	1	6
a2b3c1- a2b3c1	2	0
a2b3c1- a7b4c1	5	0
a2b3c1- a2b5b2	1	1
a2b3c1- a5b5c2	1	1
a2b3c1- a4b5c1	2	3
a2b3c1- a4b7c2	1	0
a2b3c1- X	3	3
a2b5b2 - a4b5c1	1	2
a2b5b2- X	1	0
a5b5c2-a4b5c1	0	2
a5b5c2- X	0	1
a6b5c1- a6b5c1	2	3
a6b5c1- a10b4c1	2	8
a6b5c1- -a2b3c1	7	6
a6b5c1- a7b4c1	2	0
a6b5c1- a2b5c2	0	1
a6b5c1- a5b5c2	0	2
a6b5c1- a4b5c1	1	4
a6b5c1- X	2	3
a7b4c1- a7b4c1	0	2
a7b4c1- a2b5c2	1	1

Table 6.35 **Number of TNFabc 3-locus haplotype combinations in control and acute pancreatitis patient groups** (continued...)

TNFabc haplotype combinations	Controls n=107 n	Study patients n=135 n
a7b4c1- a1b5c2	0	1
a7b4c1-a1b5c2	0	1
a7b4c1 a4b5c1	1	0
a7b4c1-a4b7c2	0	1
a7b4c1- X	3	2
a11b4c1-a11b4c1	1	5
a11b4c1-a6b5c1	10	10
a11b4c1-a10b4c1	4	3
a11b4c1-a2b3c1	3	5
a11b4c1-a7b4c1	1	4
a11b4c1-a2b5c2	3	2
a11b4c1-a5b5c2	1	2
a11b4c1-a1b5c2	0	1
a11b4c1-a4b5c1	3	1
a11b4c1-a4b7c2	2	1
a11b4c1-X	2	7
a10b4c1-a10b4c1	1	0
a10b4c1-a2b3c1	2	4
a10b4c1-a2b5c2	1	0
a10b4c1-a5b5c2	1	0
a10b4c1-a4b5c1	0	0
a10b4c1-X	2	3
a4b5c1-a4b5c1	1	1
a4b5c1-a4b7c2	0	1
a4b5c1-X	2	3
X-X	3	0

Table 6.35 Number of TNFabc 3-locus haplotype combinations in control and acute pancreatitis patient groups

Table displays actual number of TNFabc microsatellite 3-locus haplotype combinations in control and patient groups.
Haplotype analysis using 11 putative haplotypes previously characterised in Caucasian populations and confirmed in homozygous cell lines.
As can be seen, the numbers are too small for meaningful comparison, this is especially so between the patient groups. The data relating to haplotype combination frequency in the patient groups is therefore shown in appendix 1.
X unable to determine haplotype.

6.7 Putative secretor status of TNFabc 3-locus microsatellite haplotypes

Data concerning genetic variability in the TNF region affecting TNFα secretion levels by *in vitro* LPS stimulated monocytes has been extensively reviewed, re-evaluated and investigated by Weissensteiner and Lanchbury (1997). They have proposed associations between frequent Caucasian extended haplotypes involving the TNFabc microsatellite system and TNFα response. Combined experimental data was used to infer putative secretor status of extended haplotypes involving the TNFabc microsatellites from associations of MHC markers in cell line and population studies.

Table 6.36 details haplotypes determined by these authors as having a high, intermediate, or low TNFα secretor status. TNFα responses refer to experimental data in LPS stimulated monocytes.

TNFα response phenotype in LPS stimulated monocytes	TNFabc 3-locus haplotype
High	a2b1c2
	a2b3c1
	a2b5c2
	a5b5c2
	a1b5c2
	a4b7c2
Intermediate	a5b5c1
	a7b4c1
Low	a11b4c1
	a10b4c1
	a4b5c1

Table 6.36 Proposed associations between TNFabc 3-locus haplotypes and TNFα secretor response

Details proposed associations between TNFabc 3-locus haplotypes and TNFα secretor response in LPS stimulated monocytes.
Weissensteiner and Lanchbury (1997) have proposed associations between frequent Caucasian extended haplotypes involving the TNFabc microsatellite system and TNFα response. Combined experimental data was used to infer putative secretor status of extended haplotypes involving the TNFabc microsatellites from associations of MHC markers in cell line and population studies.

6.7 Putative secretor status of TNFabc 3-locus microsatellite haplotypes (continued)

The proposed associations detailed in Table 6.36 have been used to analyse the populations of controls and acute pancreatitis patients. Table 6.37 details frequencies of haplotype groups proposed as high, low, or intermediate putative secretor status in control and patient subjects and patients groups according to disease severity according to the Atlanta classification and organ failure scores. No significant differences between patient and control groups are seen with regard to frequency of high, intermediate, or low secretor status. This type of analysis has it's limitations, as it does not consider what an individual's other haplotype is, or what effect it may have upon TNFα secretor phenotype.

Table 6.38 shows frequencies of combinations of haplotype groups proposed as high, low, or intermediate putative secretor status in control and patient subjects and patients groups according to disease severity according to the Atlanta classification and organ failure scores. No significant differences between patient and control groups are seen with regard to frequency of high, intermediate, or low secretor status combinations. This type of analysis also has limitations, as no data exists as to whether there are dominant functional haplotypes, and what they may be.

Table 6.39 shows frequencies of haplotype groups proposed as high, low, or intermediate putative secretor status in normal control, alcoholic 'control', and patient according to aetiology. There was a trend towards reduced frequency of the high secretor phenotype frequency in the alcoholic pancreatitis group. This only achieved conventional statistical significance when comparison was made with the alcoholic 'control' group. Alcoholic pancreatitis vs. normal control (19 vs. 35.5%, χ^2 =4.32, p=0,038, p_c=0.15), alcoholic pancreatitis vs. other aetiology (19 vs. 36.0%, χ^2 =5.32, p=0,017, p_c=0.068), alcoholic pancreatitis vs. alcoholic 'controls' (19 vs. 45.5%, χ^2=7.85, p=0.0051, p_c=0.02). This mirrors the negative association of the a2 allele with alcoholic pancreatitis, as the a2 allele is classified as having high secretor status.

As mentioned above, this type of analysis has it's limitations, as it does not consider what an individual's other haplotype is, or what effect it may have upon TNFα secretor phenotype. Table 6.38 shows frequencies of combinations of haplotype groups proposed as high, low, or intermediate putative secretor status in normal control, alcoholic 'control', and patient according to aetiology.

There was a trend toward reduction in frequency of the High-Low secretor status combination in alcoholic pancreatitis patients. It does not achieve statistical significance. Alcoholic pancreatitis vs. normal controls (4.8 vs. 23.4%, Fishers exact test, $p=0.04$, $p_c=0.44$), alcoholic pancreatitis vs. other aetiology (4.8 vs. 25.4%, Fishers exact test, $p=0.026$, $p_c=0.25$), alcoholic pancreatitis vs. alcoholic 'controls' (4.8 vs. 27.3%, Fishers exact test, $p=0.038$, $p_c=0.38$).

As mentioned above this type of analysis has it's limitations as no data exists as to whether there are dominant functional haplotypes, and what they may be.

Putative **haplotype secretor status**	**Controls** n=107 %	**Study patients** n=135 %	**Mild** n=97 %	**Severe** n=38 %	**Local complication** n=21 %	**OFS ≥ 2** n=35 %	**OFS ≥ 3** n=18 %	**OFS ≥ 4** n=12 %
High (H)	**35.5** (76)	**33.3** (90)	**31.4** (61)	**38.2** (29)	**38.1** (16)	**37.1** (26)	**41.7** (15)	**45.8** (11)
Intermediate (I)	**22.0** (47)	**23.3** (63)	**24.2** (47)	**21.1** (16)	**26.2** (11)	**20.0** (14)	**27.8** (10)	**25.0** (6)
Low (L)	**31.8** (68)	**32.6** (88)	**33.5** (65)	**30.3** (23)	**31.0** (13)	**30.0** (21)	**30.6** (11)	**29.2** (7)
Undetermined (X)	**10.7** (23)	**10.7** (29)	**10.8** (21)	**10.5** (8)	**4.8** (2)	**12.9** (9)	**0.0** (0)	**0.0** (0)
Total number of chromosomes	214	270	194	76	42	70	36	24

Table 6.37 Frequency of putative haplotype secretor status in controls and acute pancreatitis patients grouped according to disease severity

Table shows putative haplotype secretor status frequency in controls and patients, and patients grouped according to disease severity as:

- Mild or Severe (Atlanta classification)
- Occurrence of a local complication (pancreatic necrosis, abscess or pseudocyst)
- Organ failure scores (OFS), calculated according to method outlined in section 5.1.4 (Kingsnorth et al, 1995).

Haplotype analysis using 11 putative haplotypes previously characterised in Caucasian populations and confirmed in homozygous cell lines. Haplotypes have been determined as having high, low, or intermediate (TNFα) secretor phenotypes in response to LPS stimulation (Weissensteiner and Lanchbury 1997). An undetermined secretor status is due to being unable to assign a putative haplotype.

For each haplotype the **% frequency** and the actual number of haplotypes in each specific secretor group (in brackets) is given.

Comparisons were made for each secretor phenotype between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made. No significant differences between patient and control groups are seen.

Putative haplotype secretor status combination	Controls n=107 %	Study patients n=135 %	Mild n=97 %	Severe n=38 %	Local complication n=21 %	OFS ≥ 2 n=35 %	OFS ≥ 3 n=18 %	OFS ≥ 4 n=12 %
H-H	**13.1**	**9.6**	**9.3**	**10.5**	**4.8**	**8.6**	**16.7**	**25.0**
	(14)	(13)	(9)	(4)	(1)	(3)	(3)	(3)
H-I	**16.8**	**17.8**	**17.5**	**18.4**	**28.6**	**22.9**	**27.8**	**33.3**
	(18)	(24)	(17)	(7)	(6)	(8)	(5)	(4)
H-L	**23.4**	**22.2**	**20.6**	**26.3**	**38.1**	**20.0**	**22.2**	**8.3**
	(25)	(13)	(20)	(10)	(8)	(7)	(4)	(1)
H-X	**4.7**	**7.4**	**6.2**	**10.5**	**0.0**	**14.3**	**0.0**	**0.0**
	(5)	(10)	(6)	(4)	(0)	(5)	(0)	(0)
I-I	**3.7**	**3.7**	**3.1**	**5.3**	**4.8**	**2.9**	**5.6**	**0.0**
	(4)	(5)	(3)	(2)	(1)	(1)	(1)	(0)
I-L	**15.0**	**17.8**	**19.6**	**13.2**	**14.3**	**11.4**	**16.7**	**16.7**
	(16)	(24)	(19)	(5)	(3)	(4)	(3)	(2)
I-X	**4.7**	**3.7**	**5.2**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
	(5)	(5)	(5)	(0)	(0)	(0)	(0)	(0)
L-L	**9.3**	**7.4**	**8.2**	**5.3**	**0.0**	**8.6**	**11.1**	**16.7**
	(10)	(10)	(8)	(2)	(0)	(3)	(1)	(2)
L-X	**6.5**	**10.4**	**10.3**	**10.5**	**9.5**	**11.4**	**0.0**	**0.0**
	(7)	(14)	(10)	(4)	(2)	(4)	(0)	(0)
X-X	**2.8**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
	(3)	(0)	(0)	(0)	(0)	(0)	(0)	(0)

Table 6.38 Frequency of putative haplotype secretor status combination in controls and acute pancreatitis patients grouped according to disease severity

Table shows putative haplotype secretor status combination frequency in controls and patients, and patients grouped according to disease severity as:

- Mild or Severe (Atlanta classification)
- Occurrence of a local complication (pancreatic necrosis, abscess or pseudocyst)
- Organ failure scores **(OFS),** calculated according to method outlined in section 5.1.4, (Kingsnorth et al, 1995).

Haplotype analysis using 11 putative haplotypes previously characterised in Caucasian populations and confirmed in homozygous cell lines. Haplotypes have been determined as having high, low, or intermediate (TNFα) secretor status in response to LPS stimulation (Weissensteiner and Lanchbury 1997).
For each 'secretor status' haplotype combination the **% frequency** and the actual number in each specific secretor group (in brackets) is given.
An undetermined secretor status is due to being unable to assign a putative haplotype.
H- High TNFα secretor status
L- Low TNFα secretor status
I- Intermediate TNFα secretor status
X-Undetermined TNFα secretor status
Comparisons were made for each secretor group between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made. No significant differences between patient and control groups are seen.

Putative haplotype secretor status	Controls n=107 %	Study patients n=135 %	Alcohol n=21 %	Idiopathic n=37 %	Gallstones n=77 %	Alcoholic 'controls' n=33 %
High (H)	**35.5** (76)	**33.3** (90)	**19.0** [1] (8)	**33.8** (25)	**37.0** (57)	**45.5** (30)
Intermediate (I)	**22.0** (47)	**23.3** (63)	**33.3** (14)	**27.0** (20)	**18.8** (29)	**19.7** (13)
Low (L)	**31.8** (68)	**32.6** (88)	**33.3** (14)	**32.4** (24)	**32.5** (50)	**28.8** (19)
Undetermined (X)	**10.7** (23)	**10.7** (29)	**14.3** (6)	**6.8** (5)	**11.7** (18)	**6.1** (4)
Total number of chromosomes	214	270	42	74	154	66

Table 6.39 Frequency of putative haplotype secretor status in controls and patients grouped according to disease aetiology of acute pancreatitis

Table shows putative haplotype secretor status frequency in controls, alcoholic 'controls', and patients, and patients grouped disease aetiology
Alcoholic pancreatitis if daily consumption of ≥ 80g alcohol day^{-1}
Gallstones if radiological or ERCP evidence of cholelithiasis
Idiopathic if no identifiable aetiology

Haplotype analysis using 11 putative haplotypes previously characterised in Caucasian populations and confirmed in homozygous cell lines. Haplotypes have been determined as having high, low, or intermediate (TNFα) secretor phenotypes in response to LPS stimulation (Weissensteiner and Lanchbury 1997).
For each group the **% frequency** and the actual number of haplotypes in each specific secretor group (in brackets) is given.
An undetermined secretor status is due to being unable to assign a putative haplotype.

Comparisons were made for each secretor group between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.

1. There was a trend towards reduced frequency of the high secretor status frequency in the alcoholic pancreatitis group. This only achieved statistical significance when comparison was made with the alcoholic 'control' group.
   Alcoholic pancreatitis vs. normal control, χ^2 =4.32, p=0,038, p_c=0.15
   Alcoholic pancreatitis vs. other aetiology, χ^2 =5.32, p=0,017, p_c=0.068
   Alcoholic pancreatitis vs. alcoholic 'controls', χ^2=7.85, p=0.0051, p_c=0.02

Putative haplotype secretor status combination	Controls n=107 %	Study patients n=135 %	Alcohol n=21 %	Idiopathic n=37 %	Gallstones n=77 %	Alcoholic 'controls' n=33 %
H-H	**13.1** (14)	**9.6** (13)	**4.8** (1)	**5.4** (2)	**13.0** (10)	**21.2** (7)
H-I	**16.8** (18)	**17.8** (24)	**19.0** (4)	**27.0** (10)	**13.0** (10)	**15.2** (5)
H-L	**23.4** (25)	**22.2** (13)	**4.8** [1] (1)	**27.0** (10)	**24.7** (19)	**27.3** (9)
H-X	**4.7** (5)	**7.4** (10)	**4.8** (1)	**2.7** (1)	**10.4** (8)	**6.1** (2)
I-I	**3.7** (4)	**3.7** (5)	**4.8** (1)	**2.7** (1)	**3.9** (3)	**0.0** (0)
I-L	**15.0** (16)	**17.8** (24)	**33.3** (7)	**16.2** (6)	**14.3** (11)	**18.2** (6)
I-X	**4.7** (5)	**3.7** (5)	**4.8** (1)	**5.4** (2)	**2.6** (2)	**6.1** (2)
L-L	**9.3** (10)	**7.4** (10)	**4.8** (1)	**8.1** (3)	**7.8** (6)	**6.1** (2)
L-X	**6.5** (7)	**10.4** (14)	**19.0** (4)	**5.4** (2)	**10.4** (8)	**0.0** (0)
X-X	**2.8** (3)	**0.0** (0)	**0.0** (0)	**0.0** (0)	**0.0** (0)	**0.0** (0)

Table 6.40 **Frequency of putative haplotype secretor status combination in controls and acute pancreatitis patients grouped according to disease severity**

(Footnotes next page...)

Table 6.40 Frequency of putative haplotype secretor status combination in controls and acute pancreatitis patients grouped according to disease severity

Table shows putative haplotype secretor status combination frequency in controls and patients, and patients grouped disease aetiology
Alcoholic pancreatitis if daily consumption of ≥ 80g alcohol day^{-1}
Gallstones if radiological or ERCP evidence of cholelithiasis
Idiopathic if no identifiable aetiology

Haplotype analysis using 11 putative haplotypes previously characterised in Caucasian populations and confirmed in homozygous cell lines. Haplotypes have been determined as having high, low, or intermediate (TNFα) secretor status in response to LPS stimulation (Weissensteiner and Lanchbury 1997).
For each 'secretor status' haplotype combination the **% frequency** and the actual number in each specific secretor group (in brackets) is given.
An undetermined secretor status is due to being unable to assign a putative haplotype.
H- High TNFα secretor status
L- Low TNFα secretor status
I- Intermediate TNFα secretor status
X-Undetermined TNFα secretor status
1. Trend toward reduction in frequency of H-L combination in alcoholic pancreatitis patients. Does not achieve statistical significance
   Alcohol group vs. normal controls, Fishers exact test, $p=0.04$, $p_c=0.44$
   Alcohol group vs. other aetiology, Fishers exact test, $p=0.026$, $p_c=0.25$
   Alcoholic pancreatitis vs. alcoholic 'controls', Fishers exact test, $p=0.038$, $p_c=0.38$

6.8 TNF –308 locus typing

Allelic and genotype frequencies at the TNF –308 locus within the TNFα promoter were measured in 99 controls and 131 (97.0%) of patients. A PCR-SSP assay is shown in Figure 6.5.

Table 6.41 shows allelic frequencies of the –308 locus in patient and normal control subjects. No significant differences between patient and control groups are seen.

Table 6.42 shows genotype frequencies of the –308 locus in patient and normal control subjects. No significant differences between patient and control groups are seen.

The expected frequencies for TNF –308 genotypes were calculated. A 2 x n analysis showed that frequencies of heterozygotes and homozygotes in both study and normal control populations were in Hardy Weinberg equlilibrium. This is shown in Table 6.43.

Tables 6.44 and 6.45 show allelic and genotype frequencies at the -308 locus in patient groups stratified according to disease severity classified according to the Atlanta classification and the occurrence of a local complication. Tables 6.46 and 6.47 show allelic and genotype frequencies at the -308 locus in patient groups stratified according to organ failure score.

There are no significant differences in the allelic or genotype frequencies of the TNF –308 locus between the above patient groups.

Tables 6.48 and 6.49 show allelic and genotype frequencies at the -308 locus in patient groups according to the aetiology of their disease. Tables 6.49 and 6.51 also show allelic and genotype frequencies at the -308 locus in patient groups according to the aetiology of their disease and alcoholic 'controls'. Again, there are no significant differences in the allelic or genotype frequencies of the TNF –308 locus between the groups.

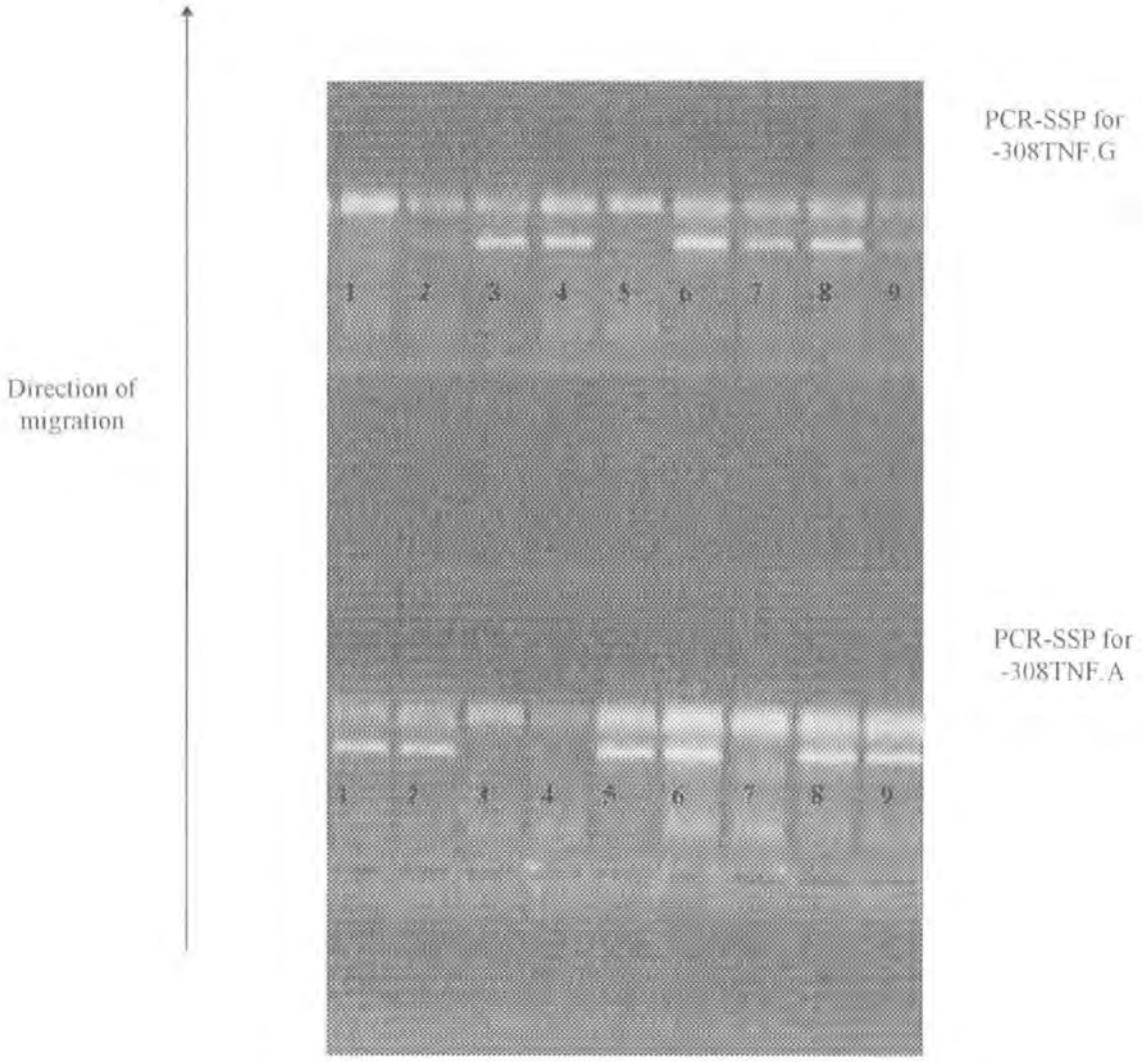

Figure 6.5 PCR-SSP typing of the TNF-308 polymorphism

Amplification of an 836 base pair (bp) fragment of the TNF promoter region was performed in two PCR-SSP reactions.
Primers for a 256 bp control amplicon from exon 15 of the adenomatous polyposis coli gene was included in the same reaction. Gels were electrophoresed for 20 minutes at 200V. The gels were then photographed under UV light (320nm) and scored for the presence or absence of an allele specific band providing a PCR control band was present.
Lanes 1-9 show 9 samples each typed for both the -308 TNF.G and the -308TNF.A allele.
Lane 1: TNF.AA
Lane 2: TNF.GA
Lane 3: TNF.GG
Lane 4: TNF.GG
Lane 5: TNF.AA
Lane 6: TNF.GA
Lane 7: TNF.GG
Lane 8: TNF.GA
Lane 9: TNF.GA

TNF –308 allele	Controls n=99 %	Study patients n=131 %
G	**78.3** (155)	**84.4** (221)
A	**21.7** (43)	**15.6** (41)
Total alleles (n)	198	262

Table 6.41 TNF –308 allele frequencies in normal controls and acute pancreatitis patients

This table displays TNF –308 allele frequencies in control and patient subjects. For each allele the **% frequency** and the actual number of alleles (in brackets) is given.
n represents the number of subjects in each group
(n) represents the number of alleles in each group.
Comparisons were made for each allele between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.
No significant differences between patient and control groups are seen.

TNF –308 genotype	Controls n=99 %	Study patients n=131 %
GG	**63.6** (63)	**71.0** (93)
GA	**29.3** (29)	**26.7** (35)
AA	**7.1** (7)	**2.3** (3)
Total	99	131

Table 6.42 TNF –308 genotype frequencies in normal controls and acute pancreatitis patients

This table displays TNF –308 genotype frequencies in control and patient subjects. For each genotype the **% frequency** and their actual number (in brackets) is given.
n represents the number of subjects in each group
Comparisons were made for each genotype between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made. No significant differences between patient and control groups are seen.

-308 Genotype	Expected		Observed		χ^2
	n	Freq.	n	Freq.	
Controls					
GG	60.7	61.3	63	63.6	0.1
GA	33.6	34.0	29	29.3	0.6
AA	4.7	4.7	7	7.1	n/a [1]
Patients					
GG	93.3	71.2	93	73.3	0.0
GA	34.5	26.3	35	26.7	0.0
AA	3.2	2.4	3	2.3	n/a [1]

Table 6.43 Frequency (%) of TNF –308 genotypes in patient and control subjects

Showing expected and observed frequencies of TNF –308 genotypes in patient and normal control subjects. Demonstrates that distribution conforms to Hardy-Weinberg equilibrium.
1. Note that as expected value is less than 5, χ^2 test is not valid.

TNF –308 allele	Controls n=99 %	Mild n=94 %	Severe n=37 %	Local complication n=21 %
G	**78.3** (155)	**84.6** (159)	**83.8** (62)	**83.3** (35)
A	**21.7** (43)	**15.4** (29)	**16.2** (12)	**16.7** (7)
Total alleles (n)	198	188	74	42

Table 6.44 TNF –308 allele frequencies in normal controls and acute pancreatitis patients grouped according to (local and systemic) disease severity

This table displays TNF –308 allele frequencies in control and patient sub-groups. For each allele the **% frequency** and the actual number of alleles (in brackets) is given.

n represents the number of subjects in each group

(n) represents the number of alleles in each group.

Mild and severe disease severity groups are according to the Atlanta convention classification (Bradley, 1993), described in section 5.1.4.

Local complication is defined as occurrence of pancreatic necrosis, abscess or pseudocyst.

Comparisons were made for each allele between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.

No significant differences between patient and control groups are seen.

TNF –308 genotype	Controls n=99 %	Mild n=94 %	Severe n=37 %	Local complication n=21 %
GG	**63.6** (63)	**71.3** (67)	**70.3** (26)	**71.4** (15)
GA	**29.3** (29)	**26.6** (25)	**27.0** (10)	**23.8** (5)
AA	**7.1** (7)	**2.1** (2)	**2.7** (1)	**4.8** (1)
Total	99	37	74	21

Table 6.45 TNF –308 genotype frequencies in normal controls and acute pancreatitis patients grouped according to (local and systemic) disease severity

This table displays TNF –308 genotype frequencies in control and patient sub-groups. For each genotype the **% frequency** and their actual number (in brackets) is given.
n represents the number of subjects in each group
Mild and severe disease severity groups are according to the Atlanta convention classification (Bradley, 1993), described in section 5.1.4.
Local complication is defined as occurrence of pancreatic necrosis, abscess or pseudocyst.
Comparisons were made between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.
No significant differences between patient and control groups are seen.

TNF –308 allele	Controls n=99 %	OFS ≥ 2 n=33 %	OFS ≥ 3 n=18 %	OFS ≥ 4 n=12 %
G	**78.3** (155)	**84.8** (56)	**86.1** (31)	**83.3** (20)
A	**21.7** (43)	**15.2** (10)	**13.9** (5)	**16.7** (4)
Total alleles (n)	198	66	36	24

Table 6.46 TNF –308 allele frequencies in normal controls and acute pancreatitis patients grouped according to (systemic) disease severity as assessed by organ failure score

This table displays TNF –308 allele frequencies in control and patient group subjects. For each allele the **% frequency** and the actual number of alleles (in brackets) is given.

n represents the number of subjects in each group

(n) represents the number of alleles in each group.

OFS Organ failure score

OFS calculated according to method described in section 5.1.4 (Kingsnorth et al, 1995).

Comparisons were made for each allele between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.

No differences are seen in the frequency of individual alleles between patient groups or between patient groups and controls.

TNF –308 genotype	Controls n=99 %	OFS ≥ 2 n=33 %	OFS ≥ 3 n=18 %	OFS ≥ 4 n=12 %
GG	**63.6** (63)	**72.7** (24)	**77.8** (14)	**75.0** (9)
GA	**29.3** (29)	**24.2** (8)	**16.7** (3)	**16.7** (2)
AA	**7.1** (7)	**3.0** (1)	**5.6** (1)	**8.3** (1)
Total	99	33	18	12

Table 6.47 TNF –308 genotype frequencies in normal controls and acute pancreatitis patients grouped according to (systemic) disease severity as assessed by organ failure score

This table displays TNF-308 genotype frequencies in control and patient group subjects. For each genotype the **% frequency** and their actual number (in brackets) is given.
n represents the number of subjects in each group
OFS Organ failure score
OFS calculated according to method described in section 5.1.4 (Kingsnorth et al, 1995).
Comparisons were made between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.
No differences are seen in the gene frequency between patient groups or between patient groups and controls.

TNF –308 allele	Controls n=99 %	Alcohol n=21 %	Idiopathic n=36 %	Gallstones n=74 %
G	**78.3** (155)	**88.1** (37)	**88.9** (64)	**81.1** (120)
A	**21.7** (43)	**11.9** (5)	**11.1** (8)	**18.9** (28)
Total alleles (n)	198	42	72	148

Table 6.48 TNF –308 allele frequencies in normal controls and acute pancreatitis patients grouped according to aetiology of acute pancreatitis

This table displays TNF -308 allele frequencies in control and patient group subjects. For each allele the **% frequency** and the actual number of alleles (in brackets) is given.

n represents the number of subjects in each group

(n) represents the number of alleles in each group.

Alcoholic pancreatitis if daily consumption of ≥ 80g alcohol day^{-1}

Gallstones if radiological or ERCP evidence of cholelithiasis

Idiopathic if no identifiable aetiology

Comparisons were made for each allele between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.

No differences are seen in the frequency of individual alleles between patient groups or between patient groups and controls.

TNF –308 genotype	Controls n=99 %	Alcohol n=21 %	Idiopathic n=36 %	Gallstones n=74 %
GG	**63.6** (63)	**76.2** (16)	**77.8** (28)	**66.2** (49)
GA	**29.3** (29)	**23.8** (5)	**22.2** (8)	**29.7** (22)
AA	**7.1** (7)	**0.0** (0)	**0.0** (0)	**4.1** (3)
Total	99	21	36	74

Table 6.49 TNF –308 genotype frequencies in normal controls and acute pancreatitis patients grouped according to aetiology of acute pancreatitis

This table displays TNF -308 genotype frequencies in control and patient group subjects.
For each genotype the **% frequency** and their actual number (in brackets) is given.
n represents the number of subjects in each group
Alcoholic pancreatitis if daily consumption of ≥ 80g alcohol day^{-1}
Gallstones if radiological or ERCP evidence of cholelithiasis
Idiopathic if no identifiable aetiology
Comparisons were made between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.
No differences are seen in gene frequency between patient groups or between patient groups and controls.

TNF –308 allele	Controls n=99 %	Alcohol 'controls' n=33 %	Alcohol n=21 %	Not alcohol n=110 %
G	**78.3** (155)	**78.8** (52)	**88.1** (37)	**83.6** (184)
A	**21.7** (43)	**21.2** (14)	**11.9** (5)	**16.4** (36)
Total alleles (n)	198	66	42	220

Table 6.50 TNF –308 allele frequencies in normal controls, alcohol-induced and non alcohol-induced acute pancreatitis patients, and alcoholic 'controls'

This table displays TNF –308 allele frequencies in both control and patient group subjects. For each allele the **%** frequency and the actual number of alleles (in brackets) is given.

n represents the number of subjects in each group

(n) represents the number of alleles in each group.

Alcoholic pancreatitis if daily consumption of ≥ 80g alcohol day^{-1}

Non-alcohol induced pancreatitis includes:

- Gallstones if radiological or ERCP evidence of cholelithiasis
- Idiopathic if no identifiable aetiology

Comparisons were made for each allele between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.

No differences are seen in the frequency of individual alleles between patient groups or between patient groups and controls.

TNF –308 genotype	Controls n=99 %	Alcohol 'controls' n=33 %	Alcohol n=21 %	Not alcohol n=110 %
GG	**63.6** (63)	**66.7** (22)	**76.2** (16)	**70.0** (77)
GA	**29.3** (29)	**24.2** (8)	**23.8** (5)	**27.3** (30)
AA	**7.1** (7)	**9.1** (3)	**0.0** (0)	**2.7** (3)
Total	99	33	21	110

Table 6.51 TNF –308 genotype frequencies in normal controls, alcohol-induced and non alcohol-induced acute pancreatitis patients, and alcoholic 'controls'

This table displays TNF –308 genotype frequencies in both control and patient group subjects. For each genotype the **% frequency** and their actual number (in brackets) is given.

n represents the number of subjects in each group

Alcoholic pancreatitis if daily consumption of ≥ 80g alcohol day^{-1}

Non-alcohol induced pancreatitis includes:

- Gallstones if radiological or ERCP evidence of cholelithiasis
- Idiopathic if no identifiable aetiology

Comparisons were made between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.

No differences are seen in gene frequency between patient groups or between patient groups and controls.

6.9 TNF –308 / TNFabc microsatellite haplotypes.

Alleles at the –308 locus have been shown to be in linkage disequilibrium with TNFabc 3-locus microsatellite haplotypes. This has been discussed in section 4.1.4.3 (TNF region linkage and extended MHC haplotypes) and detailed in table 4.3.

Analysis of patient and normal control populations in this study was also undertaken in this regard.

Table 6.52 shows expected and observed frequencies of TNF –308 / TNFabc microsatellite haplotypes in normal controls. Only associations with the –308.G allele are shown in this table. There was a negative association between allele –308.G and the a2b3c1 haplotype (χ^2 = 8.84, p=0.003). There was an association between allele –308.G and the a6b5c1 haplotype (χ^2 = 8.20, p=0.004). There was also a weaker association between allele –308.G and the a11b4c1 haplotype (χ^2=4.02, p=0.045).

These associations were also present in the patient group, shown in table 6.53. Again, there was a negative association between the –308.G allele and the a2b3c1 haplotype (χ^2 = 13.1, p=0.0003), and associations between the –308.G allele and a6b5c1 (χ^2=6.0, p=0.014), and a11b4c1 (χ^2 = 8.3, p=0.004). There was also an association between –308.G and the a2b1c2 haplotype (χ^2=5.8, p=0.016), and a negative association between the –308.G allele and the a4b5c1 haplotype (χ^2=5.4, p=0.02). Both these latter two were not seen in the normal controls. All but two of these associations have been previously reported in Caucasian populations, as detailed in table 4.3, in section 4.1.4.3 (TNF region linkage and extended MHC haplotypes). The a6b5c1 and a4b5c1 associations are not reported in the literature reviewed in this work.

It was not possible to determine haplotypes involving the –308.A allele, as not enough homozygotes were present.

The issue of haplotypes involving the –308.A allele is addressed in tables 6.54 and 6.55 (controls) and 6.56 and 6.57 (patients). As the majority of genotypes with the –308.A allele

(frequency 21.7%) are heterozygotes, these tables demonstrate the association of an –308.A allele containing genotype with a TNFabc genotype containing the microsatellite haplotype TNFa2b3c1 or a4b5c1 in normal controls and patients. These associations were tested because of the reported associations between allele -308.A and TNFa2b3c1, and due to the negative association found in this study of the –308.G allele with the haplotype a4b5c1.

In normal controls a –308.GA or –308.AA genotype was associated with a 3-locus microsatellite genotype containing an a2b3c1 haplotype (χ^2 =19.2, p<0.0001). A weaker, non significant, association was found between –308.GA and –308.AA genotypes and a 3-locus microsatellite genotype containing an a4b5c1 haplotype (Fisher's exact test, p=0.08). Likewise in the patient group, a –308.GA or –308.AA genotype was associated with a 3-locus microsatellite genotype containing an a2b3c1 haplotype (χ^2 =35.4, p<0.0001). The association between –308.GA and –308.AA genotypes and a 3-locus microsatellite genotype containing an a4b5c1 haplotype was significant in the patient group (χ^2 =23.3, p<0.0001). Although these finding do not prove allelic associations, because they involve genotypes, it strongly suggests allelic linkage.

-308.G / TNFabc haplotype	Expected		Observed		
	n	Freq.	n	Freq.	χ^2
G - a2b1c2	14.8	10.3	19	13.2	1.19
G - a2b3c1	17.4	12.1	5	3.5	8.84 [1]
G - a2b5c2	5.2	3.6	8	5.6	1.51
G - a6b5c1	16.4	11.4	28	19.4	8.20 [2]
G - a7b4c1	6.8	4.7	8	5.6	0.18
G - a11b4c1	18.4	12.8	27	18.8	4.02 [3]
G - a10b4c1	9.5	6.6	15	10.4	3.18
G - a4b5bc1	6.9	4.8	4	2.8	1.20
G - other	3.6	2.5	3	2.1	0.10
G - X	13.2	9.2	15	10.4	0.25

Table 6.52 Frequency (%) of TNF –308.G / TNFabc microsatellite haplotypes in normal controls

Table showing expected and observed frequencies of TNF –308 / TNFabc microsatellite haplotypes.
X – unable to determine haplotype
1. $\chi^2 = 8.84$, p=0.003, (negative association)
2. $\chi^2 = 8.20$, p=0.004
3. χ^2=4.02, p=0.045

Haplotype combinations able to be determined in 144/214 chromosomes (67.3%)
It was not possible to determine haplotypes involving the –308.A allele, as not enough homozygotes were present.
The issue of haplotypes involving the –308.A allele is addressed in tables 6.54 and 6.55.

-308.G / TNFabc haplotype	Expected		Observed		
	n	Freq.	n	Freq.	χ^2
G - a2b1c2	21.0	10.5	32	16	5.8 [1]
G - a2b3c1	15.0	7.5	1	0.5	13.1 [2]
G - a2b5c2	6.0	3.0	7	3.5	0.7
G - a6b5c1	25.6	12.8	38	19.0	6.0 [3]
G - a7b4c1	9.0	4.5	17	8.5	3.8
G - a11b4c1	27.0	13.5	42	21.0	8.3 [4]
G - a10b4c1	12.0	6.0	18	9.0	3.0
G - a4b5bc1	10.6	5.3	3	1.5	5.4 [5]
G - other	7.6	3.8	11	5.5	1.5
G - X	16.6	8.3	22	11.0	1.8

Table 6.53 Frequency (%) of TNF –308.G / TNFabc microsatellite haplotypes in study patients

Table showing expected and observed frequencies of TNF –308 / TNFabc microsatellite haplotypes in study patients.
X – unable to determine haplotype
1. χ^2=5.8, p=0.016
2. χ^2 = 13.1, p=0.0003 (negative association)
3. χ^2=6.0, p=0.014
4. χ^2 = 8.3, p=0.004
5. χ^2=5.4, p=0.02 (negative association)

Haplotype combinations able to be determined in 200/270 chromosomes (74.1%)
Patient subgroups are too small for meaningful analysis, as expected frequencies are less than 5 with most haplotypes. The exception is the group with gallstone –induced acute pancreatitis, but analysis gives very similar results to those seen with the study group as a whole, so data is not presented.
It was not possible to determine haplotypes involving the –308.A allele, as not enough homozygotes were present.
The issue of haplotypes involving the –308.A allele is addressed in tables 6.56 and 6.57.

-308.A / TNFabc genotype	Expected		Observed		
	n	Freq.	n	Freq.	χ^2
G/A . A – x . a2b3c1	9.5	10.1	23	24.5	19.20 [1]
G/A . A – x . other	24.0	26.0	17	18.1	2.04

Table 6.54 Frequency (%) of –308.A allele occurring with a TNFabc genotype with at least one copy of the a2b3c1 haplotype in normal controls

As the majority of genotypes with the –308.A allele (frequency 21.7%) are heterozygotes, this table demonstrates the association of an –308.A allele with a TNFabc genotype containing the microsatellite haplotype TNFa2b3c1 in normal controls.
x/other – any genotype not containing a2b31c1
1. χ^2 =19.2, p<0.0001

-308.A / TNFabc genotype	Expected		Observed		
	n	Freq.	n	Freq.	χ^2
G/A . A – x . a4b5c1	4.0	4.0	10	10.1	9.3 [1]
G/A . A – x . other	31.7	32.0	26	26.3	1.0

Table 6.55 Frequency (%) of –308.A allele occurring with a TNFabc genotype with at least one copy of the a4b5c1 haplotype in normal controls

As the majority of genotypes with the –308.A allele (frequency 21.7%) are heterozygotes, this table demonstrates the association of an –308.A allele with a TNFabc genotype containing the microsatellite haplotype TNFa4b5c1 in normal controls.
x/other – any genotype not containing a4b5c1
1. χ^2 =9.3, p=0.002
   It must be noted that as the expected value is <5, the χ^2 statistic is not valid.
   Fisher's exact test, p=0.08.

-308.A / TNFabc genotype	Expected		Observed		
	n	Freq.	n	Freq.	χ^2
G/A . A – x . a2b3c1	7.6	5.8	24	18.3	35.4 [1]
G/A . A – x . other	30.4	23.2	15	11.4	7.8 [2]

Table 6.56 Frequency (%) of –308.A allele occurring with a TNFabc genotype with at least one copy of the a2b3c1 haplotype in study patients

As the majority of genotypes with the –308.A allele (frequency 21.7%) are heterozygotes, this table demonstrates the association of an –308.A allele with a TNFabc genotype containing the microsatellite haplotype TNFa2b3c2 in study patients.
x/other – any genotype not containing a2b31c1
1. χ^2 =35.4, p<0.0001
2. χ^2 =7.8, p=0.005 (Negative association)

-308.A / TNFabc genotype	Expected		Observed		
	n	Freq.	n	Freq.	χ^2
G/A . A – x . a4b5c1	5.1	3.9	16	12.2	23.3 [1]
G/A . A – x . other	32.9	25.1	23	17.5	3.0

Table 6.57 Frequency (%) of –308.A allele occurring with a TNFabc genotype with at least one copy of the a4b5c1 haplotype in study patients

As the majority of genotypes with the –308.A allele (frequency 21.7%) are heterozygotes, this table demonstrates the association of an –308.A allele with a TNFabc genotype containing the microsatellite haplotype TNFa4b5c1 in study patients.
x/other – any genotype not containing a4b5c1
1. χ^2 =23.3, p<0.0001

6.10 TNF locus / HLA-DQB1 associations

As discussed in section 4.1.4.3 (TNF region linkage and extended MHC haplotypes) and detailed in table 4.3, alleles at the TNF loci are in linkage disequilibrium with MHC class I and II alleles. As HLA-DQB1 typing was available in 70/107 (65.4%) of controls, associations between the –308 and TNFabc microsatellite loci were tested.

Table 6.58 and 6.59 show that no associations were found between HLA-DQB1 alleles and –308.G alleles. It was not possible to determine haplotypes involving the –308.A allele, as not enough homozygotes were present.

Because of the reported linkage between the –308.G allele and the HLA-DQB1*02 allelic group and the *0201 allele and the TNFb3 allele, the association between the 3-locus haplotype a2b3c1, and the 0201 allele was tested (a2b3c1 associated with –308.A in this population). Because of the predominance of heterozygotes at both loci, it was only possible to do this by looking for associations between genotypes occurring in individuals. As discussed above this does not prove allelic associations, but does strongly suggest them.

Table 6.60 shows that there is an association between HLA-DQB1*0201 containing genotypes and 3-locus TNFa2b3c1 containing genotypes (Fisher's exact test, p=0.0015).

-308.G / HLA-DQB1 haplotype	Expected		Observed		
	n	Freq.	n	Freq.	χ^2
G - * 0201	16.3	18.5	16	18.2	0.0
G - * 0301	12.3	14.0	11	12.5	0.2
G - * 0302	9.4	10.6	10	11.4	0.0
G - * 0501	7.8	8.9	8	9.1	0.0
G - * 0601	7.4	8.4	10	11.4	1.6

Table 6.58 Frequency (%) of TNF –308.G / HLA-DQB1 haplotypes in normal controls

Table showing expected and observed frequencies of TNF –308.G / HLA-DQB1 haplotypes. No associations are seen. It was possible to determine haplotypes between the two loci in 88 chromosomes.
It was not possible to determine haplotypes involving the –308.A allele, as not enough homozygotes were present.

-308.G / HLA-DQB1 haplotype	Expected		Observed		
	n	Freq.	n	Freq.	χ^2
G - * 02	16.3	18.5	16	18.2	0.0
G - * 03	26.6	30.2	28	31.8	0.1
G - * 05	9.9	11.2	11	12.5	0.1
G - * 06	10.8	12.3	15	17.0	1.6
G - * 01/*04	5.5	6.3	10	11.4	3.7 [1]

Table 6.59 Frequency (%) of TNF –308.G / HLA-DQB1 allelic group haplotypes in normal controls

Table showing expected and observed frequencies of TNF –308.G / HLA-DQB1allelic group haplotypes. No associations are seen. It was possible to determine haplotypes between the two loci in 88 chromosomes.
1. $\chi^2 = 3.7$, p=0.054.
It was not possible to determine haplotypes involving the –308.A allele, as not enough homozygotes were present.

HLA-DQB1 / TNFabc genotype	Expected		Observed		χ^2
	n	Freq.	n	Freq.	
X .*0201 – x . a2b3c1	4.5	6.4	19	27.1	46.7 [1]
X .*0201 – x . other	11.6	16.5	14	20.0	0.5

Table 6.60 Frequency (%) of a TNFabc genotype with at least one copy of the a2b3c1 haplotype occurring with HLA-DQB1 genotype with a copy of the *0201 allele in normal controls

As the majority of genotypes at both loci are heterozygotes, this table demonstrates the association of an HLA-DQB1*0201 allele containing genotype with a TNFabc genotype containing the microsatellite haplotype TNFa2b3c1 in normal controls. It was possible to determine genotype combinations between the 2 loci in 70 individuals.

x/other – any TNF haplotype except a2b3c1

X – any HLA-DQB1 allele except *0201

1. χ^2 =46.7, 2 d.f., p<0.0001
   It must be noted that as the expected value is <5, the χ^2 statistic is not valid.
   Fisher's exact test (rounding expected value up to 5.0), p=0.0015.

This strongly suggests linkage between HLA-DQB1*0201 and TNFa2b3c1.

6.11 IL-10.G microsatellite alleles

Allelic frequencies of the IL-10.G microsatellite were measured in 100% of patients and a group of 136 normal controls. 11 of 15 previously described alleles were observed in the population studied (Figure 6.6). Table 6.61 shows the allelic frequencies in normal control and acute pancreatitis subjects. Table 6.62 shows allelic frequencies in patient groups stratified according to disease severity classified according to the Atlanta classification and the occurrence of a local complication. Tables 6.63 shows alleles inpatient groups stratified according to organ failure score.

There are no statistically significant differences in the allelic frequencies IL-10.G locus when comparing all acute pancreatitis patients with controls or when patients grouped according to disease course (mild vs. severe, mild vs. OFS≥2, mild vs. OFS≥3, mild vs. OFS≥4) were compared. There was a slight increased frequency of IL-10.G13 in the severe group compared to those patients with mild acute pancreatitis, however the difference does not reach statistical significance (26.3 vs. 17%, χ^2 =3.0, p=0.08). This was mirrored by an increased frequency (23.8%) of the IL-10.G allele in the group who had a local complication.

The distribution of all alleles between controls and patients was also not different (analysis excluding alleles with control frequencies less than 5), χ^2=8.04, 10 d.f., p=0.625.

Patients were stratified according to aetiology of acute pancreatitis comparing between groups and with normal controls and alcoholic 'controls', and allelic frequencies are shown in tables 6.64 and 6.65. There was a reduction in the frequency of the IL-10.G13 allele in patients with alcoholic pancreatitis compared to normal controls (4.8 vs 21.3%, χ^2 =6.46, p=0.011, p_c=0.12), non-alcoholic pancreatitis (4.8 vs. 22.2%, χ^2 =6.97, p= .008,p_c=0.088), and the group of alcoholic 'controls' (4.8 vs. 22.8%, χ^2=6.25, p=0.012, p_c=0.13). None of these remained significant after correction for multiple comparisons.

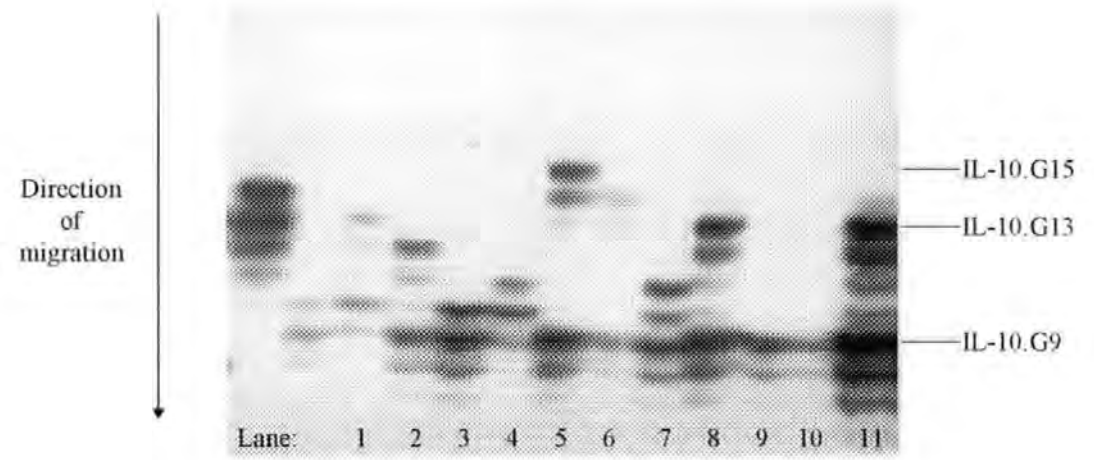

Figure 6.6 IL-10.G microsatellite autoradiograph

Amplification of a region containing the microsatellite was performed as described in section 5.5. Amplification products (6µl) mixed with 3µl of Stop solution containing Formamide (Amersham Life Science, Buckinghamshire, UK) are separated on a 6% polyacrylamide gel with 8M urea at 1700V for 2.5 hours, and alleles revealed with autoradiography.

IL-10.G has 15 previously descibed alleles. Alleles were assigned by the identification of the most common allele in Europid populations, allele 9. Identification of alleles was also confirmed by running with a radiolabelled 123 base pair ladder, as allele 4 amplified using this assay is 123 bp in length. Individual alleles differ by one base pair repeat.

Alleles IL-10.G9, IL-10.G13, and IL-10.G15 are labelled in this figure. Genotypes for each of the samples shown in lanes 1-9 are as follows:

Lane1: 10,13
Lane2: 9,12
Lane3: 9,10
Lane4: 10,11
Lane5: 9,15
Lane6: 9,14
Lane7: 9,11
Lane8: 9,13
Lane9: 9,9
Lane10: 9,9
Lane11: 9,13

IL-10.G allele	Controls n=136 %	Study patients n=135 %
4	**0.4** (1)	**0.0** (0)
5	**0.0** (0)	**0.4** (1)
7	**2.9** (8)	**1.1** (3)
8	**1.8** (5)	**3.3** (9)
9	**43.8** (119)	**41.9** (113)
10	**7.7** (21)	**7.4** (20)
11	**8.1** (22)	**11.1** (30)
12	**4.8** (13)	**7.4** (20)
13	**21.3** (58)	**19.6** (53)
14	**8.8** (24)	**6.3** (17)
15	**0.4** (1)	**1.5** (4)
Total alleles(n)	272	270

Table 6.61 IL-10.G microsatellite allele frequencies in normal controls and acute pancreatitis patients

This table displays IL-10.G microsatellite allele frequencies in control and patient subjects. For each allele the **% frequency** and the actual number of alleles (in brackets) is given.

n represents the number of subjects in each group

(n) represents the number of alleles in each group.

Comparisons were made for each allele between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.

No statistically different differences in individual alleles are seen between the control and patient subjects.

The distribution of all alleles between controls and patients was also not different (analysis excluding alleles with control frequencies less than 5), χ^2=8.04, 10 d.f., p=0.625.

IL-10.G allele	Controls n=136 %	Mild n=97 %	Severe n=38 %	Local complication n=21 %
4	**0.4** (1)	**0.0** (0)	**0.0** (0)	**0.0** (0)
5	**0.0** (0)	**0.5** (1)	**0.0** (0)	**0.0** (0)
7	**2.9** (8)	**1.0** (2)	**1.3** (1)	**2.4** (1)
8	**1.8** (5)	**4.1** (8)	**1.3** (1)	**2.4** (1)
9	**43.8** (119)	**41.8** (73)	**42.1** (32)	**40.5** (17)
10	**7.7** (21)	**8.8** (17)	**3.9** (3)	**4.8** (2)
11	**8.1** (22)	**12.4** (24)	**7.9** (6)	**7.1** (3)
12	**4.8** (13)	**8.2** (16)	**5.3** (4)	**7.1** (3)
13	**21.3** (58)	**17.0** (33)	**26.3** [1] (20)	**23.8** (10)
14	**8.8** (24)	**5.2** (10)	**9.2** (7)	**7.1** (3)
15	**0.4** (1)	**1.0** (2)	**2.6** (2)	**4.8** (2)
Total alleles(n)	272	194	76	42

Table 6.62 IL-10.G microsatellite allele frequencies in normal controls and acute pancreatitis patients grouped according to (local and systemic) disease severity

This table displays IL-10.G microsatellite allele frequencies in control and patient group subjects. For each allele the **% frequency** and the actual number of alleles (in brackets) is given.

n represents the number of subjects in each group

(n) represents the number of alleles in each group.

Mild and severe disease severity groups are according to the Atlanta convention classification (Bradley, 1993), described in section 5.1.4.

Local complication is defined as occurrence of pancreatic necrosis, abscess or pseudocyst.

Comparisons were made for each allele between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons.

1. Increased frequency of IL-10.G13 in severe vs. mild group, however does not reach statistical significance, $\chi^2 = 3.0$, $p=0.08$

IL-10.G allele	Controls n=136 %	OFS ≥ 2 n=35 %	OFS ≥ 3 n=18 %	OFS ≥ 4 n=12 %
4	**0.4** (1)	**0.0** (0)	**0.0** (0)	**0.0** (0)
5	**0.0** (0)	**0.0** (0)	**0.0** (0)	**0.0** (0)
7	**2.9** (8)	**0.0** (0)	**0.0** (0)	**0.0** (0)
8	**1.8** (5)	**1.4** (1)	**0.0** (0)	**0.0** (0)
9	**43.8** (119)	**41.4** (29)	**50.0** (18)	**50.0** (12)
10	**7.7** (21)	**2.9** (2)	**2.8** (1)	**4.2** (1)
11	**8.1** (22)	**11.4** (8)	**5.6** (2)	**4.2** (1)
12	**4.8** (13)	**5.7** (4)	**2.8** (1)	**4.2** (1)
13	**21.3** (58)	**27.1** (19)	**25.0** (9)	**25.0** (6)
14	**8.8** (24)	**8.6** (6)	**13.9** (5)	**12.5** (3)
15	**0.4** (1)	**1.4** (1)	**0.0** (0)	**0.0** (0)
Total alleles(n)	272	70	36	24

Table 6.63 IL1-.G microsatellite allele frequencies in normal controls and acute pancreatitis patients grouped according to (systemic) disease severity as assessed by organ failure score

This table displays IL-10.G microsatellite allele frequencies in control and patient group subjects. For each allele the **% frequency** and the actual number of alleles (in brackets) is given.

n represents the number of subjects in each group

(n) represents the number of alleles in each group.

OFS Organ failure score

OFS calculated according to method described in section 5.1.4 (Kingsnorth et al, 1995).

Comparisons were made for each allele between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.

No differences are seen in the frequency of individual alleles between patient groups or between patient groups and controls.

IL-10.G allele	Controls n=136 %	Alcohol n=21 %	Idiopathic n=37 %	Gallstones n=77 %
4	**0.4** (1)	**0.0** (0)	**0.0** (0)	**0.0** (0)
5	**0.0** (0)	**0.0** (0)	**0.0** (0)	**0.6** (1)
7	**2.9** (8)	**0.0** (0)	**1.4** (1)	**1.3** (2)
8	**1.8** (5)	**9.5** (4)	**4.1** (3)	**1.3** (2)
9	**43.8** (119)	**47.6** (20)	**36.5** (27)	**42.9** (66)
10	**7.7** (21)	**19.0** (8)	**6.8** (5)	**4.5** (7)
11	**8.1** (22)	**11.9** (5)	**12.2** (9)	**10.4** (16)
12	**4.8** (13)	**2.4** (1)	**8.1** (6)	**8.4** (13)
13	**21.3** (58)	**4.8** [1] (2)	**21.6** (16)	**22.7** (35)
14	**8.8** (24)	**2.4** (1)	**9.5** (7)	**5.8** (9)
15	**0.4** (1)	**2.4** (1)	**0.0** (0)	**1.9** (3)
Total alleles(n)	272	42	74	154

Table 6.64 IL-10.G microsatellite allele frequencies in normal controls and acute pancreatitis patients grouped according to aetiology of acute pancreatitis

This table displays IL-10.G microsatellite allele frequencies in control and patient group subjects. For each allele the **% frequency** and the actual number of alleles (in brackets) is given.

n represents the number of subjects in each group

(n) represents the number of alleles in each group.

Alcoholic pancreatitis if daily consumption of $\geq$ 80g alcohol day^{-1}

Gallstones if radiological or ERCP evidence of cholelithiasis

Idiopathic if no identifiable aetiology

Comparisons were made for each allele between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.

1. There was a trend towards reduced frequency of the IL-10.G13 allele in patients with

alcohol induced acute pancreatitis, although this did not achieve conventional statistical significance.

Controls vs. alcohol group, χ^2 =6.46, p=0.011, p_c=0.12

Alcohol vs. idiopathic and gallstone groups combined, χ^2 =6.97, p= 0.008, p_c=0.088

The reduction in the G13 allele in the alcoholic group was not accounted for by an increase in any one allele.

IL-10.G allele	Controls n=136 %	Alcohol 'controls' n=52 %	Alcohol n=21 %	Not alcohol n=114 %
4	**0.4** (1)	**0.0** (0)	**0.0** (0)	**0.0** (0)
5	**0.0** (0)	**0.0** (0)	**0.0** (0)	**0.4** (1)
7	**2.9** (8)	**1.5** (1)	**0.0** (0)	**1.3** (3)
8	**1.8** (5)	**3.0** (2)	**9.5** (4)	**2.2** (5)
9	**43.8** (119)	**40.9** (27)	**47.6** (20)	**40.8** (93)
10	**7.7** (21)	**9.1** (6)	**19.0** (8)	**5.3** (12)
11	**8.1** (22)	**13.6** (9)	**11.9** (5)	**11.0** (25)
12	**4.8** (13)	**7.6** (5)	**2.4** (1)	**8.3** (19)
13	**21.3** (58)	**22.7** (15)	**4.8** [1] (2)	**22.4** (51)
14	**8.8** (24)	**1.5** (1)	**2.4** (1)	**7.0** (16)
15	**0.4** (1)	**0.0** (0)	**2.4** (1)	**1.3** (3)
Total alleles(n)	272	66	42	228

Table 6.65 IL-10.G microsatellite allele frequencies in normal controls, alcohol-induced and non alcohol-induced acute pancreatitis patients, and alcoholic 'controls'

This table displays IL-10.G microsatellite allele frequencies in both control and patient group subjects. For each allele the **% frequency** and the actual number of alleles (in brackets) is given.

n represents the number of subjects in each group

(n) represents the number of alleles in each group.

Alcoholic pancreatitis if daily consumption of $\geq$ 80g alcohol day^{-1}

Non-alcohol induced pancreatitis includes:

- Gallstones if radiological or ERCP evidence of cholelithiasis
- Idiopathic if no identifiable aetiology

Comparisons were made for each allele between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.

1. There was a strong trend towards reduced frequency of the IL-10.G13 allele in patients with alcohol induced acute pancreatitis, although this did not achieve conventional statistical significance.
   Controls vs. alcohol group, χ^2 =6.46, p=0.011, p_c=0.12
   Alcohol vs. idiopathic and gallstone groups combined, χ^2 =6.97, p=0.008,p_c=0.088
   Alcohol vs. 'alcohol controls', χ^2=6.25, p=0.012, p_c=0.13
   The reduction in the G13 allele in the alcoholic group was not accounted for by an increase in any one allele.

6.12 IL-10.G microsatellite genotypes

Due to the number of alleles, there are many possible IL-10.G microsatellite genotypes. This limits analysis of genotype distribution because of very small numbers. IL-10.G genotypes in normal control and patients are displayed in Tables 6.66. Genotypes in patient groups stratified according to disease severity classified according to the Atlanta classification and the occurrence of a local complication are shown in Tables 6.67. No significant differences were seen between controls and patient groups or between patient groups.

Tables 6.68 show genotypes in patient groups stratified according to organ failure score. No significant differences were seen between controls and patient groups or between patient groups.

Tables 6.69 and 6.70 show IL-10.G genotypes in patient groups stratified according aetiology of acute pancreatitis, and alcoholic 'controls'. No significant differences were seen between controls and patient groups or between patient groups.

IL-10.G genotype	Controls n=136 %	Study patients n=135 %
4,9	0.7 (1)	0.0 (0)
5,11	0.0 (0)	0.7 (1)
7,9	1.5 (2)	0.7 (1)
7,10	0.7 (1)	0.0 (0)
7,11	1.5 (2)	0.0 (0)
7,12	1.5 (2)	1.5 (2)
7,13	0.7 (1)	0.0 (0)
8,9	2.2 (3)	1.5 (0)
8,10	0.0 (0)	2.2 (3)
8,11	0.0 (0)	0.7 (1)
8,12	0.0 (0)	1.5 (2)
8,13	1.5 (2)	0.0 (0)
8,14	0.0 (0)	0.7 (1)
9,9	17.6 (24)	15.6 (21)
9,10	5.9 (8)	4.4 (6)
9,11	5.9 (8)	12.6 (17)

Table 6.66 **Genotypes at the IL-10.G microsatellite locus in normal control and patient groups**

(continued.....)

IL-10.G genotype	Controls n=136 %	Study patients n=135 %
9,12	3.7 (5)	5.2 (7)
9,13	25.0 (34)	20.7 (28)
9,14	6.6 (9)	5.9 (8)
9,15	0.7 (1)	1.5 (2)
10,10	0.0 (0)	1.5 (2)
10,11	3.7 (5)	0.0 (0)
10,12	0.0 (0)	0.7 (1)
10,13	2.9 (4)	2.2 (3)
10,14	2.2 (3)	1.5 (2)
10,15	0.0 (0)	0.7 (1)
11,11	0.0 (0)	1.5 (2)
11,12	1.5 (2)	0.7 (1)
11,13	2.9 (4)	3.7 (5)
11,14	0.7 (1)	0.7 (1)
12,12	0.0 (0)	1.5 (2)
12,13	0.7 (1)	1.5 (2)

Table 6.66 **Genotypes at the IL-10.G microsatellite locus in normal control and patient groups**

(continued.....)

IL-10.G genotype	Controls n=136 %	Study patients n=135 %
12,14	**2.2** (3)	**0.7** (1)
13,13	**2.2** (3)	**4.4** (6)
13,14	**4.4** (6)	**2.2** (3)
14,14	**0.7** (1)	**0.0** (0)
14,15	**0.0** (0)	**0.7** (1)
Total	136	135

Table 6.66 Genotypes at the IL-10.G microsatellite locus in normal control and patient groups

This table displays IL-10.G microsatellite genotype frequencies in control and patient subjects. For each genotype the **% frequency** and the actual number of genotypes (in brackets) is given.

Comparisons were made for each genotype between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.

No significant differences were seen in genotype frequencies between patient and control groups.

IL-10.G genotype	Controls n=136 %	Mild n=97 %	Severe n=38 %	Local complication n=21 %
4,9	**0.7** (1)	**0.0** (0)	**0.0** (0)	**0.0** (0)
5,11	**0.0** (0)	**1.0** (1)	**0.0** (0)	**0.0** (0)
7,9	**1.5** (2)	**0.0** (0)	**2.6** (1)	**4.8** (1)
7,10	**0.7** (1)	**0.0** (0)	**0.0** (0)	**0.0** (0)
7,11	**1.5** (2)	**0.0** (0)	**0.0** (0)	**0.0** (0)
7,12	**1.5** (2)	**2.1** (2)	**0.0** (0)	**0.0** (0)
7,13	**0.7** (1)	**0.0** (0)	**0.0** (0)	**0.0** (0)
8,9	**2.2** (3)	**1.0** (1)	**2.6** (1)	**4.8** (1)
8,10	**0.0** (0)	**3.1** (3)	**0.0** (0)	**0.0** (0)
8,11	**0.0** (0)	**1.0** (1)	**0.0** (1)	**0.0** (0)
8,12	**0.0** (0)	**2.1** (2)	**0.0** (0)	**0.0** (0)
8,13	**1.5** (2)	**0.0** (0)	**0.0** (0)	**0.0** (0)
8,14	**0.0** (0)	**1.0** (1)	**0.0** (0)	**0.0** (0)
9,9	**17.6** (24)	**17.5** (17)	**10.5** (4)	**9.5** (2)
9,10	**5.9** (8)	**6.2** (6)	**0.0** (0)	**0.0** (0)
9,11	**5.9** (8)	**11.3** (11)	**15.8** (6)	**14.3** (3)

Table 6.67 **Genotypes at the IL-10.G microsatellite locus in normal control and acute pancreatitis patients grouped according to (local and systemic) disease severity**

(continued.....)

IL-10.G genotype	Controls n=136 %	Mild n=97 %	Severe n=38 %	Local complication n=21 %
9,12	**3.7** (5)	**6.2** (6)	**2.6** (1)	**0.0** (0)
9,13	**25.0** (34)	**18.6** (18)	**26.3** (10)	**23.8** (5)
9,14	**6.6** (9)	**4.1** (4)	**10.5** (4)	**9.5** (2)
9,15	**0.7** (1)	**1.0** (1)	**2.6** (1)	**4.8** (1)
10,10	**0.0** (0)	**2.1** (2)	**0.0** (0)	**0.0** (0)
10,11	**3.7** (5)	**0.0** (0)	**0.0** (0)	**0.0** (0)
10,12	**0.0** (0)	**0.0** (0)	**2.6** (1)	**4.8** (1)
10,13	**2.9** (4)	**3.1** (3)	**0.0** (0)	**0.0** (0)
10,14	**2.2** (3)	**1.0** (1)	**2.6** (1)	**0.0** (0)
10,15	**0.0** (0)	**0.0** (0)	**2.6** (1)	**4.8** (1)
11,11	**0.0** (0)	**2.1** (2)	**0.0** (0)	**0.0** (0)
11,12	**1.5** (2)	**1.0** (1)	**0.0** (0)	**0.0** (0)
11,13	**2.9** (4)	**5.2** (5)	**0.0** (0)	**0.0** (0)
11,14	**0.7** (1)	**1.0** (1)	**0.0** (0)	**0.0** (0)
12,12	**0.0** (0)	**2.1** (2)	**0.0** (0)	**0.0** (0)
12,13	**0.7** (1)	**1.0** (1)	**2.6** (1)	**4.8** (1)

Table 6.67 **Genotypes at the IL-10.G microsatellite locus in normal control and acute pancreatitis patients grouped according to (local and systemic) disease severity**

(continued.....)

IL-10.G genotype	Controls n=136 %	Mild n=97 %	Severe n=38 %	Local complication n=21 %
12,14	**2.2** (3)	**0.0** (0)	**2.6** (1)	**4.8** (1)
13,13	**2.2** (3)	**2.1** (2)	**10.5** (4)	**9.5** (2)
13,14	**4.4** (6)	**2.1** (2)	**2.6** (1)	**0.0** (0)
14,14	**0.7** (1)	**0.0** (0)	**0.0** (0)	**0.0** (0)
14,15	**0.0** (0)	**1.0** (1)	**0.0** (0)	**0.0** (0)
Total	136	97	38	21

Table 6.67 Genotypes at the IL-10.G microsatellite locus in normal control and acute pancreatitis patients grouped according to (local and systemic) disease severity

This table displays IL-10.G microsatellite genotype frequencies in control and patient groups. For each genotype the **% frequency** and the actual number of genotypes (in brackets) is given.
Mild and severe disease severity groups are according to the Atlanta convention classification (Bradley, 1993), described in section 5.1.4.
Local complication is defined as occurrence of pancreatic necrosis, abscess or pseudocyst.
Comparisons were made for each genotype between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.

IL-10.G genotype	Controls n=136 %	OFS ≥ 2 n=35 %	OFS ≥ 3 n=18 %	OFS ≥ 4 n=12 %
4,9	**0.7** (1)	**0.0** (0)	**0.0** (0)	**0.0** (0)
5,11	**0.0** (0)	**0.0** (0)	**0.0** (0)	**0.0** (0)
7,9	**1.5** (2)	**0.0** (0)	**0.0** (0)	**0.0** (0)
7,10	**0.7** (1)	**0.0** (0)	**0.0** (0)	**0.0** (0)
7,11	**1.5** (2)	**0.0** (0)	**0.0** (0)	**0.0** (0)
7,12	**1.5** (2)	**0.0** (0)	**0.0** (0)	**0.0** (0)
7,13	**0.7** (1)	**0.0** (0)	**0.0** (0)	**0.0** (0)
8,9	**2.2** (3)	**2.9** (1)	**0.0** (0)	**0.0** (0)
8,10	**0.0** (0)	**0.0** (0)	**0.0** (0)	**0.0** (0)
8,11	**0.0** (0)	**0.0** (0)	**0.0** (0)	**0.0** (0)
8,12	**0.0** (0)	**0.0** (0)	**0.0** (0)	**0.0** (0)
8,13	**1.5** (2)	**0.0** (0)	**0.0** (0)	**0.0** (0)
8,14	**0.0** (0)	**0.0** (0)	**0.0** (0)	**0.0** (0)
9,9	**17.6** (24)	**8.6** (3)	**16.7** (3)	**16.7** (2)
9,10	**5.9** (8)	**0.0** (0)	**0.0** (0)	**0.0** (0)
9,11	**5.9** (8)	**17.1** (6)	**11.1** (2)	**8.3** (1)

Table 6.68 **IL-10.G genotype frequencies in normal controls and acute pancreatitis patients grouped according to (systemic) disease severity as assessed by organ failure score**

(continued.....)

IL-10.G genotype	Controls n=136 %	OFS ≥ 2 n=35 %	OFS ≥ 3 n=18 %	OFS ≥ 4 n=12 %
9,12	**3.7** (5)	**2.9** (1)	**0.0** (0)	**0.0** (0)
9,13	**25.0** (34)	**28.6** (10)	**33.3** (6)	**41.7** (5)
9,14	**6.6** (9)	**11.4** (4)	**22.2** (4)	**16.7** (2)
9,15	**0.7** (1)	**2.9** (1)	**0.0** (0)	**0.0** (0)
10,10	**0.0** (0)	**0.0** (0)	**0.0** (0)	**0.0** (0)
10,11	**3.7** (5)	**0.0** (0)	**0.0** (0)	**0.0** (0)
10,12	**0.0** (0)	**2.9** (1)	**0.0** (0)	**0.0** (0)
10,13	**2.9** (4)	**0.0** (0)	**0.0** (0)	**0.0** (0)
10,14	**2.2** (3)	**2.9** (1)	**5.6** (1)	**8.3** (1)
10,15	**0.0** (0)	**0.0** (0)	**0.0** (0)	**0.0** (0)
11,11	**0.0** (0)	**0.0** (0)	**0.0** (0)	**0.0** (0)
11,12	**1.5** (2)	**2.9** (1)	**0.0** (0)	**0.0** (0)
11,13	**2.9** (4)	**2.9** (1)	**0.0** (0)	**0.0** (0)
11,14	**0.7** (1)	**0.0** (0)	**0.0** (0)	**0.0** (0)
12,12	**0.0** (0)	**0.0** (0)	**0.0** (0)	**0.0** (0)
12,13	**0.7** (1)	**2.9** (1)	**5.6** (1)	**8.3** (1)

Table 6.68 **IL-10.G genotype frequencies in normal controls and acute pancreatitis patients grouped according to (systemic) disease severity as assessed by organ failure score**

(continued.....)

IL-10.G genotype	Controls n=136 %	OFS ≥ 2 n=35 %	OFS ≥ 3 n=18 %	OFS ≥ 4 n=12 %
12,14	**2.2** (3)	**0.0** (0)	**0.0** (0)	**0.0** (0)
13,13	**2.2** (3)	**8.6** (3)	**5.6** (1)	**0.0** (0)
13,14	**4.4** (6)	**2.9** (1)	**0.0** (0)	**0.0** (0)
14,14	**0.7** (1)	**0.0** (0)	**0.0** (0)	**0.0** (0)
14,15	**0.0** (0)	**0.0** (0)	**0.0** (0)	**0.0** (0)
Total	136	35	18	12

Table 6.68 IL-10.G genotype frequencies in normal controls and acute pancreatitis patients grouped according to (systemic) disease severity as assessed by organ failure score

This table displays IL-10.G microsatellite genotype frequencies in control and patient groups. For each genotype the **% frequency** and the number of genotypes (in brackets) is given.
OFS Organ failure score
OFS calculated according to method described in section 5.1.4 (Kingsnorth et al, 1995).
Comparisons were made for each genotype between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.
No significant differences were seen

IL-10.G genotype	Controls n=136 %	Alcohol n=21 %	Idiopathic n=37 %	Gallstones n=77 %
4,9	0.7 (1)	0.0 (0)	0.0 (0)	0.0 (0)
5,11	0.0 (0)	0.0 (0)	0.0 (0)	1.3 (0)
7,9	1.5 (2)	0.0 (0)	2.7 (1)	0.0 (0)
7,10	0.7 (1)	0.0 (0)	0.0 (0)	0.0 (0)
7,11	1.5 (2)	0.0 (0)	0.0 (0)	0.0 (0)
7,12	1.5 (2)	0.0 (0)	0.0 (0)	2.6 (2)
7,13	0.7 (1)	0.0 (0)	0.0 (0)	0.0 (0)
8,9	2.2 (3)	4.8 (1)	2.7 (1)	0.0 (0)
8,10	0.0 (0)	4.8 (1)	5.4 (2)	0.0 (0)
8,11	0.0 (0)	4.8 (1)	0.0 (0)	0.0 (0)
8,12	0.0 (0)	0.0 (0)	0.0 (0)	2.6 (2)
8,13	1.5 (2)	0.0 (0)	0.0 (0)	0.0 (0)
8,14	0.0 (0)	4.8 (1)	0.0 (0)	0.0 (0)
9,9	17.6 (24)	23.8 (5)	5.4 (2)	18.2 (14)
9,10	5.9 (8)	9.5 (2)	5.4 (2)	2.6 (2)
9,11	5.9 (8)	19.0 (4)	16.2 (6)	9.1 (7)

Table 6.69 **Genotypes at the IL-10.G microsatellite locus in normal control and acute pancreatitis patients grouped according to aetiology**

(continued.....)

IL-10.G genotype	Controls n=136 %	Alcohol n=21 %	Idiopathic n=37 %	Gallstones n=77 %
9,12	**3.7** (5)	**4.8** (1)	**8.1** (3)	**3.9** (3)
9,13	**25.0** (34)	**4.8** (1)	**18.9** (7)	**26.0** (20)
9,14	**6.6** (9)	**0.0** (0)	**8.1** (3)	**6.5** (5)
9,15	**0.7** (1)	**4.8** (1)	**0.0** (0)	**1.3** (1)
10,10	**0.0** (0)	**9.5** (2)	**0.0** (0)	**0.0** (0)
10,11	**3.7** (5)	**0.0** (0)	**0.0** (0)	**0.0** (0)
10,12	**0.0** (0)	**0.0** (0)	**0.0** (0)	**1.3** (1)
10,13	**2.9** (4)	**4.8** (1)	**0.0** (0)	**2.6** (2)
10,14	**2.2** (3)	**0.0** (0)	**2.7** (1)	**1.3** (1)
10,15	**0.0** (0)	**0.0** (0)	**0.0** (0)	**1.3** (1)
11,11	**0.0** (0)	**0.0** (0)	**0.0** (0)	**2.6** (2)
11,12	**1.5** (2)	**0.0** (0)	**0.0** (0)	**1.3** (1)
11,13	**2.9** (4)	**0.0** (0)	**5.4** (2)	**3.9** (3)
11,14	**0.7** (1)	**0.0** (0)	**2.7** (1)	**0.0** (0)
12,12	**0.0** (0)	**0.0** (0)	**0.0** (0)	**2.6** (2)
12,13	**0.7** (1)	**0.0** (0)	**5.4** (2)	**0.0** (0)

Table 6.69 **Genotypes at the IL-10.G microsatellite locus in normal control and acute pancreatitis patients grouped according to aetiology**

(continued.....)

IL-10.G genotype	Controls n=136 %	Alcohol n=21 %	Idiopathic n=37 %	Gallstones n=77 %
12,14	**2.2** (3)	**0.0** (0)	**2.7** (1)	**0.0** (0)
13,13	**2.2** (3)	**0.0** (0)	**5.4** (2)	**5.2** (4)
13,14	**4.4** (6)	**0.0** (0)	**2.7** (1)	**2.6** (2)
14,14	**0.7** (1)	**0.0** (0)	**0.0** (0)	**0.0** (0)
14,15	**0.0** (0)	**0.0** (0)	**0.0** (0)	**1.3** (1)
Total	136	21	37	77

Table 6.69 Genotypes at the IL-10.G microsatellite locus in normal control and acute pancreatitis patients grouped according to aetiology

This table displays TNFb microsatellite genotype frequencies in control and patient groups. For each genotype the **% frequency** and the number of genotypes (in brackets) is given.
Alcoholic pancreatitis if daily consumption of $\geq$ 80g alcohol day^{-1}
Gallstones if radiological or ERCP evidence of cholelithiasis
Idiopathic if no identifiable aetiology
Comparisons were made for each genotype between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made. No significant differences were seen

IL-10.G genotype	Controls n=136 %	Alcohol 'controls' n=33 %	Alcohol n=21 %	Not alcohol n=114 %
4,9	**0.7** (1)	**0.0** (0)	**0.0** (0)	**0.0** (0)
5,11	**0.0** (0)	**0.0** (0)	**0.0** (0)	**0.9** (1)
7,9	**1.5** (2)	**3.0** (1)	**0.0** (0)	**0.9** (1)
7,10	**0.7** (1)	**0.0** (0)	**0.0** (0)	**0.0** (0)
7,11	**1.5** (2)	**0.0** (0)	**0.0** (0)	**0.0** (0)
7,12	**1.5** (2)	**0.0** (0)	**0.0** (0)	**1.8** (2)
7,13	**0.7** (1)	**0.0** (0)	**0.0** (0)	**0.0** (0)
8,9	**2.2** (3)	**3.0** (1)	**4.8** (1)	**0.9** (1)
8,10	**0.0** (0)	**3.0** (1)	**4.8** (1)	**1.8** (2)
8,11	**0.0** (0)	**0.0** (0)	**4.8** (1)	**0.0** (0)
8,12	**0.0** (0)	**0.0** (0)	**0.0** (0)	**1.8** (2)
8,13	**1.5** (2)	**0.0** (0)	**0.0** (0)	**0.0** (0)
8,14	**0.0** (0)	**0.0** (0)	**4.8** (1)	**0.0** (0)
9,9	**17.6** (24)	**18.2** (6)	**23.8** (5)	**14.0** (16)
9,10	**5.9** (8)	**12.1** (4)	**9.5** (2)	**3.5** (4)
9,11	**5.9** (8)	**12.1** (4)	**19.0** (4)	**11.4** (13)

Table 6.70 **Genotypes at the IL-10.G microsatellite locus in normal controls, alcohol-induced and non alcohol-induced acute pancreatitis patients, and alcoholic 'controls'**

(continued.....)

IL-10.G genotype	Controls n=136 %	Alcohol 'controls' n=33 %	Alcohol n=21 %	Not alcohol n=114 %
9,12	**3.7** (5)	**6.1** (2)	**4.8** (1)	**5.3** (6)
9,13	**25.0** (34)	**9.1** (3)	**4.8** (1)	**23.7** (27)
9,14	**6.6** (9)	**0.0** (0)	**0.0** (0)	**7.0** (8)
9,15	**0.7** (1)	**0.0** (0)	**4.8** (1)	**0.9** (1)
10,10	**0.0** (0)	**0.0** (0)	**9.5** (2)	**0.0** (0)
10,11	**3.7** (5)	**0.0** (0)	**0.0** (0)	**0.0** (0)
10,12	**0.0** (0)	**0.0** (0)	**0.0** (0)	**0.9** (1)
10,13	**2.9** (4)	**3.0** (1)	**4.8** (1)	**1.8** (2)
10,14	**2.2** (3)	**0.0** (0)	**0.0** (0)	**1.8** (2)
10,15	**0.0** (0)	**0.0** (0)	**0.0** (0)	**0.9** (1)
11,11	**0.0** (0)	**0.0** (0)	**0.0** (0)	**1.8** (2)
11,12	**1.5** (2)	**0.0** (0)	**0.0** (0)	**0.9** (1)
11,13	**2.9** (4)	**15.2** (5)	**0.0** (0)	**4.4** (5)
11,14	**0.7** (1)	**0.0** (0)	**0.0** (0)	**0.9** (1)
12,12	**0.0** (0)	**0.0** (0)	**0.0** (0)	**1.8** (2)
12,13	**0.7** (1)	**6.1** (2)	**0.0** (0)	**1.8** (2)

Table 6.70 **Genotypes at the IL-10.G microsatellite locus in normal controls, alcohol-induced and non alcohol-induced acute pancreatitis patients, and alcoholic 'controls'**

(continued.....)

IL-10.G genotype	Controls n=136 %	Alcohol 'controls' n=33 %	Alcohol n=21 %	Not alcohol n=114 %
12,14	**2.2** (3)	**3.0** (1)	**0.0** (0)	**0.9** (1)
13,13	**2.2** (3)	**6.1** (2)	**0.0** (0)	**5.3** (6)
13,14	**4.4** (6)	**0.0** (0)	**0.0** (0)	**2.6** (3)
14,14	**0.7** (1)	**0.0** (0)	**0.0** (0)	**0.0** (0)
14,15	**0.0** (0)	**0.0** (0)	**0.0** (0)	**0.9** (1)
Total	136	33	21	114

Table 6.70 Genotypes at the IL-10.G microsatellite locus in normal controls, alcohol-induced and non alcohol-induced acute pancreatitis patients, and alcoholic 'controls'

This table displays IL-10.G microsatellite genotype frequencies in control and patient groups. For each genotype the **% frequency** and the number of genotypes (in brackets) is given.

n represents the number of subjects in each group

(n) represents the number of alleles in each group.

Alcoholic pancreatitis if daily consumption of $\geq$ 80g alcohol day^{-1}

Non-alcohol induced pancreatitis includes:

- Gallstones if radiological or ERCP evidence of cholelithiasis
- Idiopathic if no identifiable aetiology

Comparisons were made for each genotype between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made. No significant differences were seen.

6.13 IL-10 -1117 locus typing

IL-10 -1117 allelic and genotype frequencies were measured in 127 normal controls and 124 acute pancreatitis patients (92%). They were not measured in the group of alcoholic 'controls'. Figure 6.7 is a PCR-SSOP autoradiograph of the -1117 assay.

Table 6.71 shows allelic frequencies of the –1117 locus in patient and normal control subjects. No significant differences between patient and control groups are seen.

Table 6.72 shows genotype frequencies of the –1117 locus in patient and normal control subjects. No significant differences between patient and control groups are seen.

The expected frequencies for IL-10 –1117 genotypes were calculated. A 2 x n analysis showed that frequencies of heterozygotes and homozygotes in both study and normal control populations were in Hardy Weinberg equlilibrium. This is shown in Table 6.73.

Tables 6.74 and 6.75 show allelic and genotype frequencies at the -1117 locus in patient groups stratified according to disease severity classified according to the Atlanta classification and the occurrence of a local complication. Tables 6.76 and 6.77 show allelic and genotype frequencies at the -1117 locus in patient groups stratified according to organ failure score.

There are no significant differences in the allelic or genotype frequencies of the IL-10 –1117 locus between the above patient groups.

Tables 6.78 and 6.79 show allelic and genotype frequencies at the -1117 locus in patient groups according to the aetiology of their disease. Again, there are no significant differences in the allelic or genotype frequencies of the IL-10 –1117 locus between the groups.

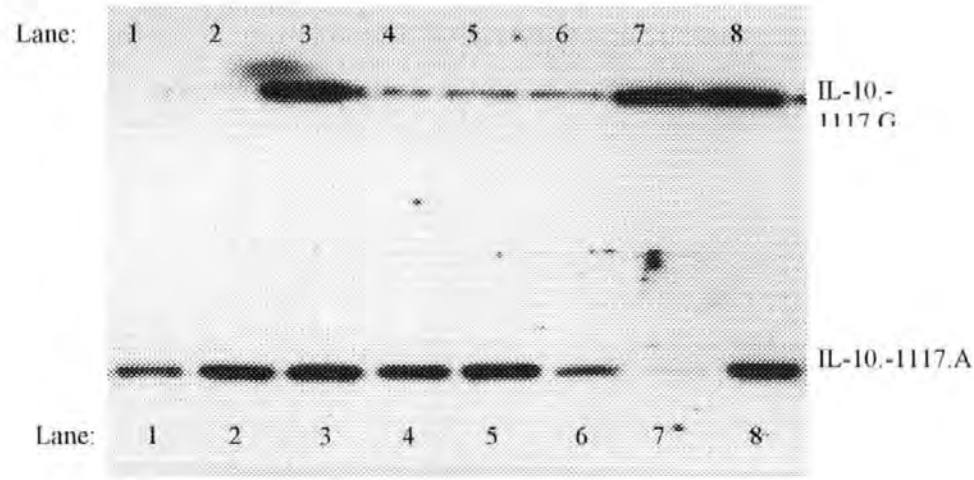

Figure 6.7 PCR-SSOP autoradiograph of the -1117 assay

Detection of the two alleles by PCR-SSOP is descibed in 5.7. Specific probes were used to detect either allele. Alleles were scored on the presence of bound probe detected by chemoluminescence. The genotypes of lanes 1-8 are scored as follows:

Lane 1: -1117.AA
Lane 2: -1117.AA
Lane 3: -1117.GA
Lane 4: -1117.AA
Lane 5: -1117.AA
Lane 6: -1117.AA
Lane 7: -1117.GG
Lane 8: -1117.GA

IL-10 –1117 allele	Controls n=127 %	Study patients n=124 %
G	**53.0** (135)	**56.0** (138)
A	**47.0** (119)	**44.0** (110)
Total alleles (n)	257	248

Table 6.71 IL-10 –1117 allele frequencies in normal controls and acute pancreatitis patients

This table displays IL-10 –1117 allele frequencies in control and patient subjects. For each allele the **% frequency** and the actual number of alleles (in brackets) is given.
n represents the number of subjects in each group
(n) represents the number of alleles in each group.
Comparisons were made for each allele between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.
No significant differences between patient and control groups are seen.

IL-10 –1117 genotype	Controls n=127 %	Study patients n=124 %
GG	**29.1** (37)	**29.8** (37)
GA	**48.0** (61)	**51.6** (64)
AA	**22.8** (29)	**18.5** (23)
Total	127	124

Table 6.72 IL-10 –1117 genotype frequencies in normal controls and acute pancreatitis patients

This table displays IL-10 –1117 genotype frequencies in control and patient subjects. For each genotype the **% frequency** and their actual number (in brackets) is given.
n represents the number of subjects in each group
Comparisons were made for each genotype between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made. No significant differences between patient and control groups are seen.

-1117 Genotype	Expected		Observed		χ^2
	n	Freq.	n	Freq.	
Controls					
GG	35.6	28.0	37	29.1	0.1
GA	63.5	50.0	61	49.2	0.0
AA	27.9	22.0	29	22.8	0.0
Patients					
GG	38.9	31.4	37	29.8	0.1
GA	61.1	49.3	64	51.6	0.1
AA	24.0	19.4	23	18.5	0.0

Table 6.73 Frequency (%) of IL-10 –1117 genotypes in patient and control subjects

Showing expected and observed frequencies of IL-10 –1117 genotypes in patient and normal control subjects. This demonstrates that the distribution conforms to Hardy-Weinberg equilibrium.

IL-10 –1117 allele	Controls n=127 %	Mild n=90 %	Severe n=34 %	Local complication n=18 %
G	**53.0** (135)	**56.0** (100)	**56.0** (38)	**53.0** (19)
A	**47.0** (119)	**44.0** (80)	**44.0** (30)	**47.0** (17)
Total alleles (n)	257	180	68	36

Table 6.74 IL-10 –1117 allele frequencies in normal controls and acute pancreatitis patients grouped according to (local and systemic) disease severity

This table displays IL-10 –1117 allele frequencies in control and patient sub-groups. For each allele the **% frequency** and the actual number of alleles (in brackets) is given.
n represents the number of subjects in each group
(n) represents the number of alleles in each group.
Mild and severe disease severity groups are according to the Atlanta convention classification (Bradley, 1993), described in section 5.1.4.
Local complication is defined as occurrence of pancreatic necrosis, abscess or pseudocyst.
Comparisons were made for each allele between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.
No significant differences between patient and control groups are seen.

IL-10 –1117 genotype	Controls n=127 %	Mild n=90 %	Severe n=34 %	Local complication n=18 %
GG	**29.1** (37)	**30.0** (27)	**29.4** (10)	**22.2** (4)
GA	**48.0** (61)	**51.1** (46)	**52.9** (18)	**61.1** (11)
AA	**22.8** (29)	**18.9** (17)	**17.6** (6)	**16.7** (3)
Total	127	90	34	18

Table 6.75 IL-10 –1117 genotype frequencies in normal controls and acute pancreatitis patients grouped according to (local and systemic) disease severity

This table displays IL-10 –1117 genotype frequencies in control and patient sub-groups. For each genotype the **% frequency** and their actual number (in brackets) is given.
n represents the number of subjects in each group
Mild and severe disease severity groups are according to the Atlanta convention classification (Bradley, 1993), described in section 5.1.4.
Local complication is defined as occurrence of pancreatic necrosis, abscess or pseudocyst.
Comparisons were made between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.
No significant differences between patient and control groups are seen.

IL-10 –1117 allele	Controls n=127 %	OFS ≥ 2 n=34 %	OFS ≥ 3 n=18 %	OFS ≥ 4 n=12 %
G	**53.0** (135)	**57.0** (39)	**67.0** (24)	**62.5** (15)
A	**47.0** (119)	**43.0** (29)	**33.0** (12)	**37.5** (9)
Total alleles (n)	257	68	36	24

Table 6.76 IL-10 –1117 allele frequencies in normal controls and acute pancreatitis patients grouped according to (systemic) disease severity as assessed by organ failure score

This table displays IL-10 –1117 allele frequencies in control and patient group subjects. For each allele the **% frequency** and the actual number of alleles (in brackets) is given.

n represents the number of subjects in each group

(n) represents the number of alleles in each group.

OFS Organ failure score

OFS calculated according to method described in section 5.1.4 (Kingsnorth et al, 1995).

Comparisons were made for each allele between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.

No differences are seen in the frequency of individual alleles between patient groups or between patient groups and controls.

IL-10 –1117 genotype	Controls n=127 %	OFS ≥ 2 n=34 %	OFS ≥ 3 n=18 %	OFS ≥ 4 n=12 %
GG	**29.1** (37)	**35.3** (12)	**50.0** (9)	**41.7** (5)
GA	**48.0** (61)	**44.1** (15)	**33.3** (6)	**41.7** (5)
AA	**22.8** (29)	**20.6** (7)	**16.7** (3)	**16.7** (2)
Total	127	33	18	12

Table 6.77 IL-10 -1117 genotype frequencies in normal controls and acute pancreatitis patients grouped according to (systemic) disease severity as assessed by organ failure score

This table displays IL-10 -1117 genotype frequencies in control and patient group subjects. For each genotype the **% frequency** and their actual number (in brackets) is given.
n represents the number of subjects in each group
OFS Organ failure score
OFS calculated according to method described in section 5.1.4 (Kingsnorth et al, 1995).
Comparisons were made between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.
No differences are seen in the gene frequency between patient groups or between patient groups and controls.

IL-10 –1117 allele	Controls n=127 %	Alcohol n=21 %	Idiopathic n=33 %	Gallstones n=70 %
G	**53.0** (135)	**57.0** (24)	**58.0** (37)	**55.0** (72)
A	**47.0** (119)	**43.0** (18)	**42.0** (27)	**45.0** (60)
Total alleles (n)	257	42	64	132

Table 6.78 IL-10 –1117 allele frequencies in normal controls and acute pancreatitis patients grouped according to aetiology of acute pancreatitis

This table displays IL-10 –1117 allele frequencies in control and patient group subjects. For each allele the **% frequency** and the actual number of alleles (in brackets) is given.

n represents the number of subjects in each group

(n) represents the number of alleles in each group.

Alcoholic pancreatitis if daily consumption of ≥ 80g alcohol day^{-1}

Gallstones if radiological or ERCP evidence of cholelithiasis

Idiopathic if no identifiable aetiology

Comparisons were made for each allele between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.

No differences are seen in the frequency of individual alleles between patient groups or between patient groups and controls.

IL-10 –1117 genotype	Controls n=127 %	Alcohol n=21 %	Idiopathic n=33 %	Gallstones n=70 %
GG	**29.1** (37)	**33.3** (7)	**34.4** (11)	**28.8** (19)
GA	**48.0** (61)	**47.6** (10)	**46.9** (15)	**51.5** (34)
AA	**22.8** (29)	**19.0** (4)	**18.8** (6)	**19.7** (13)
Total	127	21	32	66

Table 6.79 IL-10 –1117 genotype frequencies in normal controls and acute pancreatitis patients grouped according to aetiology of acute pancreatitis

This table displays IL-10 –1117 genotype frequencies in control and patient group subjects.
For each genotype the **% frequency** and their actual number (in brackets) is given.
n represents the number of subjects in each group
Alcoholic pancreatitis if daily consumption of ≥ 80g alcohol day^{-1}
Gallstones if radiological or ERCP evidence of cholelithiasis
Idiopathic if no identifiable aetiology
Comparisons were made between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.
No differences are seen in gene frequency between patient groups or between patient groups and controls.

6.14 IL-10 -854 locus typing

IL-10 -854 allelic and genotype frequencies were measured in 133 normal controls and 127 acute pancreatitis patients (94.1%). They were not measured in the group of alcoholic 'controls'.

Table 6.80 shows allelic frequencies of the –854 locus in patient and normal control subjects. No significant differences between patient and control groups are seen.

Table 6.81 shows genotype frequencies of the –854 locus in patient and normal control subjects. No significant differences between patient and control groups are seen.

The expected frequencies for IL-10 –854 genotypes were calculated. A 2 x n analysis showed that frequencies of heterozygotes and homozygotes in both study and normal control populations were in Hardy Weinberg equlilibrium. This is shown in Table 6.82.

Tables 6.83 and 6.84 show allelic and genotype frequencies at the -854 locus in patient groups stratified according to disease severity classified according to the Atlanta classification and the occurrence of a local complication. Tables 6.85 and 6.86 show allelic and genotype frequencies at the -854 locus in patient groups stratified according to organ failure score.

There are no significant differences in the allelic or genotype frequencies of the IL-10 –854 locus between the above patient groups.

Tables 6.87 and 6.88 show allelic and genotype frequencies at the -854 locus in patient groups according to the aetiology of their disease. Again, there are no significant differences in the allelic or genotype frequencies of the IL-10 –854 locus between the groups.

IL-10 –854 allele	Controls n=133 %	Study patients n=127 %
C	**74.0** (196)	**75.0** (191)
T	**26.0** (70)	**25.0** (63)
Total alleles (n)	266	254

Table 6.80 IL-10 –854 allele frequencies in normal controls and acute pancreatitis patients

This table displays IL-10 –854 allele frequencies in control and patient subjects. For each allele the **% frequency** and the actual number of alleles (in brackets) is given.
n represents the number of subjects in each group
(n) represents the number of alleles in each group.
Comparisons were made for each allele between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.
No significant differences between patient and control groups are seen.

IL-10 –854 genotype	Controls n=133 %	Study patients n=127 %
CC	**57.1** (76)	**58.3** (74)
CT	**33.1** (44)	**33.9** (43)
TT	**9.8** (13)	**7.9** (10)
Total	127	124

Table 6.81 IL-10 –854 genotype frequencies in normal controls and acute pancreatitis patients

This table displays IL-10 –854 genotype frequencies in control and patient subjects. For each genotype the **% frequency** and their actual number (in brackets) is given.
n represents the number of subjects in each group
Comparisons were made for each genotype between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made. No significant differences between patient and control groups are seen.

-854 Genotype	Expected		Observed		χ^2
	n	Freq.	n	Freq.	
Controls					
CC	72.8	54.7	76	57.1	0.1
CT	51.2	38.5	44	33.1	1.0
TT	9	6.7	13	9.8	1.8
Patients					
CC	71.4	56.3	74	58.3	0.1
CT	47.6	37.5	43	33.9	0.4
TT	7.9	6.3	10	7.9	0.6

Table 6.82 Frequency (%) of IL-10 –854 genotypes in patient and control subjects

Showing expected and observed frequencies of IL-10 –854 genotypes in patient and normal control subjects. Demonstrates that distribution conforms to Hardy-Weinberg equilibrium.

IL-10 –854 allele	Controls n=133 %	Mild n=91 %	Severe n=36 %	Local complication n=19 %
C	**74.0** (196)	**77.0** (140)	**71.0** (51)	**66.0** (25)
T	**26.0** (70)	**23.0** (42)	**29.0** (21)	**34.0** (13)
Total alleles (n)	266	182	72	38

Table 6.83 IL-10 –854 allele frequencies in normal controls and acute pancreatitis patients grouped according to (local and systemic) disease severity

This table displays IL-10 –854 allele frequencies in control and patient sub-groups. For each allele the **% frequency** and the actual number of alleles (in brackets) is given.

n represents the number of subjects in each group

(n) represents the number of alleles in each group.

Mild and severe disease severity groups are according to the Atlanta convention classification (Bradley, 1993), described in section 5.1.4.

Local complication is defined as occurrence of pancreatic necrosis, abscess or pseudocyst.

Comparisons were made for each allele between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.

No significant differences between patient and control groups are seen.

IL-10 –854 genotype	Controls n=133 %	Mild n=91 %	Severe n=36 %	Local complication n=19 %
CC	**57.1** (76)	**60.4** (55)	**52.8** (19)	**52.6** (10)
CT	**33.1** (44)	**33.0** (30)	**36.1** (13)	**26.3** (5)
TT	**9.8** (13)	**6.6** (6)	**11.1** (4)	**21.1** (4)
Total	133	91	36	19

Table 6.84 IL-10 –854 genotype frequencies in normal controls and acute pancreatitis patients grouped according to (local and systemic) disease severity

This table displays IL-10 –854 genotype frequencies in control and patient sub-groups. For each genotype the **% frequency** and their actual number (in brackets) is given.
n represents the number of subjects in each group
Mild and severe disease severity groups are according to the Atlanta convention classification (Bradley, 1993), described in section 5.1.4.
Local complication is defined as occurrence of pancreatic necrosis, abscess or pseudocyst.
Comparisons were made between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.
No significant differences between patient and control groups are seen.

IL-10 –854 allele	Controls n=133 %	OFS ≥ 2 n=34 %	OFS ≥ 3 n=18 %	OFS ≥ 4 n=12 %
C	**74.0** (196)	**74.0** (50)	**75.0** (27)	**79.2** (19)
T	**26.0** (70)	**26.0** (18)	**25.0** (9)	**20.8** (5)
Total alleles (n)	266	68	36	24

Table 6.85 IL-10 –854 allele frequencies in normal controls and acute pancreatitis patients grouped according to (systemic) disease severity as assessed by organ failure score

This table displays IL-10 –854 allele frequencies in control and patient group subjects. For each allele the **% frequency** and the actual number of alleles (in brackets) is given.

n represents the number of subjects in each group

(n) represents the number of alleles in each group.

OFS Organ failure score

OFS calculated according to method described in section 5.1.4 (Kingsnorth et al, 1995).

Comparisons were made for each allele between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.

No differences are seen in the frequency of individual alleles between patient groups or between patient groups and controls.

IL-10 –854 genotype	Controls n=133 %	OFS ≥ 2 n=34 %	OFS ≥ 3 n=18 %	OFS ≥ 4 n=12 %
CC	**57.1** (76)	**52.9** (18)	**55.6** (10)	**58.3** (7)
CT	**33.1** (44)	**41.2** (14)	**38.9** (7)	**41.7** (5)
TT	**9.8** (13)	**5.9** (2)	**5.6** (1)	**0.0** (0)
Total	133	33	18	12

Table 6.86 IL-10 –854 genotype frequencies in normal controls and acute pancreatitis patients grouped according to (systemic) disease severity as assessed by organ failure score

This table displays IL-10 –854 genotype frequencies in control and patient group subjects. For each genotype the **% frequency** and their actual number (in brackets) is given.
n represents the number of subjects in each group
OFS Organ failure score
OFS calculated according to method described in section 5.1.4 (Kingsnorth et al, 1995).
Comparisons were made between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.
No differences are seen in the gene frequency between patient groups or between patient groups and controls.

IL-10 –854 allele	Controls n=133 %	Alcohol n=21 %	Idiopathic n=35 %	Gallstones n=71 %
C	**74.0** (196)	**76.0** (32)	**68.0** (45)	**78.0** (105)
T	**26.0** (70)	**24.0** (10)	**32.0** (21)	**22.0** (29)
Total alleles (n)	266	42	66	134

Table 6.87 IL-10 –854 allele frequencies in normal controls and acute pancreatitis patients grouped according to aetiology of acute pancreatitis

This table displays IL-10 –854 allele frequencies in control and patient group subjects. For each allele the **% frequency** and the actual number of alleles (in brackets) is given.

n represents the number of subjects in each group

(n) represents the number of alleles in each group.

Alcoholic pancreatitis if daily consumption of ≥ 80g alcohol day^{-1}

Gallstones if radiological or ERCP evidence of cholelithiasis

Idiopathic if no identifiable aetiology

Comparisons were made for each allele between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.

No differences are seen in the frequency of individual alleles between patient groups or between patient groups and controls.

IL-10 –854 genotype	Controls n=133 %	Alcohol n=21 %	Idiopathic n=35 %	Gallstones n=71 %
CC	**57.1** (76)	**61.9** (13)	**51.5** (17)	**61.2** (41)
CT	**33.1** (44)	**28.6** (6)	**33.3** (11)	**34.3** (23)
TT	**9.8** (13)	**9.5** (2)	**15.2** (5)	**4.5** (3)
Total	133	21	33	67

Table 6.88 IL-10 –854 genotype frequencies in normal controls and acute pancreatitis patients grouped according to aetiology of acute pancreatitis

This table displays IL-10 –854 genotype frequencies in control and patient group subjects.
For each genotype the **% frequency** and their actual number (in brackets) is given.
n represents the number of subjects in each group
Alcoholic pancreatitis if daily consumption of ≥ 80g alcohol day^{-1}
Gallstones if radiological or ERCP evidence of cholelithiasis
Idiopathic if no identifiable aetiology
Comparisons were made between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.
No differences are seen in gene frequency between patient groups or between patient groups and controls.

6.15 IL-10 -627 locus typing

IL-10 -627 allelic and genotype frequencies were measured in 127 normal controls and 124 acute pancreatitis patients (92%). They were not measured in the group of alcoholic 'controls'.

Table 6.89 shows allelic frequencies of the –627 locus in patient and normal control subjects. No significant differences between patient and control groups are seen.

Table 6.90 shows genotype frequencies of the –627 locus in patient and normal control subjects. No significant differences between patient and control groups are seen.

The expected frequencies for IL-10 –627 genotypes were calculated. A 2 x n analysis showed that frequencies of heterozygotes and homozygotes in both study and normal control populations were in Hardy Weinberg equlilibrium. This is shown in Table 6.91.

Tables 6.92 and 6.93 show allelic and genotype frequencies at the -627 locus in patient groups stratified according to disease severity classified according to the Atlanta classification and the occurrence of a local complication. Tables 6.94 and 6.95 show allelic and genotype frequencies at the -627 locus in patient groups stratified according to organ failure score.

There are no significant differences in the allelic or genotype frequencies of the IL-10 –627 locus between the above patient groups.

Tables 6.96 and 6.97 show allelic and genotype frequencies at the -627 locus in patient groups according to the aetiology of their disease. Again, there are no significant differences in the allelic or genotype frequencies of the IL-10 –627 locus between the groups.

IL-10 –627 allele	Controls n=134 %	Study patients n=125 %
C	**63.8** (171)	**67.0** (167)
A	**36.2** (97)	**33.2** (83)
Total alleles (n)	268	250

Table 6.89 IL-10 –627 allele frequencies in normal controls and acute pancreatitis patients

This table displays IL-10 –627 allele frequencies in control and patient subjects. For each allele the **% frequency** and the actual number of alleles (in brackets) is given.
n represents the number of subjects in each group
(n) represents the number of alleles in each group.
Comparisons were made for each allele between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.
No significant differences between patient and control groups are seen.

IL-10 –627 genotype	Controls n=134 %	Study patients n=125 %
CC	**41.0** (55)	**47.2** (59)
CA	**45.5** (61)	**39.2** (49)
AA	**13.4** (18)	**13.6** (17)
Total	134	125

Table 6.90 IL-10 –627 genotype frequencies in normal controls and acute pancreatitis patients

This table displays IL-10 –627 genotype frequencies in control and patient subjects. For each genotype the **% frequency** and their actual number (in brackets) is given.
n represents the number of subjects in each group
Comparisons were made for each genotype between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.
No significant differences between patient and control groups are seen.

-627Genotype	Expected		Observed		χ^2
	n	Freq.	n	Freq.	
Controls					
CC	54.9	41.0	55	41.0	0.0
CA	61.7	46.0	61	45.6	0.0
AA	17.4	13.0	18	13.4	0.0
Patients					
CC	56.1	44.9	59	47.2	0.1
CA	55.3	44.2	49	39.2	0.7
AA	13.6	10.9	17	13.6	0.9

Table 6.91 Frequency (%) of IL-10 –627 genotypes in patient and control subjects

Showing expected and observed frequencies of IL-10 -627genotypes in patient and normal control subjects. Demonstrates that distribution conforms to Hardy-Weinberg equilibrium.

IL-10 –627 allele	Controls n=134 %	Mild n=91 %	Severe n=34 %	Local complication n=18 %
C	**63.8** (171)	**68.0** (124)	**63.0** (43)	**56.0** (20)
A	**36.2** (97)	**32.0** (58)	**36.8** (25)	**44.0** (16)
Total alleles (n)	268	182	68	36

Table 6.92 IL-10 –627 allele frequencies in normal controls and acute pancreatitis patients grouped according to (local and systemic) disease severity

This table displays IL-10 –627 allele frequencies in control and patient sub-groups. For each allele the **% frequency** and the actual number of alleles (in brackets) is given.

n represents the number of subjects in each group

(n) represents the number of alleles in each group.

Mild and severe disease severity groups are according to the Atlanta convention classification (Bradley, 1993), described in section 5.1.4.

Local complication is defined as occurrence of pancreatic necrosis, abscess or pseudocyst.

Comparisons were made for each allele between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.

No significant differences between patient and control groups are seen.

IL-10 –627 genotype	Controls n=134 %	Mild n=91 %	Severe n=34 %	Local complication n=18 %
CC	**41.0** (55)	**49.5** (45)	**41.2** (14)	**33.3** (6)
CA	**45.5** (61)	**37.4** (34)	**44.1** (15)	**44.4** (8)
AA	**13.4** (18)	**13.2** (12)	**14.7** (5)	**22.2** (4)
Total	134	91	34	18

Table 6.93 IL-10 –627 genotype frequencies in normal controls and acute pancreatitis patients grouped according to (local and systemic) disease severity

This table displays IL-10 –627 genotype frequencies in control and patient sub-groups. For each genotype the **% frequency** and their actual number (in brackets) is given.
n represents the number of subjects in each group
Mild and severe disease severity groups are according to the Atlanta convention classification (Bradley, 1993), described in section 5.1.4.
Local complication is defined as occurrence of pancreatic necrosis, abscess or pseudocyst.
Comparisons were made between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.
No significant differences between patient and control groups are seen.

IL-10 –627 allele	Controls n=134 %	OFS ≥ 2 n=33 %	OFS ≥ 3 n=18 %	OFS ≥ 4 n=12 %
C	**63.8** (171)	**68.0** (45)	**72.0** (26)	**70.8** (17)
A	**36.2** (97)	**32.0** (21)	**28.0** (10)	**29.2** (7)
Total alleles (n)	268	66	36	24

Table 6.94 IL-10 –627 allele frequencies in normal controls and acute pancreatitis patients grouped according to (systemic) disease severity as assessed by organ failure score

This table displays IL-10 –627 allele frequencies in control and patient group subjects. For each allele the **% frequency** and the actual number of alleles (in brackets) is given.
n represents the number of subjects in each group
(n) represents the number of alleles in each group.
OFS Organ failure score
OFS calculated according to method described in section 5.1.4 (Kingsnorth et al, 1995).
Comparisons were made for each allele between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.
No differences are seen in the frequency of individual alleles between patient groups or between patient groups and controls.

IL-10 –627 genotype	Controls n=134 %	OFS ≥ 2 n=33 %	OFS ≥ 3 n=18 %	OFS ≥ 4 n=12 %
CC	**41.0** (55)	**45.5** (15)	**50.0** (9)	**50.0** (6)
CA	**45.5** (61)	**45.5** (15)	**44.4** (8)	**41.7** (5)
AA	**13.4** (18)	**9.1** (3)	**5.6** (1)	**8.3** (1)
Total	134	33	18	12

Table 6.95 IL-10 –627 genotype frequencies in normal controls and acute pancreatitis patients grouped according to (systemic) disease severity as assessed by organ failure score

This table displays IL-10 –627 genotype frequencies in control and patient group subjects. For each genotype the **% frequency** and their actual number (in brackets) is given.
n represents the number of subjects in each group
OFS Organ failure score
OFS calculated according to method described in section 5.1.4 (Kingsnorth et al, 1995).
Comparisons were made between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.
No differences are seen in the gene frequency between patient groups or between patient groups and controls.

IL-10 –627 allele	Controls n=134 %	Alcohol n=21 %	Idiopathic n=34 %	Gallstones n=71 %
C	**63.8** (171)	**69.0** (29)	**63.0** (39)	**68.0** (90)
A	**36.2** (97)	**31.0** (13)	**37.0** (23)	**32.0** (42)
Total alleles (n)	268	42	62	132

Table 6.96 IL-10 –627 allele frequencies in normal controls and acute pancreatitis patients grouped according to aetiology of acute pancreatitis

This table displays IL-10 –627 allele frequencies in control and patient group subjects. For each allele the % frequency and the actual number of alleles (in brackets) is given.

n represents the number of subjects in each group

(n) represents the number of alleles in each group.

Alcoholic pancreatitis if daily consumption of ≥ 80g alcohol day^{-1}

Gallstones if radiological or ERCP evidence of cholelithiasis

Idiopathic if no identifiable aetiology

Comparisons were made for each allele between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.

No differences are seen in the frequency of individual alleles between patient groups or between patient groups and controls.

IL-10 –627 genotype	Controls n=134 %	Alcohol n=21 %	Idiopathic n=31 %	Gallstones n=66 %
CC	**41.0** (55)	**47.6** (10)	**41.9** (13)	**50.0** (33)
CA	**45.5** (61)	**42.9** (9)	**41.9** (13)	**36.4** (24)
AA	**13.4** (18)	**9.5** (2)	**16.1** (5)	**13.6** (9)
Total	134	21	31	66

Table 6.97 IL-10 –627 genotype frequencies in normal controls and acute pancreatitis patients grouped according to aetiology of acute pancreatitis

This table displays **IL-10 –627** genotype frequencies in control and patient group subjects. For each genotype the **% frequency** and their actual number (in brackets) is given.
n represents the number of subjects in each group
Alcoholic pancreatitis if daily consumption of ≥ 80g alcohol day^{-1}
Gallstones if radiological or ERCP evidence of cholelithiasis
Idiopathic if no identifiable aetiology
Comparisons were made between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.
No differences are seen in gene frequency between patient groups or between patient groups and controls.

6.16 IL-10 –1117 / 854 / 627 2 and 3-locus haplotypes

The –1117, -854, and –627 loci are situated close together within a 490 bp region in the 5' (promoter) region of the IL-10 gene. They can be expected to associate with each other in a non-random fashion forming 2 and 3-locus haplotypes. The evidence for this occurring in the study populations is detailed in Table 6.106, and discussed in section 6.17.

Table 6.98 shows frequencies of –1117 / -854 / -627 3-locus haplotypes in normal control and patient subjects.

3-locus haplotypes were assigned when an individual was homozygous at two or more loci. This was not possible in all subjects due to the high frequency of heterozygocity at all three loci. The number of subjects in the control group to whom 3-locus haplotypes could be assigned was 68 out of 127 (53.5%) in whom all 3 loci had been typed. The number of subjects in the patient group to whom 3-locus haplotypes could be assigned was 71 out of 124 (57.3%) in whom all 3 loci had been typed

A greater number of g-c-c haplotypes were able to be assigned in patient as compared to the control group, χ^2 =7.58, p=0.0059, p_c=0.047. This however, assumes less significance than at first sight, as haplotypes were able to be assigned in only just over half of patient and control subjects due to the high number of heterozygotes seen. The higher figure seen in the patient group is not confirmed on analysis of the 2-locus haplotypes (tables 6.100-102).

A greater number of a-c-a haplotypes were able to be assigned in the control as compared to the study group, χ^2 =9.8, p=0.0017, p_c=0.014. This however, assumes less significance than at first sight, as haplotypes were able to be assigned in only c. 50% of patient and control subjects due to the high number of heterozygotes seen. The higher figure seen in the patient group is not confirmed on analysis of the 2-locus haplotypes (tables 6.100-6.102).

Table 6.99 shows IL-10 –1117 / 854 / 627 3-locus haplotype frequencies in normal control and patient subjects, and patients grouped according to disease severity and aetiology as previously.

3-locus haplotypes were assigned when an individual was homozygous at two or more loci. This was not possible in all subjects so comparison between groups is of limited value due to not being to assign haplotypes in individuals heterozygous at more than one locus, so resulting in small numbers. Also the frequency of haplotypes that have been able to be assigned may not give a true estimate of actual frequencies. There is no significant difference in comparison between all subject groups beyond that discussed above and shown in Table 6.98.

Table 6.100 shows IL-10 –1117 / 854 2- locus haplotypes in normal control and patient subjects. 2-locus haplotypes were assigned when an individual was homozygous at one or more loci. This was possible in 98 of 127 (77.2%) of controls and 99 of 124 (79.8%) of patients.

No differences are seen in haplotype frequency between control and patient groups.

Table 6.101 shows IL-10 –1117 / 627 2- locus haplotypes in normal control and patient subjects. 2-locus haplotypes were assigned when an individual was homozygous at one or more loci. This was possible in 86 of 127 (67.7%) of controls and 92 of 124 (74.2%) of patients.

No differences are seen in haplotype frequency between control and patient groups.

Table 6.102 shows IL-10 –854 / 627 2- locus haplotypes in normal control and patient subjects. 2-locus haplotypes were assigned when an individual was homozygous at one or more loci. This was possible in 92 of 127 (72.4%) of controls and 96 of 124 (77.4%) of patients.

No differences are seen in haplotype frequency between control and patient groups.

Table 6.103 shows IL-10 –1117 / 854 2-locus haplotype frequencies in normal control and patient subjects, and patients grouped according to disease severity and aetiology as previously. Comparison between groups is of limited value due to not being to assign haplotypes in individuals heterozygous at more than one locus, so resulting in small

numbers. Also the frequency of haplotypes that have been able to be assigned may not give a true estimate of actual frequencies.

No differences are seen in gene frequency between control and patient groups.

Table 6.104 shows IL-10 –1117 / 627 2-locus haplotype frequencies in normal control and patient subjects, and patients grouped according to disease severity and aetiology as previously. Comparison between groups is of limited value due to not being to assign haplotypes in individuals heterozygous at more than one locus, so resulting in small numbers. Also the frequency of haplotypes that have been able to be assigned may not give a true estimate of actual frequencies.

No differences are seen in gene frequency between control and patient groups.

Table 6.105 shows IL-10 –854 / 627 2-locus haplotype frequencies in normal control and patient subjects, and patients grouped according to disease severity and aetiology as previously. Comparison between groups is of limited value due to not being to assign haplotypes in individuals heterozygous at more than one locus, so resulting in small numbers. Also the frequency of haplotypes that have been able to be assigned may not give a true estimate of actual frequencies.

No differences are seen in gene frequency between control and patient groups.

IL-10 –1117 / 854 / 627 3-locus haplotype	Controls n=68/127 %	Study patients n=71/124 %
g-c-c	**42.6** (58)	**59.2** [1] (84)
a-c-c	**24.3** (33)	**23.2** (33)
a-t-a	**12.5** (17)	**9.2** (13)
a-c-a	**11.0** (15)	**2.1** [2] (3)
g-t-c	**2.2** (3)	**0.7** (1)
g-c-a	**3.7** (5)	**1.4** (2)
a-t-c	**2.2** (3)	**2.8** (4)
g-t-a	**1.5** (2)	**1.4** (2)
Total number of chromosomes	136	142

Table 6.98 IL-10 –1117 / 854 / 627 3-locus haplotype frequencies in normal control and patient subjects

Frequencies (%) and actual number (in brackets) of IL-10 –1117 / 854 / 627 3-locus haplotypes able to be determined in each group are shown.

n number of patients

(n) number of chromosomes

3-locus haplotypes were assigned when an individual was homozygous at two or more loci. This was not possible in all subjects so the total number of subjects is also given (n/n).

1. A greater number of g-c-c haplotypes were able to be assigned in patient as compared to the control group, χ^2 =7.58, p=0.0059, p_c=0.047. This however, assumes less significance than at first sight, as haplotypes were able to be assigned in only c. 50% of patient and control subjects due to the high number of heterozygotes seen. The higher figure seen in the patient group is not confirmed on analysis of the 2-locus haplotypes (tables 6.100-102).
2. A greater number of a-c-a haplotypes were able to be assigned in the control as compared to the study group, χ^2 =9.8, p=0.0017, p_c=0.014. This however, assumes less significance than at first sight, as haplotypes were able to be assigned in only c. 50% of patient and control subjects due to the high number of heterozygotes seen. The higher figure seen in the patient group is not confirmed on analysis of the 2-locus haplotypes (tables 6.100-102).

IL-10 –1117 / 854 / 627 3-locus haplotype	Controls n=68 %	Study patients n=71 %	Mild n=52 %	Severe n=19 %	Local complic[n] n=9 %	OFS ≥ 2 n=20 %	OFS ≥ 3 n=12 %	OFS ≥ 4 n=7 %	Alcohol n=13 %	Idiopath n=19 %	G\stones n=36 %
g-c-c	**42.6**	**59.2**	**60.6**	**55.3**	**50.0**	**60.0**	**62.5**	**50.0**	**57.7**	**55.3**	**65.3**
	(58)	(84)	(63)	(21)	(9)	(24)	(15)	(7)	(15)	(21)	(47)
a-c-c	**24.3**	**23.2**	**25.0**	**18.4**	**5.6**	**20.0**	**12.5**	**21.4**	**19.2**	**21.1**	**25.0**
	(33)	(33)	(26)	(7)	(1)	(8)	(3)	(3)	(5)	(8)	(18)
a-t-a	**12.5**	**9.2**	**6.7**	**15.8**	**27.8**	**5.0**	**4.2**	**7.1**	**11.5**	**13.2**	**5.6**
	(17)	(13)	(7)	(6)	(5)	(2)	(1)	(1)	(3)	(5)	(4)
a-c-a	**11.0**	**2.1**	**1.9**	**2.6**	**0.0**	**2.5**	**4.2**	**7.1**	**0.0**	**5.3**	**1.4**
	(15)	(3)	(2)	(1)	(0)	(1)	(1)	(1)	(0)	(2)	(1)
g-t-c	**2.2**	**0.7**	**1.0**	**0.0**	**0.0**	**2.5**	**4.2**	**0.0**	**0.0**	**2.6**	**0.0**
	(3)	(1)	(1)	(0)	(0)	(1)	(1)	(0)	(0)	(1)	(0)
g-c-a	**3.7**	**1.4**	**1.0**	**2.6**	**5.6**	**2.5**	**4.2**	**7.1**	**3.8**	**0.0**	**1.4**
	(5)	(2)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(0)	(1)
a-t-c	**2.2**	**2.8**	**2.9**	**2.6**	**5.6**	**2.5**	**4.2**	**7.1**	**7.7**	**0.0**	**1.4**
	(3)	(4)	(3)	(1)	(1)	(1)	(1)	(1)	(2)	(0)	(1)
g-t-a	**1.5**	**1.4**	**1.0**	**2.6**	**5.6**	**5.0**	**4.2**	**0.0**	**0.0**	**2.6**	**0.0**
	(2)	(2)	(1)	(1)	(1)	(2)	(1)	(0)	(0)	(1)	(0)
Total number of chromosomes	136	142	104	38	18	40	24	14	26	38	72

Table 6.99 IL-10 –1117 / 854 / 627 3-locus haplotype frequencies in normal control and patient subjects, and patients grouped according to disease severity and aetiology as previously.

Frequencies (%) and actual number (in brackets) of IL-10 –1117 / 854 / 627 3-locus haplotypes able to be determined in each group are shown.
3-locus haplotypes were assigned when an individual was homozygous at two or more loci. This was not possible in all subjects so the total number of patients is also given (n/n). The difference between control and patient groups as previously noted in Table 6.96 is shown, but comparison between groups is limited due to the small number in each group.

IL-10 –1117 / 854 2-locus haplotype	Controls n=98/127 %	Study patients n=99/124 %
g-c	**42.9** (84)	**51.0** (101)
a-c	**36.7** (72)	**30.3** (60)
a-t	**15.8** (31)	**14.1** (28)
g-t	**4.6** (9)	**4.5** (9)
Total number of chromosomes	196	198

Table 6.100 IL-10 –1117 / 854 2- locus haplotypes in normal control and patient subjects

Frequencies (%) and actual number (in brackets) of IL-10 –1117 / 854 2-locus haplotypes able to be determined in each group are shown.
n number of patients
(n) number of chromosomes
2-locus haplotypes were assigned when an individual was homozygous at one or more loci. This was not possible in all subjects so the total number of patients is also given (n/n).
Comparisons were made between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.
No differences are seen in gene frequency between control and patient groups.

IL-10 –1117 / 627 2-locus haplotype	Controls n=86/127 %	Study patients n=92/124 %
g-c	**43.0** (74)	**48.9** (90)
a-a	**27.9** (48)	**17.9** (33)
a-c	**23.3** (40)	**24.5** (45)
g-a	**5.8** (10)	**8.7** (16)
Total number of chromosomes	172	184

Table 6.101 IL-10 –1117 / 627 2- locus haplotypes in normal control and patient subjects

Frequencies (%) and actual number (in brackets) of IL-10 –1117 / 854 2-locus haplotypes able to be determined in each group are shown.
n number of patients
(n) number of chromosomes
2-locus haplotypes were assigned when an individual was homozygous at one or more loci. This was not possible in all subjects so the total number of patients is also given (n/n).
Comparisons were made between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.
No differences are seen in gene frequency between control and patient groups.

IL-10 –854 / 627 2-locus haplotype	Controls n=92/127 %	Study patients n=96/124 %
c-c	**64.1** (118)	**67.7** (130)
c-a	**17.9** (33)	**14.6** (28)
t-a	**13.6** (24)	**13.5** (26)
t-c	**4.9** (9)	**4.2** (8)
Total number of chromosomes	184	192

Table 6.102 IL-10 –854 / 627 2- locus haplotypes in normal control and patient subjects

Frequencies (%) and actual number (in brackets) of IL-10 –1117 / 854 2-locus haplotypes able to be determined in each group are shown.
n number of patients
(n) number of chromosomes
2-locus haplotypes were assigned when an individual was homozygous at one or more loci. This was not possible in all subjects so the total number of patients is also given (n/n).
Comparisons were made between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.
No differences are seen in gene frequency between control and patient groups.

IL-10 –1117 / 854 2-locus haplotype	Controls n=98 %	Study patients n=99 %	Mild n=52 %	Severe n=19 %	Local complic[n] n=9 %	OFS ≥ 2 n=20 %	OFS ≥ 3 n=12 %	OFS ≥ 4 n=7 %	Alcohol n=13 %	Idiopath n=19 %	G\stones n=36 %
g-c	**42.9** (84)	**51.0** (101)	**51.4** (74)	**50.0** (27)	**50.0** (13)	**50.0** (25)	**60.0** (18)	**55.6** (10)	**55.6** (20)	**54.8** (23)	**49.1** (55)
a-c	**36.7** (72)	**30.3** (60)	**31.3** (45)	**27.8** (15)	**23.1** (6)	**32.0** (16)	**20.0** (6)	**27.8** (5)	**25.0** (9)	**21.4** (9)	**32.1** (36)
a-t	**15.8** (31)	**14.1** (28)	**13.2** (19)	**16.7** (9)	**23.1** (6)	**10.0** (5)	**10.0** (3)	**11.1** (2)	**16.7** (6)	**14.3** (6)	**15.2** (17)
g-t	**4.6** (9)	**4.5** (9)	**4.2** (6)	**5.6** (3)	**3.8** (1)	**8.0** (4)	**10.0** (3)	**5.6** (1)	**2.8** (1)	**9.5** (4)	**3.6** (4)
Total number of chromosomes	196	198	144	54	26	50	30	18	36	42	112

Table 6.103 IL-10 –1117 / 854 2-locus haplotype frequencies in normal control and patient subjects, and patients grouped according to disease severity and aetiology as previously.

Frequencies (%) and actual number (in brackets) of IL-10 –1117 / 854 2-locus haplotypes able to be determined in each group are shown.

n number of patients

(n) number of chromosomes

2-locus haplotypes were assigned when an individual was homozygous at one or more loci. This was not possible in all subjects .

Comparisons were made between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.

No differences are seen in gene frequency between control and patient groups.

IL-10 –1117 / 627 2-locus haplotype	Controls n=86 %	Study patients n=92 %	Mild n=52 %	Severe n=19 %	Local complic[n] n=9 %	OFS ≥ 2 n=20 %	OFS ≥ 3 n=12 %	OFS ≥ 4 n=7 %	Alcohol n=13 %	Idiopath n=19 %	G\stones n=36 %
g-c	**43.0** (74)	**48.9** (90)	**49.3** (68)	**47.8** (22)	**50.0** (9)	**55.6** (25)	**60.7** (17)	**50.0** (8)	**50.0** (16)	**54.8** (23)	**49.0** (51)
a-a	**27.9** (48)	**17.9** (33)	**16.7** (23)	**21.7** (10)	**11.1** (2)	**15.6** (7)	**10.7** (3)	**12.5** (2)	**15.6** (5)	**16.7** (7)	**17.3** (18)
a-c	**23.3** (40)	**24.5** (45)	**26.1** (36)	**19.6** (9)	**22.2** (4)	**15.6** (7)	**17.9** (5)	**25.0** (4)	**25.0** (8)	**19.0** (8)	**26.0** (27)
g-a	**5.8** (10)	**8.7** (16)	**8.0** (11)	**10.9** (5)	**16.7** (3)	**13.3** (6)	**10.7** (3)	**12.5** (2)	**9.4** (3)	**9.5** (4)	7.7 (8)
Total number of chromosomes	172	184	138	46	18	45	28	16	32	42	104

Table 6.104 IL-10 –1117 / 627 2-locus haplotype frequencies in normal control and patient subjects, and patients grouped according to disease severity and aetiology as previously.

Frequencies (%) and actual number (in brackets) of IL-10 –1117 / 627 2-locus haplotypes able to be determined in each group are shown.
n number of patients
(n) number of chromosomes
2-locus haplotypes were assigned when an individual was homozygous at one or more loci. This was not possible in all subjects.
Comparisons were made between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.
No differences are seen in gene frequency between control and patient groups.

IL-10 –854 / 627 2-locus haplotype	Controls n=92 %	Study patients n=96 %	Mild n=52 %	Severe n=19 %	Local complic[n] n=9 %	OFS ≥ 2 n=20 %	OFS ≥ 3 n=12 %	OFS ≥ 4 n=7 %	Alcohol n=13 %	Idiopath n=19 %	G\stones n=36 %
c-c	**64.1** (118)	**67.7** (130)	**68.8** (99)	**64.6** (31)	**50.0** (15)	**72.7** (32)	**73.1** (19)	**68.8** (11)	**67.6** (23)	**62.5** (30)	**72.0** (72)
c-a	**17.9** (33)	**14.6** (28)	**15.3** (22)	**12.5** (6)	**16.7** (5)	**11.4** (5)	**11.5** (3)	**18.8** (3)	**11.8** (4)	**10.4** (5)	**15.0** (15)
t-a	**13.6** (24)	**13.5** (26)	**11.8** (17)	**18.8** (9)	**26.7** (8)	**11.4** (5)	7.7 (3)	**6.3** (1)	**17.6** (6)	**20.8** (10)	**11.0** (11)
t-c	**4.9** (9)	**4.2** (8)	**4.2** (6)	**4.2** (2)	**6.7** (2)	**4.5** (2)	7.7 (2)	**6.3** (1)	**2.9** (1)	**6.3** (3)	**2.0** (2)
Total number of chromosomes	184	192	144	48	30	44	26	16	34	48	100

Table 6.105 IL-10 –854 / 627 2-locus haplotype frequencies in normal control and patient subjects, and patients grouped according to disease severity and aetiology as previously.

Frequencies (%) and actual number (in brackets) of IL-10 –854 / 627 2-locus haplotypes able to be determined in each group are shown.

n number of patients

(n) number of chromosomes

2-locus haplotypes were assigned when an individual was homozygous at one or more loci. This was not possible in all subjects.

Comparisons were made between patient and control groups using 2x2 contingency tables and the χ^2 test. P values were corrected for the number of comparisons made.

No differences are seen in gene frequency between control and patient groups

6.17 IL-10.G / -1117 / -854 / -627 haplotypes

The IL-10.G locus is also close to the 3 bi-allelic polymorphisms at –1117, -854, and –627. It starts only c. 34 bp from the –1117 locus. Haplotypes can also be expected to exist between the 4 loci. Table 6.106 shows frequencies of the 3 locus (3 bi-allelic polymorphisms), and 4-locus haplotypes (3 bi-allelic polymorphisms and IL-10.G locus) in normal controls and provides evidence that allelic associations occur.

It was possible to determine IL-10.G / -1117 2-locus haplotypes in 75/124 (60.5%) of subjects, -1117 / 854 / 627 3-locus haplotypes in 71/124 subjects (57.3%), and IL-10.G / -1117 / 854 / 627 3-locus haplotypes in 43/124 subjects (34.7%).

An allelic association (haplotype) is seen to occur between IL-10.G9 and –1117.A (χ^2 =9.5, p=0.002).

The –1117 / -854 / -627 3-locus haplotype G-C-C is confirmed to exist at higher frequency than could be expected by non-random association of alleles (χ^2 =16.9, p<0.0001), as is the A-T-A haplotype (χ^2 =20.2, p<0.00001). The haplotypes A-C-C and A-C-A occur as frequently as expected from random association, whilst the 3-locus combinations G-T-C (χ^2=6.8, p=0.009), G-C-A (χ^2 =10.3, p=0.001), A-T-C (χ^2=5.5, p=0.019), and G-T-A (χ^2=3.3, p=0.067) occur much less frequently than can be expected from random association confirming that linkage disequilibrium occurs between the 3 loci to produce the increased frequency of the haplotypes G-C-C and A-T-A.

Haplotypes also exist between IL-10.G and the 3 bi-allelic loci. IL-10.G9 is seen to associate with the 3-locus haplotype A-T-A (Fishers exact test, p<0.0001).

In the patient group (Table 6.107), similar associations are seen. However, no association is seen between IL-10.G9 and -1117.A. The 3-locus haplotype A-C-A is seen at a lower frequency than is expected (χ^2=10.2, p=0.001), and the IL.G9 association with the 3-locus haplotype A-T-A, although present at slightly higher frequency, does not approach statistical significance (Fishers exact test, p=0.19). The A-T-A haplotype is present at lower

frequency in study patients compared to controls, which may account for this. It is not possible to compare 4-locus associations between patient groups, as numbers are too small.

IL-10.G / -1117 / -854 / -627 haplotypes				Expected		Observed		χ^2
IL-10.G	-1117	-854	-627	n	Freq.	n	Freq.	
9	A	-	-	27.0	20.6	43	32.8	9.5 [1]
9	G	-	-	30.4	23.2	27	20.6	0.4
13	A	-	-	13.0	10.0	12	9.2	0.1
13	G	-	-	14.8	11.3	18	13.7	0.7
X	A	-	-	21.5	16.4	30	22.9	3.4
X	G	-	-	24.2	18.5	11	8.4	7.2
-	G	C	C	34.0	25.0	58	42.6	16.9 [2]
-	A	C	C	30.3	22.3	33	24.3	0.3
-	A	T	A	6.0	4.4	17	12.5	20.2 [3]
-	A	C	A	17.0	12.5	15	11.0	0.2
-	G	T	C	12.0	8.8	3	2.2	6.8 [4]
-	G	C	A	19.0	14.0	5	3.7	10.3 [5]
-	A	T	C	10.6	8.0	3	2.2	5.5 [6]
-	G	T	A	6.7	4.9	2	1.5	3.3
9	G	C	C	11.6	18.7	13	19.7	0.2
9	A	C	C	6.6	10.6	0	0.0	6.5 [7]
9	A	T	A	3.4	5.5	15	22.7	p<0.003 [8]
9	Other haplotype			5.9	9.0	5	7.6	0.15
13	G	C	C	5.6	9.1	10	15.2	3.46
13	Other haplotype			8.0	12.2	5	7.6	1.1
X	G	C	C	9.8	14.9	7	10.6	0.8
X	Other haplotype			13.2	20.0	8	12.1	2.0

Table 6.106 IL-10.G / -1117 / -854 / -627 haplotype frequencies (%) in normal controls

Shows frequency (%) of IL-10.G / -1117 / -854 / -627 haplotype in the 5' flanking region of the IL-10 gene on chromosome 1 in normal controls.

X – IL-10.G allele, not G9 or G13

1. χ^2 =9.5, p=0.002
2. χ^2 =16.9, p<0.0001
3. χ^2 =20.2, p<0.00001
4. χ^2=6.8, p=0.009 (negative association)
5. χ^2 =10.3, p=0.001 (negative association)
6. χ^2=5.5, p=0.019 (negative association)
7. χ^2=6.5, p=0.01 (negative association)
8. As cell value is <5, χ^2 statistic not valid. Fishers exact test, p<0.003

Able to determine IL-10 G / -1117 2-locus haplotypes in 65/127 subjects
Able to determine -1117 / 854 / 627 3-locus haplotypes in 68/127 subjects
Able to determine IL-10 G / -1117 / 854 / 627 3-locus haplotypes in 31/127 subjects

IL-10.G / -1117 / -854 / -627 haplotypes				Expected		Observed		χ^2
IL-10.G	-1117	-854	-627	n	Freq.	n	Freq.	
9	A	-	-	27.6	18.4	29	19.3	0.1
9	G	-	-	35.3	23.5	42	28.0	1.3
13	A	-	-	12.9	8.6	11	7.3	0.3
13	G	-	-	16.5	11.0	18	12.0	0.1
X	A	-	-	25.4	16.9	23	15.3	0.2
X	G	-	-	32.4	21.6	27	18.0	0.9
-	G	C	C	39.9	28.1	84	59.2	48.7 [1]
-	A	C	C	31.4	22.1	33	23.2	0.1
-	A	T	A	5.3	3.7	13	9.2	11.2 [2]
-	A	C	A	15.6	11.0	3	2.1	10.2 [3]
-	G	T	C	13.9	9.8	1	0.7	12.0 [4]
-	G	C	A	19.7	13.9	2	1.4	15.9 [5]
-	A	T	C	10.5	7.4	4	2.8	4.0
-	G	T	A	6.5	4.6	2	1.4	3.1
9	G	C	C	21.3	24.8	28	32.2	2.1
9	A	C	C	8.4	9.7	2	2.3	4.9 [6]
9	A	T	A	3.3	3.9	7	8.0	p=0.16 [7]
9	Other haplotype			3.0	3.5	2	2.3	n/a
13	G	C	C	10	11.6	12	13.8	0.4
13	Other haplotype			6.8	7.9	5	5.7	0.5
X	G	C	C	19.6	22.8	26	29.9	2.1
X	Other haplotype			13.5	15.7	5	5.7	5.35

Table 6.107 IL-10.G / -1117 / -854 / -627 haplotype frequencies (%) in patients

Shows frequency (%) of IL-10.G / -1117 / -854 / -627 haplotype in the 5' flanking region of the IL-10 gene on chromosome 1 in patients.

X – IL-10.G allele, not G9 or G13

1. χ^2=48.7, p<10^{-5}
2. χ^2=11.2, p=0.0008
3. χ^2=10.2, p=0.0014
4. χ^2=12.0, p=0.00053
5. χ^2=15.9, p=0.00007
6. χ^2=4.9, p=0.27 (negative association)
7. Expected cell value <5, χ^2 statistic not valid. Fishers exact test, p=0.19

Able to determine IL-10.G / -1117 2-locus haplotypes in 75/124 subjects
Able to determine -1117 / 854 / 627 3-locus haplotypes in 71/124 subjects
Able to determine IL-10.G / -1117 / 854 / 627 3-locus haplotypes in 43/124 subjects

6.18 TNF locus and IL-10 locus genotype associations

Because the functions of IL-10 and TNF can be considered counter-regulatory with respect to the regulation of inflammation, it is of import to consider whether certain alleles or genotypes of each locus, which may have functional relevance by affecting TNF and IL-10 protein levels, occur in particular association in individuals. In this study, the analysis of associations of genotypes of the two cytokine genes is limited, as many loci are multi-allelic, producing many possible genotypes, so numbers of associations will be small.

6.18.1 IL-10.G microsatellite and TNFabc microsatellite 3-locus haplotype associations.

Table 6.108 shows that there is no association between IL-10.G genotypes containing allele G9 and TNFabc 3-locus genotypes containing certain haplotypes. Likewise Table 6.109 shows no association between IL-10.G genotypes containing allele G13 and TNFabc 3-locus genotypes. It is not possible to analyse individual genotype associations as most subjects are heterozygous at all 4 microsatellite loci. Furthermore it is pertinent to mention that the IL-10.G genotype G9,13 is present in 25% of control subjects, so there is some crossover between these two analyses.

The same analysis was done for the acute pancreatitis patients, IL-10.G genotypes containing allele G9 are shown in table 6.110 for patients classified as having mild pancreatitis. It was not possible to ascertain associations involving the G13 allele because of the small numbers involved. Likewise analysis of patients with severe disease was not possible due to the small numbers involved.

IL-10.G9 containing genotype / TNFabc association	Expected		Observed		χ^2
	n	Freq.	n	Freq.	
G 9 – a2b1c2	19.1	9.2	20	9.6	0.0
G 9- a2b3c1	22.4	10.8	21	10.1	0.1
G 9- a2b5c2	6.9	3.3	8	3.9	0.2
G 9- a6b5c1	21.0	10.1	18	8.7	0.4
G 9x- a7b4c1	9.4	4.5	9	4.3	0.0
G 9x- a11b4c1	23.9	11.5	23	11.1	0.0
G 9x- a10b4c1	12.3	5.9	12	5.8	0.0
G 9x- a4b5c1	8.9	4.3	9	4.3	0.0
G 9x- X	17.1	8.2	19	9.1	0.5

Table 6.108 Association of IL-10.G microsatellite genotype containing allele G9 with TNFabc haplotype in normal controls

Shows a lack of an association between possession of genotype containing allele 9 of IL-10.G and possession of a copy of the TNFabc 3-locus haplotype in normal controls.
X – undetermined TNFabc haplotype
Only those combinations with an expected number of >5 are included.

IL-10.G13 containing genotype / TNFabc association	Expected		Observed		χ^2
	n	Freq.	n	Freq.	
G 13x – a2b1c2	11.0	5.6	13	6.6	0.4
G 13x- a2b3c1	13.0	6.6	13	6.6	0.0
G 13x- a6b5c1	12.2	6.2	13	6.6	0.1
G 13x- a11b4c1	13.9	7.1	11	5.6	0.6
G 13x- a10b4c1	7.1	3.6	8	4.1	0.1
G 13x- a4b5c1	5.1	2.6	6	3.0	0.2
G 13x- X	9.9	5.0	10	5.1	0.0

Table 6.109 Association of IL-10.G microsatellite genotype containing allele G13 with TNFabc haplotype in normal controls

Shows a lack of an association between possession of genotype containing allele 13 of IL-10.G and possession of a copy of the TNFabc 3-locus haplotype in normal controls.
X – undetermined TNFabc haplotype
Only those combinations with an expected number of >5 are included.

IL-10.G9 containing genotype / TNFabc association	Expected		Observed		χ^2
	n	Freq.	n	Freq.	
G 9 – a2b1c2	14.7	7.8	20	10.6	1.9
G 9- a2b3c1	10.5	5.6	13	6.9	0.6
G 9- a6b5c1	19.8	10.5	23	12.2	0.5
G 9x- a7b4c1	6.8	3.6	4	2.1	1.2
G 9x- a11b4c1	22.6	12.0	24	12.8	0.1
G 9x- a10b4c1	7.3	3.9	8	4.3	0.1
G 9x- a4b5c1	8.5	4.5	10	5.3	0.3
G 9x- X	10.1	5.4	11	5.9	0.1

Table 6.110 Association of IL-10.G microsatellite genotype containing allele G9 with TNFabc haplotype in patients classified as having mild pancreatitis

Shows a lack of an association between possession of genotype containing allele 9 of IL-10.G and possession of a copy of the TNFabc 3-locus haplotype in patients classified as having mild pancreatitis.
X – undetermined TNFabc haplotype
Only those combinations with an expected number of >5 are included.

6.18.2 IL-10.G microsatellite and TNF –308 genotype associations.

A similar analysis was undertaken to look for an association between IL-10.G genotypes containing allele G9 or G13 and TNF –308 genotypes. Table 6.111 shows a lack of any such association in normal control subjects, whilst table 6.112 shows the same in patient subjects.

IL-10.G9 or G13 containing genotype / TNF-308 genotype association	Expected		Observed		χ^2
	n	Freq.	n	Freq.	
IL-10.G 9x - GG	41.4	44.5	40	43.0	0.0
IL-10.G 9x - GA	18.8	20.3	20	21.5	0.1
IL-10.G 9x - GA\AA	23.6	25.4	24	25.8	0.0
IL-10.G 13x - GG	23.9	25.7	25	26.9	0.1
IL-10.G 13x - GA	11.0	11.8	15	16.1	1.5
IL-10.G 13x - GA\AA	13.7	14.7	16	17.2	0.4

Table 6.111 Association between IL-10.G9 and .G13 containing genotype and TNF –308 genotypes in normal controls

Shows lack of any association between IL-10.G9 and .G13 containing genotypes with TNF –308 genotypes in normal controls

IL-10.G9 or G13 containing genotype / TNF-308 genotype association	Expected		Observed		χ^2
	n	Freq.	n	Freq.	
IL-10.G 9x - GG	62.9	48.4	61	46.9	0.1
IL-10.G 9x - GA	23.7	18.2	24	18.5	0.0
IL-10.G 9x - GA\AA	25.6	19.7	26	20.0	0.0
IL-10.G 13x - GG	32.1	24.7	31	23.8	0.0
IL-10.G 13x - GA	12.1	9.3	11	8.5	0.1
IL-10.G 13x - GA\AA	13.1	10.1	13	10.0	0.0

Table 6.112 Association between IL-10.G9 and .G13 containing genotype and TNF –308 genotypes in study patients

Shows frequency (%) of IL-10.G9 and IL-10.G13 containing genotypes occurring with TNF -308 genotypes.
There is no association between IL-10.G9 and.G13 containing genotypes and TNF –308 genotypes in study patients.

6.18.3 TNFabc microsatellite putative secretor status combinations and IL-10.G microsatellite genotype association.

Because it is possible to assign a smaller number of putative secretor status 'genotypes' to the TNFabc 3-locus genotypes instead of using the 3-locus genotype itself (which has many different genotypes), an association was looked for between putative secretor 'genotypes' and IL-10.G genotypes containing allele 9 or 13. Tables 6.113 and 6.114 show there is no such association in normal controls, and tables 6.115 and 6.116 show the same in the patient subjects. It was not possible to do this analysis in patient subgroups as numbers were too small.

TNFabc putative secretor genotypes and IL-10.G9 containing genotypes	Expected		Observed		χ^2
	n	Freq.	n	Freq.	
HH – IL-10.G9x	8.9	9.2	9	9.3	0.0
HI - IL-10.G9x	11.3	11.7	8	8.2	1.0
HL - IL-10.G9x	15.9	16.4	21	21.6	1.6
IL - IL-10.G9x	10.2	10.5	9	9.3	0.1
LL - IL-10.G9x	6.3	6.5	5	5.2	0.3
Other - IL-10.G9x	13.3	13.7	17	17.5	1.0

Table 6.113 Association between TNFabc microsatellite 'putative secretor' combinations and IL-10.G microsatellite genotypes containing allele G9 in normal controls

Shows the frequency (%) of genotype combinations occurring in normal controls and demonstrates an absence of any association.
Haplotype analysis using 11 putative haplotypes previously characterised in Caucasian populations and confirmed in homozygous cell lines. Haplotypes have been determined as having high, low, or intermediate (TNFα) secretor phenotypes in response to LPS stimulation (Weissensteiner and Lanchbury 1997).
H- High TNFα secretor phenotype
L- Low TNFα secretor phenotype
I- Intermediate TNFα secretor phenotype
X-Undetermined TNFα secretor phenotype

Other – HX, II, IX, and LX

TNFabc putative secretor genotypes and IL-10.G13 containing genotypes	Expected		Observed		χ^2
	n	Freq.	n	Freq.	
HH – IL-10.G13x	5.1	5.3	3	3.1	0.9
HI - IL-10.G13x	6.6	6.8	6	6.2	0.1
HL - IL-10.G13x	9.2	9.5	14	14.4	2.5
IL - IL-10.G13x	5.9	6.1	8	8.2	0.7
Other - IL-10.G13x	11.3	11.6	10	10.3	0.1

Table 6.114 Association between TNFabc microsatellite 'putative secretor' haplotypes and IL-10.G microsatellite genotypes containing allele G13 in normal controls

Shows the frequency (%) of genotype combinations occurring in normal controls and demonstrates an absence of any association.
Haplotype analysis using 11 putative haplotypes previously characterised in Caucasian populations and confirmed in homozygous cell lines. Haplotypes have been determined as having high, low, or intermediate (TNFα) secretor phenotypes in response to LPS stimulation (Weissensteiner and Lanchbury 1997).
H- High TNFα secretor phenotype
L- Low TNFα secretor phenotype
I- Intermediate TNFα secretor phenotype
X-Undetermined TNFα secretor phenotype

Other – HX, II, IX, LL, and LX

TNFabc putative secretor genotypes and IL-10.G9 containing genotypes	Expected		Observed		χ^2
	n	Freq.	n	Freq.	
HH – IL-10.G9x	8.3	6.5	12	9.4	1.6
HI - IL-10.G9x	15.4	12.1	14	11.0	0.1
HL - IL-10.G9x	19.2	15.1	21	16.5	0.2
HX - IL-10.G9x	6.4	5.0	5	3.9	0.3
IL - IL-10.G9x	15.4	12.1	17	13.4	0.2
LL - IL-10.G9x	6.4	5.0	8	6.3	0.4
LX - IL-10.G9x	9.0	7.1	8	6.3	0.1
Other - IL-10.G9x	6.4	5.0	5	3.9	0.3

Table 6.115 Association between TNFabc microsatellite 'putative secretor' haplotypes and IL-10.G microsatellite genotypes containing allele G9 in study patients

Shows the frequency (%) of genotype combinations occurring in normal controls and demonstrates an absence of any association.
Haplotype analysis using 11 putative haplotypes previously characterised in Caucasian populations and confirmed in homozygous cell lines. Haplotypes have been determined as having high, low, or intermediate (TNFα) secretor phenotypes in response to LPS stimulation (Weissensteiner and Lanchbury 1997).
H- High TNFα secretor phenotype
L- Low TNFα secretor phenotype
I- Intermediate TNFα secretor phenotype
X-Undetermined TNFα secretor phenotype

Other –II and IX

TNFabc putative secretor genotypes and IL-10.G13 containing genotypes	Expected		Observed		χ^2
	n	Freq.	n	Freq.	
HI - IL-10.G13x	7.9	6.2	10	7.9	0.6
HL - IL-10.G13x	9.8	7.7	10	7.9	0.0
IL - IL-10.G13x	7.9	6.2	4	3.1	1.9
Other - IL-10.G13x	18.8	14.8	23	18.1	0.9

Table 6.116 Association between TNFabc microsatellite 'putative secretor' haplotypes and IL-10.G microsatellite genotypes containing allele G13 in study patients

Shows the frequency (%) of genotype combinations occurring in normal controls and demonstrates an absence of any association.
Haplotype analysis using 11 putative haplotypes previously characterised in Caucasian populations and confirmed in homozygous cell lines. Haplotypes have been determined as having high, low, or intermediate (TNFα) secretor phenotypes in response to LPS stimulation (Weissensteiner and Lanchbury 1997).
H- High TNFα secretor phenotype
L- Low TNFα secretor phenotype
I- Intermediate TNFα secretor phenotype
X-Undetermined TNFα secretor phenotype

Other – HH, HX, II, IX, LL, and LX

6.18.4 TNF –308 genotype and IL-10 –1117, -854, and –627 genotype associations.

An association was looked for between TNG –308 genotypes and genotypes occurring at the 3 bi-allelic loci within the IL-10 promoter region.

Tables 6.117, 6.118, and 6.119 show there is no such association in normal controls, and tables 6.120, 6.121, and 6.122 show there is no such association in study subjects. It was not possible to do this analysis in patient subgroups as numbers are too small.

TNF -308 genotype / IL-10 -1117 genotype	Expected		Observed		χ^2
	n	Freq.	n	Freq.	
GG - GG	13.6	14.5	15	16.0	0.1
GG - GA	27	30.5	30	31.9	0.1
GG - AA	17.4	18.5	18	19.1	0.0
GA - GG	6.3	6.7	3	3.2	1.7
GA - GA	13.3	14.1	16	17.0	0.5
GA - AA	8.0	8.5	6	6.4	0.5
AA - GG / GA / AA	-	-	6	6.4	n/a

Table 6.117 Association between TNF -308 and IL-10. -1117 genotypes in normal controls

Shows a lack of any association between genotypes of the TNF -308 and IL-10 -1117 loci. Expected frequencies for individual TNF.AA associations are not shown as they are <5.

TNF -308 genotype / IL-10 -854 genotype	Expected		Observed		χ^2
	n	Freq.	n	Freq.	
GG - CC	33.9	36.4	36	38.7	0.1
GG - CT	19.6	21.1	17	18.3	0.4
GG - TT	5.6	6.0	5	5.4	0.1
GA - CC	15.5	16.7	14	15.1	0.2
GA - CT	9.0	9.7	10	10.8	0.1
GA - TT	2.7	2.9	2	2.2	n/a
AA - CC / CT / TT	-	-	6	6.4	n/a

Table 6.118 Association between TNF -308 and IL-10. -854 genotypes in normal controls

Shows a lack of any association between genotypes of the TNF -308 and IL-10 -854 loci. Expected frequencies for individual TNF.AA associations are not shown as they are <5.

TNF -308 genotype / IL-10 -627 genotype	Expected		Observed		χ^2
	n	Freq.	n	Freq.	
GG - CC	24.0	26.1	27	29.3	0.4
GG - CA	26.7	29.0	27	29.3	0.0
GG - AA	7.8	8.5	6	6.5	0.4
GA - CC	11.0	12.0	10	10.9	0.1
GA - CA	12.2	13.3	11	12.0	0.1
GA - AA	3.7	4.0	5	5.4	n/a
AA - CC / CA / AA	-	-	6	6.4	n/a

Table 6.119 Association between TNF -308 and IL-10. -627 genotypes in normal controls

Shows a lack of any association between genotypes of the TNF -308 and IL-10 -627 loci. Expected frequencies for individual TNF.AA associations are not shown as they are <5.

TNF -308 genotype / IL-10 -1117 genotype	Expected		Observed		χ^2
	n	Freq.	n	Freq.	
GG - GG	23.3	21.2	24	21.8	0.0
GG - GA	40.3	36.6	39	35.5	0.0
GG - AA	14.4	13.1	15	13.6	0.0
GA - GG	8.8	8.0	8	7.3	0.1
GA - GA	15.2	13.8	18	16.4	0.5
GA - AA	5.4	4.9	4	3.6	04
AA - GG / GA / AA	-	-	2	1.8	n/a

Table 6.120 Association between TNF -308 and IL-10. -1117 genotypes in study patients

Shows a lack of any association between genotypes of the TNF -308 and IL-10 -1117 loci. Expected frequencies for individual TNF.AA associations are not shown as they are <5. This type of analysis is not possible for the patient subgroups as expected numbers are small.

TNF -308 genotype / IL-10 -854 genotype	Expected		Observed		χ^2
	n	Freq.	n	Freq.	
GG - CC	46.0	41.4	51	45.9	0.5
GG - CT	31.4	28.3	21	18.9	3.4
GG - TT	6.2	5.6	7	6.3	0.1
GA - CC	17.3	15.6	11	9.9	2.3
GA - CT	11.9	10.7	16	14.5	1.4
GA - TT	2.3	2.1	3	2.7	n/a
AA - CC / CT / TT	-	-	6	6.4	n/a

Table 6.121 Association between TNF -308 and IL-10. -854 genotypes in study patients

Shows a lack of an association between genotypes of the TNF -308 and IL-10 -854 loci. Expected frequencies for individual TNF.AA associations are not shown as they are <5. This type of analysis is not possible for the patient subgroups as expected numbers are small.

TNF -308 genotype / IL-10 -627 genotype	Expected		Observed		χ^2
	n	Freq.	n	Freq.	
GG - CC	33.5	33.5	41	41.0	1.3
GG - CA	27.8	27.8	17	17.0	4.2
GG - AA	9.7	9.7	10	10.0	0.0
GA - CC	12.6	12.6	11	11.0	0.2
GA - CA	10.4	10.4	14	14.0	1.2
GA - AA	3.6	3.6	5	5.0	n/a
AA - CC / CA / AA	-	-	2	2.0	n/a

Table 6.122 Association between TNF -308 and IL-10. -627 genotypes in study patients

Shows a lack of an association between genotypes of the TNF -308 and IL-10 -854 loci. Expected frequencies for individual TNF.AA associations are not shown as they are <5. This type of analysis is not possible for patient subgroups as expected numbers are small.

6.18.5 TNFabc microsatellite putative secretor status combinations and IL-10 –1117, -854, and –627 genotype associations

Because it is possible to assign a smaller number of putative secretor status 'genotypes' to the TNFabc 3-locus genotypes instead of using the 3-locus genotype itself (which has many different genotypes), an association was looked for between putative secretor 'genotypes' and IL-10 –1117, -854, and –627 genotypes. Even by assigning putative secretor status 'genotypes' numbers are too small for meaningful comparison. Therefore an association was looked for between high, intermediate, and low secretor status containing 'genotypes and IL-10 –1117, -854, and –627 genotypes. This type of analysis has it's limitations, as it does not consider what an individual's other haplotype is, or what effect it may have upon TNFα secretor phenotype. Also no data exists as to whether there are dominant functional haplotypes, and what they may be. Table 6.123 shows there is no association between putative secretor status 'genotypes' containing copies of haplotypes assigned as having a high secretor status and any of the genotypes at the –1117, -854, and –627 loci in normal controls. Similarly no association is seen with putative secretor status 'genotypes' containing copies of haplotypes assigned as having a intermediate or low secretor status and any of the three IL-10 bi-allelic loci (tables 6.124 and 6.125). Tables 6.126, 6.127, and 6.128 show similar findings in study patient subjects.

TNFabc 'high secretor' containing genotypes and IL-10 –1117 – 854, and - 627 genotypes	Expected		Observed		χ^2
	n	Freq.	n	Freq.	
<u>- 1117</u>					
Hx - GG	13.9	7.8	8	4.4	2.5
Hx - GA	29.4	16.3	35	19.4	1.1
Hx - AA	17.8	9.9	18	10.0	0.0
<u>- 854</u>					
Hx - CC	36.1	19.4	40	21.5	0.4
Hx - CT	11.9	6.4	18	9.7	3.1
Hx - TT	6.1	3.3	5.0	2.7	0.2
<u>- 627</u>					
Hx - CC	26.8	14.0	27	14.1	0.0
Hx - CA	29.7	15.5	36	18.8	1.3
Hx - AA	8.8	4.6	16	8.3	5.9

Table 6.123 Showing frequency (%) of the association of TNFabc 'putative secretor' containing genotypes with IL-10 – 1117 genotypes in normal controls

Shows a lack of any association between TNFabc ' high putative secretor' containing genotypes and IL-10 –1117, - 854, and – 627 genotypes. It was not possible to compare putative secretor 'genotypes' as expected numbers are too small. The total $\chi^2 = 11.0$, p=0.2, (8 d.f), indicating normal distribution in this analysis, so it is unlikely an association is seen with H-I-L 'genotypes'.

Haplotype analysis using 11 putative haplotypes previously characterised in Caucasian populations and confirmed in homozygous cell lines. Haplotypes have been determined as having high, low, or intermediate (TNFα) secretor phenotypes in response to LPS stimulation (Weissensteiner and Lanchbury 1997).

Hx- High secretor haplotype with any other haplotype

Lx- Low secretor haplotype with any other haplotype

Ix– Intermediate secretor haplotype with any other haplotype

TNFabc 'intermediate secretor' containing genotypes and IL-10 –1117, – 854, and - 627 genotypes	Expected		Observed		χ^2
	n	Freq.	n	Freq.	
- 1117					
Ix - GG	8.6	4.8	7	3.9	0.3
Ix - GA	18.8	10.0	21	11.7	0.3
Ix - AA	11.0	6.1	11	6.1	0.0
- 854					
Ix - CC	21.0	11.6	19	0.6	0.2
Ix - CT	12.5	6.9	13	7.2	0.0
Ix - TT	3.8	2.1	4	2.2	n/a
- 627					
Ix - CC	16.5	8.6	18	9.4	0.1
Ix - CA	18.3	9.6	18	9.4	0.0
Ix - AA	5.4	2.8	6	3.1	0.1

Table 6.124 Showing frequency (%) of the association of TNFabc intermediate putative secretor containing genotypes with IL-10 – 1117 genotypes in normal controls

Shows a lack of any association between TNFabc ' intermediate putative secretor' containing genotypes and IL-10 –1117, - 854, and – 627 genotypes. It was not possible to compare putative secretor 'genotypes' as expected numbers are too small. The total χ^2 = 1.0, p>0.9, (8 d.f), indicating normal distribution in this analysis, so it is unlikely an association is seen with H-I-L 'genotypes'.
Haplotype analysis using 11 putative haplotypes previously characterised in Caucasian populations and confirmed in homozygous cell lines. Haplotypes have been determined as having high, low, or intermediate (TNFα) secretor phenotypes in response to LPS stimulation (Weissensteiner and Lanchbury 1997).
Hx- High secretor haplotype with any other haplotype
Lx- Low secretor haplotype with any other haplotype
Ix– Intermediate secretor haplotype with any other haplotype

TNFabc 'low secretor' containing genotypes and IL-10 –1117, – 854, and - 627 genotypes	Expected		Observed		χ^2
	n	Freq.	n	Freq.	
- 1117					
Lx – GG	12.7	7.1	13	7.2	0.0
Lx – GA	26.8	14.9	29	16.1	0.2
Lx - AA	16.2	9.0	16	8.9	0.0
- 854					
Lx – CC	31.9	17.7	32	17.8	0.0
Lx – CT	18.5	10.3	26	14.4	3.0
Lx - TT	5.5	3.1	7	3.9	0.4
- 627					
Lx – CC	24.4	12.7	22	11.5	0.2
Lx – CA	27.1	14.1	21	10.9	1.4
Lx - AA	8.1	4.2	6	3.1	0.5

Table 6.125 Showing frequency (%) of the association of TNFabc low putative secretor containing genotypes with IL-10 – 1117 genotypes in normal controls

Shows a lack of any association between TNFabc ' low putative secretor' containing genotypes and IL-10 –1117, - 854, and – 627 genotypes. It was not possible to compare putative secretor 'genotypes' as expected numbers are too small. The total $\chi^2 = 5.7$, $p>0.6$, (8 d.f), indicating normal distribution in this analysis, so it is unlikely an association is seen with H-I-L 'genotypes'.

Haplotype analysis using 11 putative haplotypes previously characterised in Caucasian populations and confirmed in homozygous cell lines. Haplotypes have been determined as having high, low, or intermediate (TNFα) secretor phenotypes in response to LPS stimulation (Weissensteiner and Lanchbury 1997).

Hx- High secretor haplotype with any other haplotype

Lx- Low secretor haplotype with any other haplotype

Ix– Intermediate secretor haplotype with any other haplotype

7 Discussion

This study is the first to characterise TNF and IL-10 polymorphisms in patients with acute pancreatitis. It also documents allelic, gene, and haplotype frequencies across the polymorphic TNF and IL-10 loci, and provides description of linkage in the IL-10 promoter region between haplotypes of the 3 locus bi-allelic system and alleles 9 and 13 of the IL-10.G microsatellite.

In addition it provides considerable demographic data upon a group of 135 patients with acute pancreatitis and enables the examination of this data in relation to aetiology and disease outcome.

7.1 TNF loci

Allele and gene frequencies of the TNFabc microsatellite loci and the -308 locus in the population of Europids studied are similar to those in previously published work (Crouau-Roy et al., 1993). TNF microsatellite 3-locus haplotype analysis was performed using all 11 haplotypes described in European populations (see section 4.1.4.3), and haplotype frequencies are also similar to those previously published (Crouau-Roy et al., 1993).

As previously discussed, alleles at the TNF loci are in linkage disequilibrium with each other and with class I and II alleles. Various previously described associations are seen in the normal population studied in this work. These include the negative association of the TNF -308.G allele with the TNF a2b3c1 haplotype and the association of the -308.G allele with the TNFa11 b4c1 haplotype. A new association, not previously reported, was seen between the -308.G allele and the TNFa6b5c1 haplotype in the normal population.

This work also shows that there is an association between HLA-DQB1*0201 containing genotypes and 3-locus TNFa2b3c1 containing genotypes in normal controls. As the analysis was performed using genotypes it does not prove linkage, but does strongly suggest it.

7.2 IL-10 loci

Allelic frequencies at the three bi-allelic loci (-1117, -854, and -627) and the IL-10.G

microsatellite allele frequencies were in the main comparable with the previously published frequencies (Turner et al, 1997; Eskdale et al, 1997). Only the allele frequencies at position -627 were significantly different from these, 627*C and 627*A occurring in 62% and 38% of the study population compared to 79%and 21% of the previously characterised population ($\chi^2 = 16.2$, $p < 0.001$). However, the observed gene frequencies in the study population did not significantly differ from the expected, so Hardy-Weinberg equilibrium was established. Heterozygotes and homozygotes were detected at all 4 loci.

Eight three locus haplotypes of the bi-allelic polymorphisms at positions -1117, -854, and -627 were identified, in comparison to previously published work which only identified 3 haplotypes (Turner et al, 1997). The 4 most common haplotypes accounted for 91% of total haplotypes.

The –1117 / -854 / -627 3-locus haplotype G-C-C has been confirmed to exist at higher frequency than could be expected by non-random association of alleles, as is the A-T-A haplotype. The haplotypes A-C-C and A-C-A occur as frequently as expected from random association, whilst the 3-locus combinations G-T-C, G-C-A, A-T-C, and G-T-A occur much less frequently than can be expected from random association confirming that linkage disequilibrium occurs between the 3 loci to produce the increased frequency of the haplotypes G-C-C and A-T-A.

Haplotypes also exist between IL-10.G and the 3 bi-allelic loci. IL-10.G9 is seen to associate with the 3-locus haplotype A-T-A. No associations are seen with the IL-10.G13 allele.

This study has provided the first evidence of linkage in the IL-10 promoter region between haplotypes of the 3 locus bi-allelic system and alleles 9 and 13 of the IL-10.G microsatellite. The study of cytokine gene polymorphisms in autoimmune and inflammatory diseases is used to identify useful disease associated genetic markers, and to provide insight into pathogenic mechanisms of disease at the molecular level. To ensure that all possible genetic

markers are studied for a particular gene locus the accurate characterisation of allelic, genotypic, and haplotype distribution within normal healthy populations is a prerequisite for studies of this type.

The characterisation of more haplotypes and linkage across the four polymorphic sites in the IL-10 locus described in this work will aid the analysis of disease in relation to the human IL-10 promoter, as it identifies increased sequence polymorphism, so providing a more powerful tool for population genetic analysis.

7.3 IL-10 and TNF locus associations

The analysis of associations of genotypes of the two cytokine genes was limited in this study, as many of the loci studied are multi-allelic. This produces many possible genotypes, so numbers of associations will be small.

As expected no associations were seen between loci in the region of the TNF gene and loci of IL-10 in normal controls.

7.4 Acute pancreatitis patients

Two recognised systems for classification of disease outcome were used in this study. The Atlanta convention classification (Bradley., 1993) and an organ failure scoring system were both used to stratify patients, as not all those classified under the Atlanta system as having severe disease have organ failure, and it also does not distinguish between differing severity of organ failure.

Age distribution has been shown to be different in those classified as having severe disease (Atlanta) compared to those with mild disease. The same was true for those with an OFS≥2, which is comparable with published data (de Beaux et al., 1995) showing morbidity and mortality is related to age. However, no difference in distribution of age was seen between patients with mild disease and those with higher degrees of organ failure as evidenced by an OFS ≥3 or ≥4. This may be due to relatively small numbers in the latter two groups.

The proportion of patients in this study with gallstone associated acute pancreatitis is 57%,

which is slightly higher than previously reported in UK populations, although wide variation has been shown in studies, from 30 to 50% (Thompson et al., 1987; Corfield et al., 1985; Mann et al., 1994; De Beaux et al., 1995). There is also wide variability in reported incidence of alcohol induced acute pancreatitis in the UK, from 11 to 38% (Thompson et al., 1987; Corfield et al., 1985; De Beaux et al., 1995; Mann et al., 1994). The incidence of 15.6% found in the population studied in this work falls within this range, although one cannot directly compare studies, as different epidemiological studies have no common defining criteria for when alcohol is considered the main aetiology . Only one published study has set an alcohol consumption level (consumption of $\geq$80 g day^{-1}) at which subjects were defined as having alcoholic acute pancreatitis (Wilson et al., 1985). This criteria has been adopted in this study. Furthermore, no mention is made in other published studies of any attempts to distinguish those with alcohol induced chronic pancreatitis from those with alcohol induced acute disease.

In the population studied idiopathic pancreatitis accounted for 27.4% of patients, which is high compared to other studies (14% and 23% of cases of acute pancreatitis (Mann et al., 1994; Thompson et al., 1987; De Beaux et al., 1995; Corfield et al., 1985)). In this study the exclusion of subjects from the alcohol-induced group who consumed less than 80g day^{-1} but had no evidence of gallstones helped increase the proportion in the idiopathic group. In this study greater numbers of patients with idiopathic disease were seen than expected in the groups with severe disease (Atlanta) and an OFS$\geq$3 . 17 of 38 patients with severe disease had idiopathic disease, as did 11 of 18 patients with an OFS$\geq$3. The group with idiopathic disease only accounted for 27.4% of all patients. Although this was only a trend, as the difference did not approach statistical significance, it is of interest to note that a previous study reports mortality rates as being higher in those with idiopathic disease (de Beaux et al., 1995) compared to gallstone associated disease (3 vs. 15%, p=0.03). Subjects with alcohol induced pancreatitis are shown to have a significantly younger

distribution of age than those with idiopathic and gallstone. The alcohol induced pancreatitis group were also predominantly male. The reasons for this difference in age and sex distribution are unclear. It cannot just be due to the fact that young men start excessive drinking behaviour (and therefore develop alcohol associated diseases) early in life as patients with alcoholic pancreatitis were also significantly younger than the group of alcoholic 'controls', and there was also a significant difference in sex distribution between these two latter groups. However, there was a significant difference in sex distribution between the alcohol 'control' group and the normal controls, indicating that alcohol induced liver and pancreatic disease is more prevalent in males. This may only mean that alcoholism is more prevalent in the male sex, which cannot be tested in this study, as no group of disease free alcoholics was available for comparison. Previously published data, however, confirms that alcoholism is more prevalent in males compared to females (77 vs. 33%), although the incidence in women in the Western world is increasing (Sherlock and Dooley, 1993).

7.5 Immunogenetic studies in acute pancreatitis patients

The importance of both pro-inflammatory and regulatory cytokines in the pathogenesis of acute pancreatitis is well established.

Animal models have shown that TNF plays an important role in the early disease pathogenesis. Increased TNF expression has been demonstrated in these animal models (Gukovskaya et al, 1997; Norman et al, 1995a; Fu et al, 1997), and TNF antagonism is known to ameliorate local and systemic markers of disease (Gukovskaya et al, 1997; Hughes et al, 1996; Norman et al, 1996b). Since TNF production in individuals has been shown to be genetically influenced (Poicot et al, 1993), it was hypothesised that polymorphism of the TNF gene locus may influence disease susceptibility and course in acute pancreatitis.

The regulatory cytokine, IL-10, is undoubtedly important in the pathogenesis of acute

pancreatitis. Unlike TNF, it acts to reduce inflammation, and as a part of this reduces macrophage secretion of TNF (Fiorentino et al, 1991). As could be expected, experimental data with animals suggests that IL-10 acts to suppress the inflammatory process associated with acute pancreatitis (Van Laethem et al., 1995; Rongione et al., 1997; Kusske et al., 1996a). As a locus within the IL-10 promoter has been shown to influence IL-10 production (Turner et al, 1997), it can be hypothesised that IL-10 promoter region polymorphisms may influence the development or severity of acute pancreatitis.

This study shows that the TNF and IL-10 gene polymorphisms studied are not determinants of disease severity in acute pancreatitis. However, alleles of both TNF and IL-10 microsatellite polymorphisms are differently distributed in patients with alcohol-induced acute pancreatitis compared to normal controls and a group of patients with alcoholic liver disease. These findings are independent of one another.

In agreement with the findings of this study, characterisation of the TNF -308 polymorphism in patients with severe post-operative sepsis, a condition thought to have a similar pathogenesis to acute pancreatitis, reveals no association with disease severity (Stuber et al, 1996b). There are many other studies, already reviewed in section 4.1.4.5 (Table 4.4), that investigate associations between the TNF -308.G allele and susceptibility to and severity of various acute and chronic inflammatory and infective disorders in which TNF is thought to play an important role. As previously indicated, there is often no consistent finding between different studies of the same disease, and most associations were found to be due to linkage of the -308 locus with HLA types known to be linked with the disease in question. Where this wasn't the case HLA associations often were not investigated.

TNF microsatellites have also been investigated in various inflammatory and infective disorders as detailed in section 5.1.4.5 (Table 4.5). No one allele or haplotype has been identified as being consistently associated with the development of severity of disease, and it

is interesting to note that it is also the case with TNF microsatellites that differences in allelic or haplotype frequency were not independent of HLA associations, due to linkage between loci.

Previous studies have demonstrated associations in various populations between serologically typed HLA-B and C antigens and chronic pancreatitis of alcoholic origin. These include HLA-Bw40, HLA-B13, HLA-Bw39, and HLA-Cw52. Our results provide further evidence to suggest that the MHC is involved in the development of alcoholic pancreatitis.

There are several possible explanations to explain the results reported. If the TNF locus is itself associated with the development of acute pancreatitis following excessive alcohol consumption, then specific alleles or haplotypes may be influencing TNF regulation. There is some evidence that certain microsatellite alleles in the TNF locus may have a functional role (Poicot et al, 1993). The a2 and c2 alleles are associated with higher TNF production from stimulated lymphocytes in vitro. The a2 allele is found at a markedly reduced frequency in the group of patients with alcohol-induced pancreatitis. Although this finding does not reach conventional statistical significance, it strongly suggests that the a2 allele is negatively associated with the development of pancreatic inflammation following excessive alcohol consumption. It appears that this negative association is specific to the pancreas, as the group of alcoholic liver disease patients also had different allelic frequencies compared to the alcoholics with pancreatic disease. The significance of these findings is unclear, as animal models suggest that TNF upregulates the inflammatory process that occurs in the development of pancreatitis, and the a2 allele that has been associated with higher TNF production is markedly reduced in frequency.

It may be that TNF microsatellites are linked to as yet undefined genes within the MHC that play a major role in conferring protection from alcoholic pancreatitis than TNF itself. As the MHC has a high degree of linkage disequilibrium these genes may even be some distance

away from the TNF locus. The number of as yet undefined gene loci within the MHC upon chromosome 6 preclude speculation as to the which linked genes may be involved, as there are many genes within the MHC that are involved in a multitude of diverse immune processes, a lot of them immune. Within the TNF region itself are genes speculated to encode for inhibitors of transcription factors, and others of unknown function including the LST-1 gene (Trowsdale and Campbell., 1998).

We have shown that the bi-allelic polymorphisms of the IL-10 promoter have no association with susceptibility or severity of acute pancreatitis. We also investigated the potentially more informative microsatellite, IL-10.G, and although no association reaches conventional statistical significance, it appears that the IL-10.G13 microsatellite allele, like the TNFa2 allele, is present at reduced frequency in those with alcoholic acute pancreatitis. These findings are independent of one another.

At present the importance of the IL-10.G microsatellite in regulating IL-10 production is under investigation, enabling limited interpretation of the results presented in this work. However, characterisation of the IL-10 promoter region in which the IL-10 polymorphisms described here occur, show that there are many transcription sites close to these polymorphisms, which may be influenced by them. For example, there are two potential NFκB/REL sites 60 and 80bp immediately upstream of IL10.G (Eskdale et al, 1997b), which may be involved in cytokine mediated modulation of IL-10 expression, so influencing the pathogenesis of inflammatory disorders such as human acute pancreatitis.

Overall, because of the failure of the findings in this study to achieve conventional statistical significance, it appears that the associations demonstrated between these cytokine gene polymorphisms and alcoholic pancreatitis have some limitation. It may be that the loci themselves are not important determinants of disease susceptibility, but that sites close to or in linkage disequilibrium with them induce susceptibility to disease. Further studies are required to assess the functional importance of these polymorphisms and their effect upon

susceptibility to alcoholic pancreatitis.

Should it be possible to identify loci that are more closely associated with alcoholic pancreatitis it may be possible to use genetic typing to predict clinical outcome in alcoholics and to use the information to be able to give patients a more accurate assessment of risk of pancreatic disease in the event of continued excessive alcohol intake.

Once the functional importance of any disease associated locus is fully identified then it may be possible to assess the effects of replacing deficient proteins, or antagonising excess proteins in the event of either being pivotal in disease pathogenesis, in individuals identified by genetic testing.

7.6 Suggested further studies arising from this work

1. In-vitro studies upon the functional relevance of the TNF and IL-10 polymorphisms.
2. Characterisation of the TNF and IL-10 polymorphisms in a group of pancreatic and liver disease free alcoholics, and comparison with the group of alcohol-induced pancreatitis patients.
3. Characterisation of the TNF and IL-10 polymorphisms in a group of patients with chronic pancreatitis.
4. Characterisation of other pro-inflammatory cytokine polymorphisms in patients with acute pancreatitis.
5. Search for other susceptibility genes involved in alcoholic acute pancreatitis. These might include chemokine genes or alcohol metabolising enzyme genes.

A1 TNF microsatellite 3-locus genotypes in patient subgroups

TNFabc haplotype combinations	Controls n=107	Study patients n=135	Mild n=97	Severe n=38	Local complic[n] n=21
a2b1c2- a2b1c2	2	2	2	0	0
a2b1c2- a11b4c1	4	3	3	0	0
a2b1c2- a6b5c1	4	7	5	2	2
a2b1c2- a10b4c1	4	3	1	2	1
a2b1c2- a2b3c1	5	3	2	1	0
a2b1c2- a7b4c1	1	4	2	2	1
a2b1c2- a2b5c2	2	3	2	1	1
a2b1c2- a5b5c2	0	1	1	0	0
a2b1c2- a1b5c2	1	0	0	0	0
a2b1c2- a4b5c1	2	1	1	0	0
a2b1c2- a4b7c2	0	2	1	0	0
a2b1c2- X	1	6	4	3	0
a2b3c1- a2b3c1	2	0	0	0	0
a2b3c1- a7b4c1	5	0	0	0	0
a2b3c1- a2b5b2	1	1	0	1	1
a2b3c1- a5b5c2	1	1	1	0	0
a2b3c1- a4b5c1	2	3	2	1	0
a2b3c1- a4b7c2	1	0	0	0	0
a2b3c1- X	3	3	2	1	0
a2b5b2 - a4b5c1	1	2	1	1	1
a2b5b2- X	1	0	0	0	0
a5b5c2-a4b5c1	0	2	0	2	2
a5b5c2- X	0	1	0	1	0
a6b5c1- a6b5c1	2	3	2	1	1
a6b5c1- a10b4c1	2	8	4	4	2
a6b5c1- -a2b3c1	7	6	5	1	1
a6b5c1- a7b4c1	2	0	0	0	0
a6b5c1- a2b5c2	0	1	0	1	0

Table A1 **Number of TNFabc 3-locus haplotype combinations in control and acute pancreatitis patients grouped according to (local and systemic) disease severity**

(continued...)

TNFabc haplotype combinations	Controls n=107	Study patients n=135	Mild n=97	Severe n=38	Local complic[n] n=21
a6b5c1- a5b5c2	0	2	1	1	1
a6b5c1- a4b5c1	1	4	4	0	0
a6b5c1- X	2	3	3	0	0
a7b4c1- a7b4c1	0	2	1	1	0
a7b4c1- a2b5c2	1	1	1	0	0
a7b4c1-a1b5c2	0	1	1	0	0
a7b4c1 a4b5c1	1	0	0	0	0
a7b4c1-a4b7c2	0	1	1	0	0
a7b4c1- X	3	2	1	1	1
a11b4c1-a11b4c1	1	5	5	0	0
a11b4c1-a6b5c1	10	10	9	1	1
a11b4c1-a10b4c1	4	3	2	1	0
a11b4c1-a2b3c1	3	5	3	2	2
a11b4c1-a7b4c1	1	4	4	0	0
a11b4c1-a2b5c2	3	2	2	0	0
a11b4c1-a5b5c2	1	2	2	0	0
a11b4c1-a1b5c2	0	1	0	1	1
a11b4c1-a4b5c1	3	1	0	1	0
a11b4c1-a4b7c2	2	1	1	0	0
a11b4c1-X	2	7	4	3	2
a10b4c1-a10b4c1	1	0	0	0	0
a10b4c1-a2b3c1	2	4	4	0	0
a10b4c1-a2b5c2	1	0	0	0	0
a10b4c1-a5b5c2	1	0	0	0	0
a10b4c1-a4b5c1	0	0	0	0	0
a10b4c1-X	2	3	2	1	0
a4b5c1-a4b5c1	1	1	1	0	0
a4b5c1-a4b7c2	0	1	1	0	0
a4b5c1-X	2	3	3	0	0
X-X	3	0	0	0	0

Table A1 **Number of TNFabc 3-locus haplotype combinations in control and acute pancreatitis patients grouped according to (local and systemic) disease severity**

(footnotes continued...)

Table A1 **Number of TNFabc 3-locus haplotype combinations in control and acute pancreatitis patients grouped according to (local and systemic) disease severity**

Shows actual number of TNFabc microsatellite 3-locus combinations in normal controls and study patients, patients grouped according to severity of disease as assessed by the Atlanta classification, and occurrence of a local complication.
Local complic^{n} Local complication
Mild and **severe** disease severity groups are according to the Atlanta convention classification (Bradley, 1993), described in section 5.1.4.
Local complication is defined as occurrence of pancreatic necrosis, abscess or pseudocyst
n represents the number of subjects in each group
X denotes unable to determine haplotype
Haplotype analysis using 11 putative haplotypes previously characterised in Caucasian populations and confirmed in homozygous cell lines.

TNFabc haplotype combinations	Controls n=107	Study patients n=135	OFS ≥ 2 n=35	OFS ≥ 3 n=18	OFS ≥ 4 n=12
a2b1c2- a2b1c2	2	2	0	0	0
a2b1c2- a11b4c1	4	3	0	0	0
a2b1c2- a6b5c1	4	7	3	2	2
a2b1c2- a10b4c1	4	3	1	1	0
a2b1c2- a2b3c1	5	3	1	1	1
a2b1c2- a7b4c1	1	4	2	2	1
a2b1c2- a2b5c2	2	3	1	1	1
a2b1c2- a5b5c2	0	1	0	0	0
a2b1c2- a1b5c2	1	0	0	0	0
a2b1c2- a4b5c1	2	1	0	0	0
a2b1c2- a4b7c2	0	2	1	0	0
a2b1c2- X	1	6	2	0	0
a2b3c1- a2b3c1	2	0	0	0	0
a2b3c1- a7b4c1	5	0	0	0	0
a2b3c1- a2b5b2	1	1	1	1	1
a2b3c1- a5b5c2	1	1	0	0	0
a2b3c1- a4b5c1	2	3	1	1	0
a2b3c1- a4b7c2	1	0	0	0	0
a2b3c1- X	3	3	1	0	0
a2b5b2 - a4b5c1	1	2	0	0	0
a2b5b2- X	1	0	0	0	0
a5b5c2-a4b5c1	0	2	2	1	1
a5b5c2- X	0	1	1	0	0
a6b5c1- a6b5c1	2	3	0	0	0
a6b5c1- a10b4c1	2	8	3	3	2
a6b5c1- -a2b3c1	7	6	1	0	0
a6b5c1- a7b4c1	2	0	0	0	0
a6b5c1- a2b5c2	0	1	1	0	0
a6b5c1- a5b5c2	0	2	1	1	1
a6b5c1- a4b5c1	1	4	1	0	0

Table A2 **Number of TNFabc 3-locus haplotype combinations in normal controls and acute pancreatitis patients grouped according to (systemic) disease severity as assessed by organ failure score**

(continued...)

TNFabc haplotype combinations	Controls n=107	Study patients n=135	OFS ≥ 2 n=35	OFS ≥ 3 n=18	OFS ≥ 4 n=12
a6b5c1- X	2	3	0	0	0
a7b4c1- a7b4c1	0	2	1	1	0
a7b4c1- a2b5c2	1	1	0	0	0
a7b4c1-a1b5c2	0	1	0	0	0
a7b4c1 a4b5c1	1	0	0	0	0
a7b4c1-a4b7c2	0	1	0	0	0
a7b4c1- X	3	2	0	0	0
a11b4c1-a11b4c1	1	5	1	0	0
a11b4c1-a6b5c1	10	10	0	0	0
a11b4c1-a10b4c1	4	3	1	1	1
a11b4c1-a2b3c1	3	5	1	0	0
a11b4c1-a7b4c1	1	4	0	0	0
a11b4c1-a2b5c2	3	2	0	0	0
a11b4c1-a5b5c2	1	2	1	0	0
a11b4c1-a1b5c2	0	1	0	0	0
a11b4c1-a4b5c1	3	1	1	1	1
a11b4c1-a4b7c2	2	1	0	0	0
a11b4c1-X	2	7	3	0	0
a10b4c1-a10b4c1	1	0	0	0	0
a10b4c1-a2b3c1	2	4	1	1	0
a10b4c1-a2b5c2	1	0	0	0	0
a10b4c1-a5b5c2	1	0	0	0	0
a10b4c1-a4b5c1	0	0	0	0	0
a10b4c1-X	2	3	1	0	0
a4b5c1-a4b5c1	1	1	0	0	0
a4b5c1-a4b7c2	0	1	0	0	0
a4b5c1-X	2	3	0	0	0
X-X	3	0	0	0	0

Table A2 **Number of TNFabc 3-locus haplotype combinations in normal controls and acute pancreatitis patients grouped according to (systemic) disease severity as assessed by organ failure score**

(footnotes continued...)

Table A2 Number of TNFabc 3-locus haplotype combinations in normal controls and acute pancreatitis patients grouped according to (systemic) disease severity as assessed by organ failure score

Shows actual number of TNFabc microsatellite 3-locus combinations in normal controls and study patients, and patients grouped for severity of disease as assessed by organ failure score

OFS Organ failure score, calculated according to method described in section 5.1.4 (Kingsnorth 1995)

n represents the number of subjects in each group

X denotes unable to determine haplotype

Haplotype analysis using 11 putative haplotypes previously characterised in Caucasian populations and confirmed in homozygous cell lines.

TNFabc haplotype combinations	Controls n=107	Alcohol n=21	Idiopath. n=37	G\stones n=77	Alcohol controls n=33
a2b1c2- a2b1c2	2	2	0	1	1
a2b1c2- a11b4c1	4	3	0	1	2
a2b1c2- a6b5c1	4	7	1	4	2
a2b1c2- a10b4c1	4	3	0	2	1
a2b1c2- a2b3c1	5	3	0	1	2
a2b1c2- a7b4c1	1	4	0	3	1
a2b1c2- a2b5c2	2	3	0	0	3
a2b1c2- a5b5c2	0	1	0	0	1
a2b1c2- a1b5c2	1	0	0	0	0
a2b1c2- a4b5c1	2	1	0	1	0
a2b1c2- a4b7c2	0	2	0	0	2
a2b1c2- X	1	6	0	1	5
a2b3c1- a2b3c1	2	0	0	0	0
a2b3c1- a7b4c1	5	0	0	0	0
a2b3c1- a2b5b2	1	1	0	0	1
a2b3c1- a5b5c2	1	1	1	0	0
a2b3c1- a4b5c1	2	3	0	2	1
a2b3c1- a4b7c2	1	0	0	0	0
a2b3c1- X	3	3	1	0	2
a2b5b2 - a4b5c1	1	2	0	0	2
a2b5b2- X	1	0	0	0	0
a5b5c2-a4b5c1	0	2	0	1	1
a5b5c2- X	0	1	0	0	1
a6b5c1- a6b5c1	2	3	1	0	2
a6b5c1- a10b4c1	2	8	2	2	4
a6b5c1- -a2b3c1	7	6	2	1	3
a6b5c1- a7b4c1	2	0	0	0	0
a6b5c1- a2b5c2	0	1	0	0	1
a6b5c1- a5b5c2	0	2	0	1	1
a6b5c1- a4b5c1	1	4	0	2	2
a6b5c1- X	2	3	1	0	2

Table A3 **Number of TNFabc 3-locus haplotype combinations in normal controls and acute pancreatitis patients grouped according to aetiology of acute pancreatitis** (continued...)

TNFabc haplotype combinations	Controls n=107	Alcohol n=21	Idiopath. n=37	G\stones n=77	Alcohol controls n=33
a7b4c1- a7b4c1	0	2	0	1	1
a7b4c1- a2b5c2	1	1	0	0	1
a7b4c1-a1b5c2	0	1	1	0	0
a7b4c1 a4b5c1	1	0	0	0	0
a7b4c1-a4b7c2	0	1	0	1	0
a7b4c1- X	3	2	0	1	1
a11b4c1-a11b4c1	1	5	1	1	3
a11b4c1-a6b5c1	10	10	4	2	4
a11b4c1-a10b4c1	4	3	0	1	2
a11b4c1-a2b3c1	3	5	0	0	5
a11b4c1-a7b4c1	1	4	2	1	1
a11b4c1-a2b5c2	3	2	0	0	2
a11b4c1-a5b5c2	1	2	0	1	1
a11b4c1-a1b5c2	0	1	0	1	0
a11b4c1-a4b5c1	3	1	0	1	0
a11b4c1-a4b7c2	2	1	0	0	1
a11b4c1-X	2	7	3	1	3
a10b4c1-a10b4c1	1	0	0	0	0
a10b4c1-a2b3c1	2	4	1	1	2
a10b4c1-a2b5c2	1	0	0	0	0
a10b4c1-a5b5c2	1	0	0	0	0
a10b4c1-a4b5c1	0	0	0	0	0
a10b4c1-X	2	3	0	0	3
a4b5c1-a4b5c1	1	1	0	0	1
a4b5c1-a4b7c2	0	1	0	0	1
a4b5c1-X	2	3	0	1	2
X-X	3	0	0	0	0

Table A3 Number of TNFabc 3-locus haplotype combinations in normal controls and acute pancreatitis patients grouped according to aetiology of acute pancreatitis

Shows actual number of TNFabc microsatellite 3-locus combinations in normal controls, 'alcoholic controls', and patients grouped according to aetiology of acute pancreatitis.
G/stones Gallstones
X denotes unable to determine haplotype
Alcoholic pancreatitis if daily consumption of ≥ 80g alcohol day^{-1}
Gallstones if radiological or ERCP evidence of cholelithiasis

Idiopathic if no identifiable aetiology
Haplotype analysis using 11 putative haplotypes previously characterised in Caucasian populations and confirmed in homozygous cell lines.

LIST OF ABBREVIATIONS

ACh	Acetylcholine (ACh)
ADH	Alcohol dehydrogenase
AIDS	Aquired immune deficiency syndrome
ALDH	Aldehyde dehydrogenase
APACHE II	Acute Physiology and Chronic Health Evaluation II
APC	Antigen presenting cell
APP	Acute phase proteins
ARDS	Adult respiratory distress syndrome
ARMS	Amplification refractory mutation system
ATP	Adenosine tri-phophate
bp	Base pairs
cAMP	Cyclic AMP
CCK	Cholecystokinin (CCK)
CD	Cluster of differentiation / Crohn's disease
CDE	Choline-deficient ethionine-supplemented (diet)
cDNA	Complimentary deoxyribonucleic acid
CMI	Cell mediated immunity
CRP	C-reactive protein
CT	Computerised tomography
CTL	Cytotoxic T lymphocyte
DIC	Disseminated Intravascular Coagulation
DIG	Digoxygenin
DNA	Deoxyribonucleic acid
EDTA	Ethylene diamine tetra-acetic acid
ER	Endoplasmic reticulum

ERCP	Endoscopic retrograde cholangio-pancreatography
FAEE's	Fatty acid ethyl esters
GM-CSF	Granulocyte-macrophage colony stimulating factor
GTP	Glycerine tri-phosphate
HLA	Human leucocyte antigen
HP	Hereditary pancreatitis
HsP	Heat shock protein
IAPP	Islet cell amyloid polypeptide
IBD	Inflammatory bowel disease
ICE	Interleukin-1 converting enzyme
IDDM	Insulin dependent diabetes mellitus
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
IL-10R	IL-10 receptor
IL-1ra	IL-1 receptor antagonist
KDa	Kilodalton
KIR	Killer cell inhibitory receptor
KPa	Kilopascal
LT	Lymphotoxin
LMP	Large multifunctional protease
LPS	Lipopolysaccharide
MAC	Membrane attack complex
MBP	Mannose binding protein
MHC	Major histocompatibility complex
mmHg	Millimetres of mercury

MODS	Multiple organ dysfunction syndrome
mRNA	Messenger ribonucleic acid
MSOF	Multisytem organ failure
NFκB	Nuclear factor kappa beta
NIDDM	Non-insulin dependant diabetes mellitus
NK	Natural killer (cell)
NO	Nitric oxide
NOS	Nitric oxide synthase
OFR	Oxygen free radicals
PAF	Platelet activating factor
PaO_2	Partial pressure of oxygen
PBMC's	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PNK	Polynucleotide kinase
PP	Pancreatic polypeptide
PSP	pancreatic stone protein
PSTI	Pancreatic secretory trypsin inhibitor
RER	Rough endoplasmic reticulum
RAG	Recombination-activation-genes
RF	Rheumatoid factor
RING	Really interesting new gene
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
SIRS	Systemic inflammatory response syndrome
SLE	Systemic lupus erythamatosus
SSC	Sodium chloride & sodium citrate

SSOP	Sequence specific oligonucleotide probing
SSP	Sequence specific primers
TAP	Trypsinogen activation peptide / Transporter associated with antigen presentation
TBE	Tris- boric acid- ethylene diamine tetra-acetic acid
TCR	T cell receptor
TGF	Transforming growth factor
T_H	T helper (cell)
TMAC	Tetra-methyl-ammonium chloride
TNF	Tumour necrosis factor
TNFR	TNF receptor
UC	Ulcerative colitis
USS	Ultrasound scan
VIP	Vasoactive intestinal polypeptide (VIP)
VNTR	Variable number of tandem repeats

REFERENCES

Abraham, L.A., Marley, J.V., Nedaspasov, S.A., Cambon-Thomsen, A., Crouau-Roy, B., Dawkins, R.L., and Giphart, M.J. (1993). Microsatellite, Restriction-Fragment-Length Polymorphism, and sequence-Specific Oligonucleotide Typing of the Tumour Necrosis Factor Region. Human Immunology *38*, 17-23.

Acioli, J.M., Isobe, M., and Kawasaki, S. (1997). Early complement system activation and neutrophil priming in acute pancreatitis: participation of trypsin. Surgery *122*, 909-917.

Acosta, J.M. and Ledesma, C.L. (1974). Gallstone migration as a cause of acute pancreatitis. New England Journal of Medicine *290*, 484-487.
Acosta, J.M., Pellegrini, C.A., and Skinner, D.B. (1980). Etiology and pathogenesis of acute biliary pancreatitis. Surgery 118-125.

Adler, G., Beglinger, C., Braun, U., Reinshagen, M., Koop, I., Schafmeyer, A., Rovati, L., and Arnold, R. (1991). Interaction of the cholinergic system and cholecystokinin in the regulation of endogenous and exogenous stimulation of pancreatic secretion in humans. Gastroenterology *100*, 537-543.

Adler, G., Nelson, D.K., Katchinski, M., and Beglinger, C. (1995). Neurohormonal control of human pancreatic exocrine secretion. Pancreas *10*, 1-13.

Agematsu, K., Nagumo, H., Oguchi, Y., and et al (1998). Generation of plama cells from peripheral blood memory cells: synergistic effect of interleukin 10 and CD27/Cd70 interaction. Blood *91*, 173-180.

Alexander, H.R..., Sheppard, B.C., Jensen, J.C., and et al (1991). Treatment with recombinant human tumor necrosis factor-alpaha protects rats against the lethality, hypotension, and hypothermia of gram-negative sepsis. J Clin Invest *88*, 34-39.

Allen, J.E. and Maizels, R.M. (1997). Th1-Th2: reliable paradigm or dangerous dogma? Immunology Today *18*, 387-392.

Allison, J.P., McIntyre, B.W., and Bloch, D. (1982). Tumor-specific antigen of murine T lymphoma defined with monoclonal antibody. Journal of Immunology *129*, 2293-2299.

Andersson, R., Deng, X.M., and Wang, X.D. (1997). Role of macrophage overactivation in the development of acute pancreatitic injury in rats. Br J Surg *84*, 775-780.

Apte, M., Norton, I., Haber, P., Applegate, T., Korsten, M., McCaughan, G., Pirola, R., and Wilson, J. (1998). The effect of ethanol on pancreatic enzymes-a dietary artifact? Biochim Biophys Acta *1379*, 314-324.

Apte, M.V., Norton, I.D., Haber, P.S., McCaughan, G.W., Korsten, M.A., Pirola, R.C., and Wilson, J.S. (1996). Both ethanol and protein deficiency increase messenger RNA levels for pancreatic lithostathine. Life Sciences *58*, 485-492.

Arai, T., Hiromatsu, K., Nishimura, H., and et al (1995). Endogenous interleukin 10 prevents apoptosis in macrophages during salmonella infection. Biochem Biophys Res Commun *213*, 600-607.

Arias, A.I., Giles, B., Eiermann, T.H., Sterry, W., and Pandey, J.P. (1997). Tumor necrosis factor-alpha polymorphism in psoriasis. Exp Clin Immunogenet *14*, 226-233.

Arias, A.I., Giles, B., Eiermann, T.H., Sterry, W., and Pandey, J.P. (1997). Tumor necrosis factor-alpha polymorphism in psoriasis. Exp Clin Immunogenet *14*, 226-233.

Arnon, R., Espoti, S.D., and Zern, M.A. (1995). Molecular biological aspects of alcohol-induced liver disease. Alcoholism Clin Exp Res *19*, 247-256.

Asano, H., Kobayashi, T., Uchida, K., Hayashi, S., Yokoyama, I., Inoko, H., and Takagi,

H. (1997). Significance of tumor necrosis factor microsatellite polymorphism in renal transplantation. Tissue Antigens *50*, 484-488.

Assuma, R., Oates, T., Cochran, D., Amar, S., and Graves, D.T. (1998). IL-1 and TNF antagonists inhibit the inflammatory response and bone loss in experimental periodontitis. J Immunol *160*, 403-409.

Baeuerle, P.A. and Henkel, T. (1994). Function and activation of NFkB in the immune system. Annu Rev Immunol *12*, 141-179.

Banerjee, A.K., Galloway, S.W., and Kingsnorth, A.N. (1994). Experimental models of acute pancreatitis. Br J Surg *81*, 1096-1103.

Banks, P.A. (1997). Practice guidlines in acute pancreatitis. The American Journal of Gastroenterology *92*, 377-386.

Banks, P.A., Tenner, S., Noordhoek, E.C., Sica, G., Feng, S., and Zinner, M. (1996). Does pancreatic necrosis predict severity in patients with acute pancreatitis? Digestion *57*, 218(Abstract)

Bannister, L.H. (1995). Alimentary System. In Gray's Anatomy. P. Williams, R. Warwick, M. Dyson, and L. Bannister, eds. (New York: Churchill Livingstone), pp. 1790-1794.

Barie, P.S., Tahamont, M.V., and Malik, A.B. (1982). Prevention of increased pulmonary vascular permeability after pancreatitis by granulocyte depletion in sheep. Am rev Respir Dis *126*, 904-908.

Baumann, H. and Gauldie, J. (1994). The acute phase response. Immunology Today *15*, 74-79.

Bazzoni, F. and Beutler, B. (1996). The tumor necrosis factor ligand and receptor families. New England Journal of Medicine *334*, 1717-1725.

Bazzoni, F., Alejos, E., and Beutler, B. (1995). Chimeric tumor necrosis factor receptors with constitutive signaling activity. Proc. Natl. Acad. Sci. USA *92*, 5376-5380.

Bean, A.G., Freiburg, R.A., Andrade, s., Menon, S., and Zlotnik, A. (1993). Interleukin-10 protects mice against staphylococcal enterotoxin B-induced lethal shock. Infection and immunity *61*, 4937-4939.

Beutler, B., Milsark, I.W., and Cerami, A.C. (1985). Passive immunisation against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. Science *229*, 869-871.

Bianchi, E., Bender, J.R., Blasi, F., and Pardi, R. (1997). Through and beyond the wall: late steps in leukocyte transendothelial migration. Immunology Today *18*, 586-591.

Biragyn, A. and Nedaspasov, S.A. (1995). Lipopolysaccharide induced expression of TNF-alpha gene in the macrophage cell line ANA-1 regulated at the levels of transcriptional processivity. J Immunol *155*, 674-683.

Bird, G.L.A., Sheron, N., and Goka, A.K.J. (1990). Increased plasma tumor necrosis factor in severe alcoholic hepatitis. Ann Intern Med *112*, 917-922.

Blakemore, A.I.F., Cox, A., Gonzalez, A., Makill, J.K., Hughes, M.E., Wilson, R.M., Ward, J.D., and Duff, G.W. (1996). Interleukin-1 receptor antagonist allele (IL1RN*2) associated with nephropathy in diabetes mellitus. Hum Genet *97*, 369-374.

Bone, R.C. (1998). Toward a theory regarding the pathogenesis of the systemic inflammatory response syndrome: what we do and do not know about cytokine regulation. Crit Care Med *24*, 163-172.

Bouma, G., Xia, B., Crusius, J.B.A., Bioque, G., Koutroubakis, I., Von Blomberg, B.M.E.,

Meuwissen, S.G.M., and Pena, A.S. (1996). Distribution of four polymorphisms in the tumour necrosis factor (TNF) genes in patients with inflammatory bowel disease (IBD). Clin Exp Immunol *103*, 391-396.

Bouma, G., Xia, B., Crusius, J.B.A., Bioque, G., Koutroubakis, I., Von Blomberg, B.M.E., Meuwissen, S.G.M., and Pena, A.S. (1996). Distribution of four polymorphisms in the tumour necrosis factor (TNF) genes in patients with inflammatory bowel disease (IBD). Clin Exp Immunol *103*, 391-396.

Bradley, E.L. (1993). A clinically based classification system for acute pancreatitis. Arch Surg *128*, 586-590.

Braganza, J.M. (1991). Evolution of pancreatitis. In The pathogenesis of pancreatitis. J.M. Braganza, ed. (Manchester-New York: Manchester University Press), pp. 19-33.

Braganza, J.M., Scott, P., Bilton, D., and et al (1995). Evidence for early oxidative stress in acute pancreatitis. Int J Pancreatol *17*, 69-81.

Braquet, P., Touqui, L., and Phen, T.Y. (1987). Perspectives in Platelet Activating Factor research. Pharmacol Rev *39*, 97-145.

Braud, V.M., Allan, D.S.J., O'Callaghan, C.A., Soderstrom, K., D'Andrea, A., Ogg, G.S., Lazetic, S., Young, N.T., Bell, J.I., Phillips, J.H., Lanier, L.L., and McMichael, A.J. (1998). HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. Nature

Braunstein, J., Qiao, L., Autschbach, F., Schurmann, G., and Meuer, S. (1997). T cells of the human intestinal lamina propria are high producers of interleukin-10. Gut *41*, 215-220.

Brinkman, B.M.N., Huizinga, T.W.J., Kurban, S.S., Velde, E.A., Schreuder, G.M., Hazes, J.M., Breedveld, F.C., and Verweij, C.L. (1997). Tumour necrosis factor alpha gene polymorphisms in rheumatoid arthritis: association with susceptibility to, or severity of, disease? Br J Rheumatol *36*, 516-521.

Brinkman, B.M.N., Zuijdgeest, D., Kaijel, E.L., Breedveld, F.C., and Verweij, C.L. (1996). Relevance of the TNF? -308 promoter polymorphism in TNF? gene regulation. J Inflamm *46*, 32-41.

Bunce, M., O'Neill, C.M., Barnado, M.C.M.N., Krausa, P., Browning, M.J., Morris, P.J., and Welsh, K.I. (1995). Phototyping: comprehensive DNA typing for HLA-A, B, C, DRB1, DRB3, DRB4, DRB5, & DQB1 BY PCR with 144 primer mixes utilizing sequence-specific primers (PCR-SSP). Tissue Antigens *46*, 355-367.

Bussolati, B., Mariano, F., Montrucchio, G., Piccoli, C., and Camussi, G. (1997). Modulatory effect of interleukin-10 on the production of platelet activating factor and super oxide anions by humnan leukocytes. Immunology *90*, 440-447.

Cabrera, M., Shaw, M.A., Sharpes, C., Willaims, H., Castes, M., Convit, J., and Blackwell, J.M. (1995). Polymorphism in tumor necrosis factor genes associated with mucocutaneous leishmaniasis. J Exp Med *182*, 1259-1264.

Cabrera, M., Shaw, M.A., Sharpes, C., Willaims, H., Castes, M., Convit, J., and Blackwell, J.M. (1995). Polymorphism in tumor necrosis factor genes associated with mucocutaneous leishmaniasis. J Exp Med *182*, 1259-1264.

Carroll, M.C., Katzman, P., and Alicot, E.M. (1987). Linkage map of the human major histocompatibility complex including the tumor necrosis factor genes. Proc. Natl. Acad. Sci. *84*, 8535-8539.

Carswell, E.A., Old, L.J., Kassel, R.L., Green, S., Foire, N., and Williamson, B. (1975). An endotoxin-induced serum factor that causes necrosis of tumors. Proc. Natl. Acad. Sci. USA *72*, 3666-3670.

Cassatella, M.A. and Meda, L. (1994). J Exp Med *179*, 1695-1699.

Chao, W. and Olson, M.S. (1993). Platelet activating factor: receptors and signal transduction. Biochem J *292*, 617-629.

Chesney, J., Metz, C., Stavitsky, A.B., Bacher, M., and Bucala, R. (1998). Regulated production of type I collagen and inflammatory cytokines by peripheral blood fibrocytes. J Immunol *160*, 419-425.

Chiari, H. (1896). Ueber selbstverdaung des Menschlichen Pancreas. Zeitschrift fur Heilkunde *17*, 69-96.

Chouchane, L., Ahmed, S.B., Baccouche, S., and Remadi, S. (1997). Polymorphism in the tunor necrosis factor-a promoter region and in the heat shock protein 70 genes associated with malignant tumors. Cancer *80*, 1489-1496.

Chouchane, L., Ahmed, S.B., Baccouche, S., and Remadi, S. (1997). Polymorphism in the tunor necrosis factor-a promoter region and in the heat shock protein 70 genes associated with malignant tumors. Cancer *80*, 1489-1496.

Cohen, M.C. and Cohen, S. (1996). Cytokine function. A study in biologic diversity. Am J Clin Pathol *105*, 589-598.

Cohen, S.B.A., Crawley, J.B., Kahan, M.C., Feldmann, M., and Foxwell, B.M.J. (1997). Interleukin-10 rescues T cells from apoptotic cell death. Immunology *92*, 1-5.

Colonna, M. and Samaridis, J. (1995). Cloning of immunoglobulin-superfamily members associated with HLA-C and HLA-B recognition by human natural killer cells. Science *268*, 405-408.

Copekland, N.G., Silan, C., Kingsley, D., and et al (1991). Chromosomal location of IL-1 receptor genes. Genomics *9*, 44-50.

Corfield, A.P., Cooper, M.J., and Williamson, R.C.N. (1985). Acute Pancreatitis: a lethal disease of increasing incidence. Gut *26*, 724-729.

Cory S., Adams J.M. (1980). Delations are asssociated with somatic rearrangement of Ig heavy chain genes. Cell *19*, 37-51.

Couzigou, P., Fleury, B., Groppi, A., Iron, A., Coutelle, C., Cassaigne, A., and Begueret, J. (1990). Role of alcohol dehydrogenase polymorphism in ethanol metabolism and alcohol related diseases. In Enzymology and Molecular Biology of Carbonyl Metabolism 3. H. Weiner, ed. (New York: Plenum Press), pp. 263-270.

Crabb, D.W. (1995). Ethanol oxidising enzymes and liver disease. Progress in liver diseases *13*, 152-172.

Crawley, J.B., Williams, L.M., Mander, T., Brennan, F.M., and Foxwell, B.M.J. (1996). Interleukin 10 stimulation of phosphatidylinositol 3-kinase and p70 S6 kinase is required for the proliferative but not the antiinflammatory effects of the cytokine. J Biol Chem *271*, 16357-16362.

Crouau-Roy, B., Bouzekri, N., Carcassi, C., Clayton, J., Contu, L., and Cambon-Thomsen, A. (1996). Strong association between microsatellites and an HLA-B, DR haplotype (B18-DR3): implication for microsatellite evolution. Immunogenetics *43*, 255-260.

Crouau-Roy, B., Bouzekri, N., Carcassi, C., Clayton, J., Contu, L., and Cambon-Thomsen, A. (1996). Strong association between microsatellites and an HLA-B, DR haplotype (B18-DR3): implication for microsatellite evolution. Immunogenetics *43*, 255-260.

Crouau-Roy, B., Briant, L., Bouissou, C., Stavropoulos, C., Poicot, F., Cambon-Thomsen, A., and Clayton, J. (1993). Tumor necrosis factor microsatellites in four European

populations. Human Immunology *38*, 213-216.

Crowe, P.D., VanArsdale, T.L., Walter, B.N., and et al (1994). A lymphotoxin-?-specific receptor. Science *264*, 707-709.

Curley, P.J. (1996). Endotoxin, cellular immune dysfunction and acute pancreatitis. Annals of the Royal College of Surgeons of England *78*, 531-535.

Curley, P.J., Nestor, M., Collins, K., Saporoschetz, I., Mendez, M., Mannick, J.A., and Rodrick, M.L. (1996). Decreased Interleukin-2 production in murine acute pancreatitis: potential for immunomodulation. Gastroenterology *110*, 583-588.

Dabrowski, A. and Chwiecko, M. (1992). Oxidative stress: An early phenomenon characteristic of acute experimental pancreatitis. Int J Pancreatol *12*, 193-199.

Dabrowski, A., Gabryelewicz, A., and Chyczewski, L. (1995). The effect of platelet activating factor antagonist (BN 52021) on acute experimental pancreatitis with reference to multiorgan oxidative stress. Int J Pancreatol *17*, 173-180.

Dale, J.B. and Beachey, E.H. (1985). Epitopes of streptococcal M protein shared with cardiac myosin. J Exp Med *162*, 583-591.

D'Alfonso, S. and Momigliano Richiardi, P. (1994). A polymorphic variation in a putative regulation box of the TNFA promoter region. Immunogenetics *39*, 150-154.

D'Alfonso, S. and Momigliano, P. (1994). A new polymorphism in the TNFA gene. Hum Immunol *47*, 65A(Abstract)

Damas, P., Reuter, A., Gysen, P., Demonty, J., Lamy, M., and Franchimont, P. (1989). Tumor necrosis factor and interleukin-1 serum levels during severe sepsis in humans. Crit Care Med *17*, 975-978.

Danis, V.A., Millington, M., Hyland, V.J., and Grennan, D. (1995). Cytokine production by normal human monocytes: inter-subject variation and relationship to an IL-1 receptor antagonist (IL-1ra) gene polymorphism. Clin Exp Immunol *99*, 303-310.

Davies, M.G. and Hagen, P. (1997). Systemic inflammatory response syndrome. Br J Surg *84*, 920-935.

Day, C.P., Bashir, R., James, O.F.W., Bassendine, M.F., Crabb, D.W., Thomasson, H.R., Li, T., and Edenberg, H.J. (1991). Investigation of thr role of polymorphisms at the alcohol and aldehyde dehydrogenase loci in genetic predisposition to alcohol-related end-organ damage. Hepatology *14*, 798-801.

Day, C.P., Grove, J., Daly, A.K., Stewart, M.W., Avery, P.J., and Walker, M. (1998). Tumour necrosis factor-alpha gene promoter polymorphism and decreased insulin resistance. Diabetologia *41*, 430-434.

Day, C.P., Grove, J., Daly, A.K., Stewart, M.W., Avery, P.J., and Walker, M. (1998). Tumour necrosis factor-alpha gene promoter polymorphism and decreased insulin resistance. Diabetologia *41*, 430-434.

De Beaux, A.C., Goldie, A.S., Ross, J.A., Carter, D.C., and Fearon, K.H.C. (1996). Serum concentrations of inflammatory mediators related to organ failure in patients with acute pancreatitis. British Journal of Surgery *83*, 349-353.

De Beaux, A.C., Palmer, K.R., and Carter, D.C. (1995). Factors influencing morbidity and mortality in acute pancreatitis; an analysis of 279 cases. Gut *37*, 121-126.

De Beaux, A.C., Ross, J.A., Maingay, J.P., Fearon, K.H.C., and Carter, D.C. (1997). Proinflammatory cytokine release by peripheral blood mononuclear cells from patients with acute pancreatitis. British Journal of Surgery *83*, 1071-1075.

De Togni, P., Goellner, J., Ruddle, N.H., Streeter, P.R., and et al (1994). Abnormal development of peripheral lymphoid organs in mice deficient in lymphotoxin. Science *264*, 703-707.

de Waal Malefyt, R., Haanen, J., Spits, H., Roncarolo, M., Te Velde, A., Figdor, C., Johnson, K., Kastelein, R., Yssel, H., and de Vries, J.E. (1991). Interleukin -1(IL-10) and viral IL-10 strongly reduce antigen-specific human T-cell proliferation by diminishing the antigen-presenting capacity of monocytes via down regulation of class II major histocompatibility complex expression. Journal of Experimental Medicine *174*, 915-924.

de Waal Malefyt, R., Yssel, H., Roncarolo, M., Spits, H., and de Vries, J.E. (1992). Interleukin-10. Current Opinion in Immunology *4*, 314-320.

Debets, J.M.H., Kampmeijer, R., Van Der Linden, M.P.M.H., Buurman, W.A., and Van Der Linden, C.J. (1989). Plasma tumor necrosis factor and mortality in critically ill septic patients. Crit Care Med *17*, 489-494.

Dejbakhsh-Jones, S., Jerabek, L., Weissman, I.L., and Strober, S. (1995). Journal of Immunology *155*, 3338-3344.

Dempsey, P.W., Allison, M.E.D., Akkaraju, S., Goodnow, C.C., and Fearon, D.T. (1996). C3d of complement as a molecular adjuvant: bridging innate and acquired immunity. Science *271*, 348-350.

Deng, G.Y., McClaren, N.K., Huang, H.S., Zuang, J.P., and She, J.X. (1996). No primary association between the 308 polymorphism in the tumor necrosis factor alpha promoter region and insulin-dependent diabetes mellitus. Hum Immunol *45*, 137-142.

Denham, W., Fink, G.W., Yang, J., Ulrich, P., Tracey, K.J., and Norman, J.G. (1997a). Small molecule inhibition of tumor necrosis factor gene processing during acute pancreatitis prevents cytokine cascade progression and attenuates pancreatitis severity. Am Surg *63*, 1045-1049.

Denham, W., Yang, J., Fink, G.W., Denham, D., Carter, G., Ward, K., and Norman, J.G. (1997b). Gene targeting demonstrates additive detrimental effects of interleukin -1 and tumor necrosis factor. Gastroenterology *113*, 1741-1746.

Derr, R.F., Porta, E.A., Larkin, E.C., and Arnanda Rao, G. (1990). Is ethanol per se hepatotoxic? J Hepatol *10*, 381-386.

Dickensheets, H.L., Freeman, S.L., Smith, M.F., and Donnelly, R.P. (1997). Interleukin-10 upregulates tumor necrosis factor receptor type II (p75) gene expression in endotoxin stimulated human monocytes. Blood *90*, 4162-4171.

Diehl, A.K., Holleman, D.R., Chapman, J.B., Schwesinger, W.H., and Kurtin, W.E. (1997). Gallstone size and risk of pancreatitis. Archives of Internal Medicine *157*, 1674-1678.

Dower, S.K., Fanslow, W., Jacobs, C., Waugh, S., Sims, J.E., and Widmer, M.B. (1994). Interleukin-1 antagonists. Therapeutic Immunology *1*, 113-122.

Dunham, I., Sargent, C.A., Trowsdale, J., and Campbell, R.D. (1987). Molecular mapping of the major histocompatibility complex by pulse-field gel electrophoresis. Proc. Natl. Acad. Sci. *84*, 7237-7241.

Dunn, J.A., Li, C., Ha, T., Kao, R.L., and Browder, W. (1997). Therapeutic modification of nuclear factor kappa B biding activity and tumor necrosis factor-alpha gene expression during acute biliary pancreatitis. Am Surg *63*, 1036-1043.

Echtenacher, B., Falk, W., Mannel, D.N., and Krammer, P.H. (1990). Requirement of endogenous tumor necrosis factor/cahectin for recovery from experimental peritonitis. J Immunol *145*, 3762-3766.

Eigler, A., Sinha, B., Hartmann, G., and Endres, S. (1997). Taming TNF: strategies to restrain this pro-inflammatory cytokine. Immunology Today *18*, 487-491.

Emmanuelli, G., Montrucchio, G., Gaia, E., Dughera, L., Corvelli, G., and Gupetta, L. (1989). Experimental acute pancreatitis induced by platelet activating factor in rabbits. Am J Pathol *134*, 315-326.

Enk, A.H. and Katz, S.I. (1992). Identification and induction of keratinocyte derived IL-10. J Immunol *149*, 92-95.

Erickson, S.L., de Sauvage, F.J., Kikly, K., Carver-Moore, K., Pitts-Meek, S., Gillet, N., and et al (1994). decreased sensitivity to tumour necrosis factor but normal T-cell development in TNF receptor-2-deficient mice. Nature *372*, 560-563.

Eskdale, J. and Gallagher, G. (1995). A polymorphic dinucleotide repeat in the human IL-10 promoter. Immunogenetics *42*, 444-445.

Eskdale, J., Kube, D., and Gallagher, G. (1996). A second polymorphic dinucleotide repeat in the 5' flanking region of the human IL-10 gene. Immunogenetics *45*, 82-83.

Eskdale, J., Kube, D., Tesch, H., and Gallagher, G. (1997a). Mapping of the human IL10 gene and further characterisation of the 5' flanking sequence. Immunogenetics *46*, 120-128.

Eskdale, J., Wordsworth, P., Bowman, S., Field, M., and Gallagher, G. (1997b). Association between polymorphisms of the human IL-10 locus and systemic lupus erythematosus. Tissue Antigens *49*, 635-639.

Exley, A.R., Leese, T., Holliday, M.P., Swann, R.A., and Cohen, J. (1992). Endotoxaemia and serum tomour necrosis factor as prognostic markers in severe acute pancreatitis. Gut *33*, 1126-1128.

Fanning, G., Bunce, M., Black, C.M., and Welsh, K.I. (1997). Polymerase chain reaction haplotyping using 3' mismatches in the forward and reverse primers: application to the bi-allelic polymorphisms of tumor necrosis factor and lymphotoxin alpha. Tissue Antigens *50*, 23-31.

Genetet, B., Gosselin, M., and Gastard, J. (1979). HLA antigens in chronic alcoholic pancreatitis. Tissue Antigens *13*, 163-166.

Field, M., Gallagher, G., Eskdale, J., McGarry, F., Richards, S.D., Oh, H.H., and Campbell, C. (1997). Tumour necrosis factor locus polymorphisms in rheumatoid arthritis. Tissue Antigens *50*, 303-307.

Fink, G.W. and Norman, J.G. (1996). Intrapancreatic Interleukin -1? gene expression by specific leukocyte populations during acute pancreatitis. J Surg Res *63*, 369-373.

Fink, G.W. and Norman, J.G. (1997). Specific changes in the pancreatic expression of the interleukin 1 family of genes during experimental acute pancreatitis. Cytokine *9*, 1023-1027.

Fink, G.W., Yang, J., Carter, G., and Norman, J.G. (1997). Acute pancreatitis-induced enzyme release and necrosis are attenuated by IL-1 antagonism through an indirect mechanism. Journal of Surgical Research *67*, 94-97.

Fiorentino, D.F., Bond, M.W., and Mosmann, T.R. (1989). Two types of helper T cell. T cell clones secrete a factor that inhibits cytokine production Bh Th1 clones. J Exp Med *170*, 2081-1095.

Fiorentino, D.F., Zlotnik, A., Mosmann, T.R., Howard, M., and O'Garra, A. (1991). IL-10 inhibits cytokine production by activated macrophages. Journal of Immunology *147*, 3815-3822.

Fitz, R. (1889). Acute pancreatitis: a consideration of pancreatic haemorrhage,

haemorrhagic, supperative, and gangerenous pancreatitis, and of disseminated fat necrosis. Boston Medical and Surgical Journal *70*, 181-187.

Folsch, E., Closa, D., Prats, N., Gelpi, E., and Rosello-Catafau, J. (1998). Leukotreine generation and neutrophil infiltration after experimental acute pancreatitis. Inflammation *22*, 83-93.

Forbes, A., Schwarz, G., Mirikian, R., Drummond, V., Chan, C., Cotton, P.B., and Bottazo, G.F. (1987). HLA antigens in chronic pancreatitis. Tissue Antigens *30*, 176-183.

Formela, L.J., Wood, L.M., Whittaker, M., and Kingsnorth, A.N. (1994). Amelioration of acute pancreatitis in a rat model by a potent platelet activating factor antagonist. British Journal of Surgery *81*, 1783-1785.

Foulis, A.K., Murray, W.R., Galloway, D., McCartney, A.C., Lang, E., and Veitch, J. (1992). Endotoxaemia and complement activation in acute pancreatitis in man. Gut *23*, 656-661.

Frank, M.M. and Fries, L.F. (1991). The role of complement in inflammation and phagocytosis. Immunology Today *12*, 322-326.

Fraser, J.D., Irving, B.A., Crabtree, G.R., and Weiss, A. (1991). Regulation of Interleukin-2 gene enhancer activity by the T cell accessory molecule CD28. Science *251*, 313-316.

Frick, T.W., Fernandez-del-castillo, C., Bimmler, D., and Warshaw, A.L. (1997). Elevated calcium and activation of trypsinogen in rat pancreatic acini. Gut *41*, 339-343.

Fries, J.W., Williams, A.J., Atkins, R.C., Newman, W., Liscomb, M.F., and Collins, T. (1993). Expression of VCAM-1 and E-selectin in an in vivo model of endothelial cell activation. American Journal of Pathology *143*, 725-737.

Fu, K., Sarras, M.P., De Lisle, R.C., and Andrews, G.K. (1997). Expression of oxidative stress responses genes and cytokine genes during caerulein induced acute pancreatitis. Am J Physiol *273*, G696-G705.

Fujimoto, K., Hosotani, R., Doi, R., Wada, M., Lee, J.U., Koshiba, T., Miyamoto, Y., and Imamura, M. (1997). Role of neutrophils in cerulein induced pancreatitis in rats: possible involvement of apoptosis. Digestion *58*, 421-430.

Furukawa, M., Kimura, T., Yamaguchi, H., and et al (1994). Role of oxygen-derived free radicals in hemorrhagic pancreatitis induced by stress and cerulein in rats. Pancreas *9*, 67-72.

Gallagher, G., Eskdale, J., Oh, H.H., Richards, S.D., Campbell, D.A., and Field, M. (1997a). Polymorphisms in the TNF gene cluster and MHC serotypes in the West of Scotland. Immunogenetics *45*, 188-194.

Gallagher, G., Lindemann, M., Oh, H.H., Ferencik, S., Waldz, M.K., Schmitz, A., Richards, S.D., Eskdale, J., Field, M., and Grosse-Wilde, H. (1997b). Association of the TNFa2 microsatellite allel with the presence of colorectal cancer. Tissue Antigens *50*, 47-51.

Gallea-Robache, S., Morand, V., Millet, S., and et al (1997). A metalloproteinase inhibitor blocks the shedding of soluble cytokine receptors and processing of transmembrane cytokine precursors in human monocytic cells. Cytokine *9*, 340-346.

Galloway, S.W. and Kingsnorth, A.N. (1996). Lung injury in a microembolic model of acute pancreatitis. Pancreas *13*, 140-146.

Gamble, J.R., Harlan, J.M., Klebanoff, S.J., and Vadas, M.A. (1985). Stimulation of the adherence of neutrophils to umbilical vein endothelium by human recombinant tumor necrosis factor. Proc. Natl. Acad. Sci. USA *82*, 8667-8671.

Garcia-Merino, A., Alper, C.A., Usuku, K., Marcus-Bagley, D., Lincoln, R., Awdeh, Z., Yunis, E.J., Eisenbarth, G.S., Brink, S.J., and Hauser, S.L. (1996). Tumor necrosis factor (TNF) microsatellite haplotypes in relation to extended haplotypes, susceptibility to diseases associated with the Major Histocompatibility Complex and TNF secretion. Human Immunology *50*, 11-21.

Garcia-Merino, A., Alper, C.A., Usuku, K., Marcus-Bagley, D., Lincoln, R., Awdeh, Z., Yunis, E.J., Eisenbarth, G.S., Brink, S.J., and Hauser, S.L. (1996). Tumor necrosis factor (TNF) microsatellite haplotypes in relation to extended haplotypes, susceptibility to diseases associated with the Major Histocompatibility Complex and TNF secretion. Human Immunology *50*, 11-21.

Georgopoulos, K. (1997). Transcription factors required for lymphoid lineage commitment. Current Opinion in Immunology *9*, 222-227.

Gerard, C., Bruyns, C., Marchant, A., and et al (1993). Interleukin 10 reduces the relaeseof tumor necrosis factor and prevents lethality in experimental endotoxaemia. J Exp Med *177*, 547-550.

Gerard, C., Frossard, J., Bhatia, M., Saluja, A., Gerard, N.P., Lu, B., and Steer, M.L. (1997). Targeted disruption of the ?-chemokine receptor CCR1 protects against pancrreatitis-associated lung injury. Journal of Clinical Investigation *100*, 2022-2027.

Gesser, B., Leffers, H., Jinquan, T., and et al (1997). Identification of functional domains on human interleukin 10. Proc. Natl. Acad. Sci. USA *94*, 14620-14625.

Gjrup, I., Roikjaer, O., and Amderven, B. (1992). A double blinded multicenter trial of somatostatin in the treatment of acute pancreatitis. Surgery, Gynaecology, Obstetrics *175*, 397-400.

Goff, J.S. (1993). The effect of ethanol on the pancreatic duct sphincter of Oddi. Am J Gastroenterol *88*, 656-660.

Goldfield, A.E., Doyle, C., and Maniatis, T. (1990). Human tumor necrosis factor ? gene regulation by virud and lipopolysaccharide. Proc. Natl. Acad. Sci. USA *87*, 9769-9772.

Goris, R.J.A., te Boekhorst, T.P.A., Nuytinck, J.K.S., and Gimbrere, J.S.F. (1985). Multiple-organ failure. Arch Surg *120*, 1109-1115.

Goto, M., Matsuno, K., Yamaguchi, Y., Ezaki, T., and Ogawa, M. (1993). Proliferation kinetics of macrophage subpoulations in a rat experimental pancreatitis model. Arch Histol Cytol *56*, 75-82.

Grady, T., Liang, P., Ernst, S.A., and Logsdon, C.D. (1997). Chemokine gene expression in rat pancreatic acinar cells is an early event associated with acute pancreatitis. Gastroenterology *113*, 1966-1975.

Greenbaum, L.M. and Hirschkowitz, A. (1961). Endogenous cathepsin activates trypsinogen in extracts of dog pancreas. Proc Soc Exp Biol Med *107*, 74-76.

Grell, M., Douni, E., Wajant, H., Lohden, M., Clauss, M., Maxiener, B., and et al (1995). The transmembrane form of tumor necrosis factor is the prime activating ligand of the 80kDa tumor necrosis factor receptor. Cell *83*, 793-802.

Grewal, H.P., Mohey el-Din, A.B., Gaber, L.W., Kotb, M., and Gaber, A.O. (1994). Amelioration of the physiologic and biochemical changes of acute pancreatitisusing an anti-TNF-? polyclonal antibody. The American Journal of Surgery *167*, 214-219.

Griesbacher, T., Tiran, B., and Lembeck, F. (1993). Pathological events in experimental acute pancreatitis prevented by the bradykinin antagonist, Hoe 140. British Journal of Pharmacology *108*, 405-411.

Grimsley, C. and Ober, C. (1997). population studies of HLA-E. Evidence for selection. Hum Immunol *52*, 33-40.

Gross, V., Andreesen, R., Leser, H.G., Ceska, M., Liehl, E., Lausen, M., and et al (1992). Interleukin-8 and neutrophil activation in acute pancreatitis. European Journal of Clinical Investigation *22*, 200-203.

Grove, J., Daly, A.K., Bassendine, M.F., and Day, C.P. (1997). Association of a tumor necrosis factor promoter polymorphism with susceptibility to alcoholic steatohepatitis. Hepatology *26*, 143-146.

Grove, J., Daly, A.K., Bassendine, M.F., and Day, C.P. (1997). Association of a tumor necrosis factor promoter polymorphism with susceptibility to alcoholic steatohepatitis. Hepatology *26*, 143-146.

Guice, K.S., Oldham, K.T., and Johnson, K.S. (1988). Pancreatitis induced lung injury. Annals of Surgery *208*, 71-77.

Guice, K.S., Oldham, K.T., Caty, M.C., Johnson, K.J., and Ward, P. (1989). Neutrophil dependant oxygen radical mediated lung injury associated with acute pancreatitis. Annals of Surgery *210*, 740-747.

Guice, K.S., Oldham, K.T., Remick, D.G., Kunkel, S.L., and Ward, P.A. (1991). Anti-tumor necrosis factor antibody augments edema formation in caerulein-induced pancreatitis. Journal of Surgical Research *51*, 495-499.

Gukovskaya, A.S., Gukovsky, I., Zaninovic, V., Song, M., Sandoval, D., Gukovsky, S., and Pandol, S.J. (1997). Pancreatic acinar cells produce, release, and respond to tumour necrosis factor-a. The Journal of Clinical Investigation *100*, 1853-1862.

Gukovsky, I., Gukovskaya, A.S., and Pandol, S.J. (1998). Gastroenterology (Abstract 485)

Gullo, L., Tabacchi, P.L., Corazza, G.R., Calanca, F., Campione, O., and Labo, G. (1982). HLA-B13 and chronic calcific pancreatitis. Dig Dis Sci *27*, 214-216.

Guy, O., Robles-Diaz, G., Adrich, Z., Sahel, J., and Sarles, H. (1983). Protein content of precipitates present in pancreatic juice of alcoholic subjects and patients with chronic calcifying pancreatitis. Gastroenterology *89*, 102-107.

Haber, P., Wilson, J., Apte, M., Korsten, M., and Pirola, R. (1995). Individual susceptibility to alcoholic pancreatitis: still an enigma. J Lab Clin Med *125*, 305-312.

Hacker, U.T., Gomolka, M., Keller, E., Eigler, A., Folwaczny, C., Fricke, H., Albert, E., Loeschke, K., and Endres, S. (1997). Lack of association between an interleukin-1 receptor antagonist gene polymorphism and ulcerative colitis. Gut *40*, 623-627.

Hajeer, A.H., Lazarus, M., Turner, D.M., Mageed, R.A., Vencovsky, J., Sinnott, P.J., Hutchinson, I.V., and Ollier, W.E.R. (1998). IL-10 gene promoter polymorphisms in rheumatoid arthritis. Scand J rheumatol *27*, 142-145.

Hajeer, A.H., Worthington, J., Silman, A.J., and Ollier, W.E.R. (1996). Association of tumor necrosis factor microsatellite haplotypes with HLA-DRB1*04 bearing haplotypes in rheumatoid arthritis patients. Arthritis rheum *39*, 1109-1114.

Hale, K.K., Smith, C.G., Baker, S.L., Vanderslice, R.W., Squires, C.H., Gleason, T.M., Tucker, K.K., Kohno, T., and Russell, D.A. (1995). Multifunctional regulation of the biological effects of TNF-? by the soluble type I and type II receptors. Cytokine *7*, 26-38.

Hart, P.H., Ahern, M.J., Smith, M.D., and Finlay-Jones, J.J. (1995). Comparison of the supressive effects of interleukin 10 and interleukin 4 on synovial fluid macrophages and blood monocytes from patients with inflammatory arthritis. Immunology *84*, 536-542.

Hart, P.H., Hunt, E.K., Bonder, C.S., Watson, C.J., and Finlay-Jones, J.J. (1996). Regulation of surface and soluble TNF receptor expression on human monocytes and synovial fluid macrophages by IL-4 and IL-10. J Immunol *157*, 3672-3680.

Havell, E.A. (1989). Evidence that tumor necrosis factor has an important role in antibacterial resistance. The Journal of Immunology *143*, 2894-2899.

Hawrami, K., Hitman, G.A., Rema, M., Snehalatha, C., Viswasathan, M., Ramachandran, A., and Mohan, V. (1996). An association in non-insulin dependent Diabetes Mellitus subjects between susceptibility to retinopathy and tumour necrosis factor polymorphism. Human Immunology *46*, 49-54.

Hawrami, K., Hitman, G.A., Rema, M., Snehalatha, C., Viswasathan, M., Ramachandran, A., and Mohan, V. (1996). An association in non-insulin dependent Diabetes Mellitus subjects between susceptibility to retinopathy and tumour necrosis factor polymorphism. Human Immunology *46*, 49-54.

Hendrick (1994). Nature *308*, 149

Heresbach, D., Alizadeh, M., Dabadie, A., Le Berre, N., Columbel, J.F., Yaouanq, J., Bretagne, J.F., and Semana, G. (1997). Significance of interleukin-1 beta and interleukin-1 receptor antagonist genetic polymorphism in inflammatory bowel diseases. Am J Gastroenterol *92*, 1164-1169.

Hermann, S.M., Rickard, S., Nicaud, V., Mallet, C., Arveiler, D., Evans, A., Ruidavets, J.B., Luc, G., Barra, L., Parra, H.J., Poinier, O., and Cambien, F. (1998). Polymorphisms of the tumor necrosis factor-alpha gene, coronary heart disease and obesity. Eur J Clin Invest *28*, 59-66.

Hershey, G.K., Friedrich, M.F., Esswein, L.A., Thomas, M.L., and Chatila, T.A. (1997). The association of atopy with a gain-of-function mutation in the alpha subunit of the interleukin-4 receptor. N Engl J Med *337*, 1720-1725.

Hibbs, J.B., Taintor, R.R., Vavrin, Z., and Rachlin, E.M. (1988). Nitric oxide: a cytotoxic activated macrophage effector molecule. Biochem Biophys Res Commun *157*, 87-94.

Hirano, T., Saluja, A., Ramarao, P., Lerch, M.M., Saluja, M., and Steer, M.L. (1991). Apical secretion of lysosomal enzymes in rabbit pancreas occurs via a secretagogue regulated pathway and is increased after pancreatic duct obstruction. Journal of Clinical Investigation *87*, 865-869.

Ho, A.S. and Moore, K.W. (1994). Interleukin-10 and its receptor. Therapeutic Immunology *1*, 173-185.

Hoffmann, T.F., Leiderer, R., Waldner, H., and Messmer, K. (1996a). Bradykinin antagonists HOE-140 and CP-0597 diminish microcirculatory injury after ischaemia-reperfusion of the pancreas in rats. British Journal of Surgery *83*, 189-195.

Hoffmann, T.F., Waldner, H., and Messmer, K. (1996b). The bradykinin antagonist CP-0597 can limit the progression of postischaemic pancreatitis. Immunopharmacology *33*, 243-246.

Hohler, T., Kruger, A., Gerken, G., Schneider, P.M., Meyer zum Buschenfelde, K.H., and Rittner, C. (1997). A tumor necrosis factor-alpha (TNF-alpha) polymorphism is associated with chronic hepatitis-B infection. Clin Exp Immunol *111*, 579-582.

Honda, Z. (1991). Cloning by functional expression of platelet activating factor from guinea pig lung. Nature *349*, 342-346.

Hotamisligil, G.S., Arner, P., Caro, J.F., Atkinson, R.L., and Spiegelman, B.M. (1995). Increased adipose tissue expression of tumor necrosais factor in human obesity and insulin

resistance. Journal of Clinical Investigation *95*, 2409-2415.

Hotter, G., Closa, D., Gelpi, E., and et al (1995). Role of Xanthine oxidase and eicosanoids in development of pancreatic ischaemia-reperfusion injury. Inflammation *4*, 469-478.

Howard, M., Muchamuel, T., Andrade, s., and et al (1993). Interleukin-10 protects mice from lethal endotoxemia. J Exp Med *177*, 1205-1209.

Hughes, C.B., Gaber, L.W., Kotb, M., Mohey el-Din, A.B., Pabst, M., and Gaber, A.O. (1995). Induction of acute pancreatitis in germ-free rats: Evidence of a primary role for tumor necrosis factor-alpha. Surgery *117*, 201-205.

Hughes, C.B., Gaber, L.W., Mohey el-Din, A.B., Grewal, H.P., Kotb, M., Mann, L., and Gaber, A.O. (1996). Inhibition of TNF-? improves survival in an experimental model of acute pancreatitis. The American Surgeon *62*, 8-13.

Huizinga, T.W.J., Westendorp, R.G.J., Bollen, E.L.E.M., ., Keijsers, V., Brinkman, B.M.N., Langermans, J.A.M., Breedveld, F.C., Verweij, C.L., Van de Gaer, L., Crusius, J.B.A., Garcia-Gonzalez, A., Van Oosten, B.W., Polman, C.H., and Pena, A.S. (1997). TNF-? promoter polymorphisms, production and susceptibility to multiple sclerosis in different groups of patients. Journal of Neuroimmunology *72*, 149-153.

Huizinga, T.W.J., Westendorp, R.G.J., Bollen, E.L.E.M., ., Keijsers, V., Brinkman, B.M.N., Langermans, J.A.M., Breedveld, F.C., Verweij, C.L., Van de Gaer, L., Crusius, J.B.A., Garcia-Gonzalez, A., Van Oosten, B.W., Polman, C.H., and Pena, A.S. (1997). TNF-? promoter polymorphisms, production and susceptibility to multiple sclerosis in different groups of patients. Journal of Neuroimmunology *72*, 149-153.

Hunger, R.E., Mueller, C., Z'Graggen, K., Friess, H., and Buchler, M.W. (1997). Cytotoxic cells are activated in cellular infiltrates of alcoholic chronic pancreatitis. Gastroenterology *112*, 1656-1663.

Iimuro, Y., Bradford, B.U., Gao, W., Kadiiska, M., Mason, R.P., Stefanovic, B., Brenner, D.A., and Thurman, R.G. (1996). Detection of alpha-hydroxyethyl free radical adducts in the pancreas after chronic exposure to alcohol in the rat. Molecular Pharmacology *50*, 656-661.

Inoue, S., Nakao, A., Kishimoto, W., Murakami, H., Itoh, K., Itoh, T., Harada, A., Nonami, T., and Takagi, H. (1995). Anti-neutrophil antibody attenuates the severity of acute lung injury in rats with experimental acute pancreatitis. Arch Surg *130*, 93-98.

Isenmann, R. and Buchler, M.W. (1994). Infection and acute pancreatitis. British Journal of Surgery *81*, 1707-1708.

Ishizaka, S., Saito, S., Yoshikawa, M., Kimoto, M., and Nishiyama, T. (1996). IL-10 production in mouse hepatocytes augmented by TGF-B. Cytokine *8*, 837-843.

Itoh, K. and Hirohata, S. (1995). The role of IL-10 in human B cell activation, proliferation, and differentiation. J Immunol *154*, 4341-4350.

Jaakkola, M. and Nordback, I. (1993). Pancreatitis in Finland between 1970 and 1989. Gut *34*, 1255-1260.

Jacob, C.O., Fronek, Z., Lewis, G.D., Koo, M., Hansen, J.A., and McDevitt, H.O. (1990). Hereditable major histocompatibility complex class II-associated differences in production of tumor necrosis factor ? :Relevance to genetic predisposition to systemic lupus erythematosus. Proc. Natl. Acad. Sci. *87*, 1233-1237.

Jalleh, R.P., Gilbertson, J.A., Williamson, R.C.N., Slater, S.D., and Foster, C.S. (1993). Expression of major histocompatibility antigens in human chronic pancreatitis. Gut *34*, 1452-1457.

Janeway, C. and Travers, P. (1996). Antigen recognition by T lymphocytes. In Immunobiology: the immune system in health and disease. New York: Current Biology Ltd), pp. 4:1-4:48.

Joillet, P., Bulpa, P., and Ritz, M. (1997). Additive beneficial effects of the prone position, nitric oxide, and almatrine bismesylate on gas exchange and oxygen transport in acute respiratory distress syndrome. Critical Care Medicine *25*, 786-794.

Jones, E.Y., Stuart, D.I., and Walker, N.P.C. (1989). Structure of tumour necrosis factor. Nature *338*, 225-228.

Jongeneel, C.V., Briant, L., Udalova, I.A., Sevin, A., Nedaspasov, S.A., and Cambon-Thomsen, A. (1991). Extensive genetic polymorphism in the human tumour necrosis factor region and relation to extended HLA haplotypes. Proc. Natl. Acad. Sci. *88*, 9717-9721.

Jongeneel, C.V., Briant, L., Udalova, I.A., Sevin, A., Nedaspasov, S.A., and Cambon-Thomsen, A. (1991). Extensive genetic polymorphism in the human tumour necrosis factor region and relation to extended HLA haplotypes. Proc. Natl. Acad. Sci. *88*, 9717-9721.

Kanbe, T., Naruse, S., Kitagawa, M., Nakae, Y., and Hayakawa, T. (1996). Effects of a bradykinin receptor antagonist (HOE140) on taurochlorate-induced acute pancreatitis in rats. Pancreas *13*, 283-288.

Karimgani, I., Porter, K.A., Langevin, R.E., and Banks, P.A. (1992). Prognostic factors in sterile pancreatic necrosis. Gastroenterology *103*, 1636-1640.

Karlson, L., Surh, C.D., Sprent, J., and Peterson, P.A. (1992). An unusual class II molecule. Immunol Today *13*, 269-270.

Kazantse, G.B., Hecht, D.W., Rao, R., Fedorak, I.J., Gattuso, P., Thompson, K., Djuricin, G., and Prinz, R.A. (1994). Plasmic labelling confirms bacterial translocation in pancreatitis. American Journal of Surgery *167*, 201-207.

Keel, M., Ungethum, U., Steckholzer, U., and et al (1997). Interleukin 10 counterregulates proinflammatory cytokine induced inhibition of neutrophil apoptosis during severe sepsis. Blood *90*, 3356-3363.

Kellermeyer, R.W. and Graham, R.C. (1968). Kinins-possible physiologic and pathologic roles in man. New England Journal of Medicine *279*, 859-866.

Khoo, S.H., Pepper, L., Snowden, N., Hajeer, A.H., Valelly, P., Wilkins, E.G., Mandal, B.K., and Ollier, W.E.R. (1997). Tumour necrosis factor c2 microsatellite allele is associated with rate of HIV disease progression. AIDS *11*, 423-428.

Kim, J.M., Brannan, C.I., Copeland, N.G., Jenkins, N.A., Khan, T.A., and Moore, K.W. (1992). Structure of the mouse IL-10 gene and chromosomal localisation of the mouse and human genes. Journal of Immunology *146*, 3618-3623.

Kim, S., Davis, M., Sinn, E., Patten, P., and Hood, L. (1981). Antibody diversity; Somatic hypermutation of rearranged Vh genes. Cell 27, 573-581.

Kindler, V. and Zubler, R.H. (1997). Memory, but not naive B Lymphocytes differentiate into Ig secreting cells after Cd40 ligation and costimulation with Il-4 and the differentiation factors, IL-2,IL-10. J Immunol *159*, 2085-2090.

Kingsnorth, A.N. (1996). Platelet-Activating factor. Scand J Gastroenterol *31(S2)*, 28-31.

Kingsnorth, A.N. (1997). Role of cytokines and their inhibitors in acute pancreatitis. Gut *40*, 1-4.

Kingsnorth, A.N. and for the British Acute Pancreatitis Study Group (1997). early treatment with Lexipafant, a platelet activating factor antagonist reduces mortality in acute

pancreatitis: a double blind, randomised placebo controlled study. Gastroenterology *112*, 453A

Kingsnorth, A.N. and Sargen, K. (1998). The inflammatory response in acute pancreatitis. Current Opinion in Critical Care *4*, 121-124.

Kingsnorth, A.N., Galloway, S.W., and Formela, L.J. (1995). Randomised, double-blind phase II trial of Lexipafant, a platelet activating factor antagonist, in human acute pancreatitis. British Journal of Surgery *82*, 1414-1420.

Kleijmeer, M.J., Kelly, A., Geuze, H.J., Slot, J.W., Townsend, A., and Trowsdale, J. (1992). Location of MHC-encoded transporters in the endoplasmic reticulum and cis-golgi. Nature *357*, 342-344.

Knolle, P., Schlaak, J., Uhrig, A., Kempf, P., and Gerken, G. (1995). human kupffer cells secrete IL-10 in response to lipopolysaccharide challenge. Journal of Hepatology *22*, 226-229.

Knuchel, M.C., Spira, T.J., Neumann, A.U., Xiau, L., Rudolph, D.L., Phair, J., Wolinsky, S.M., Koup, R.A., Cohen, O.J., Folks, T.M., and Lal, R.B. (1998). Analysis of bi-allelic polymorphism in the tumor necrosis factoralpha promoter and HIV type 1 disease progression. AIDS Res *14*, 305-309.

Kornfield, S. (1986). Trafficking of lysosomal enzymes in normal and disease states. Journal of Clinical Investigation *77*, 1-6.

Kube, D., Platzer, C., Von Knethen, A., Straub, H., Bohlen, H., Hafner, M., and Tesch, H. (1995). Isolation of the human interleukin 10 promoter. Characterisation of the promoter activity in Burkitt's Lymphoma cell lines. Cytokine *7*, 1-7.

Kubes, P., Suzuki, M., and Granger, D.N. (1991). Nitric oxide: an endogenous modulator of leukocyte adhesion. Proc. Natl. Acad. Sci. USA *88*, 4651-4655.

Kusske, A.M., Rongione, A.J., and Reber, H.A. (1996b). Cytokines and acute pancreatitis. Gastroenterology *110*, 639-642.

Kusske, A.M., Rongione, A.J., Ashley, S.W., and et al (1996a). Interleukin-10 prevents death in lethal necrotizing pancreatitis in mice. Surgery *120*, 284-289.

Lankisch, P.G. and Banks, P.A. (1998a). Chronic pancreatitis: Complications. In Pancreatitis. P. Lanksich and P.A. Banks, eds. (Berlin: Springer-Verlag), pp. 115-143.

Lankisch, P.G. and Banks, P.A. (1998b). General Considerations: Anatomy. In Pancreatitis. P. Lanksich and P.A. Banks, eds. (Berlin: Springer-Verlag), pp. 15-18.

Lankisch, P.G. and Banks, P.A. (1998c). Acute pancreatitis:pathophysiology. In Pancreatitis. P. Lanksich and P.A. Banks, eds. (Berlin: Springer-Verlag), pp. 161

Lankisch, P.G. and Banks, P.A. (1998d). Chronic pancreatitis: Pathology. In Pancreatitis. P. Lanksich and P.A. Banks, eds. (Berlin: Springer-Verlag), pp. 215-217.

Lankisch, P.G. and Banks, P.A. (1998e). Acute Pancreatitis: Complications. In Pancreatitis. P. Lanksich and P.A. Banks, eds. (Berlin: Springer-Verlag), pp. 115-143.

Lankisch, P.G., Assmus, C., Pflichthofer, D., Maisonneuve, P., and Lowenfels, A.B. (1998). Risk factors for the developing chronic pancreatitis after a first attack of alcoholic acute pancreatitis. Gastroenterology A501(Abstract)

Lankisch, P.G., Droge, M., and Gottesleben, F. (1995). Drug induced acute pancreatitis: incidence and severity. Gut *37*, 565-567.

Lankisch, P.G., Pohl, U., and Otto, J. (1989). Xanthine oxidase inhibitor in acute experimental pancrreatitis in rats and mice. Pancreas *4*, 436-440.

Larvin, M. and McMahon, M.J. (1989). Apache-II score for assessment and monitoring of acute pancreatitis. Lancet *ii*, 201-205.

Larvin, M., Alexander, D.J., Switala, S.F., and McMahon, M.J. (1993). impaired mononuclear phagocyte function in patients with severe acute pancreatitis. Digestive Diseases and Sciences *38*, 18-27.

Laster, S.M., Wood, J.G., and Gooding, L.R. (1988). Tumor necrosis factor can induce both apoptic and necrotic forms of cell lysis. J Immunol *141*, 2629-2634.

Lazarus, M., Hajeer, A.H., Turner, D.M., Sinnott, P.J., Worthington, J., Ollier, W.E.R., and Hutchinson, I.V. (1997). Genetic variation in the Interleukin-10 gene promoter and systemic lupus erythematosus. J Rheumatol *24*, 2314-2317.

Le Boutellier, P. (1994). HLA class I chromosomal region, genes, and products: facts and questions. Crit Rev Immunol *14*, 89-129.

Leach, S.D., Gorelick, F.S., and Modlin, I.M. (1990). Acute pancreatitis at its centenary. Annals of Surgery *212*, 109-113.

Ledebur, H.C. and Parks, T.P. (1995). Transcriptional regulation of the intercellular adhesion molcule 1 gene by inflammatory cytokines in human endothelial cells:essential roles of a variant NFkB site and p65 homodimers. J Biol Chem *270*, 933-943.

Lee, S.P., Nicholls, J., and Park, H.Z. (1992). Biliary sludge as a cause of acute pancreatitis. New England Journal of Medicine *326*, 589-593.

Lerch, M.M., Saluja, A., Dawra, R., Ramarao, P., Saluja, M., and Steer, M.L. (1992). acute necrotizing pancreatitis in the opossum: earliest morphological changes involve acinar cells. Gastroenterology *103*, 205-213.

Lerch, M.M., Weidenbach, H., Gress, T.M., and Adler, G. (1995). Effect of kinin inhibition in experimental acute pancreatitis. American Journal of Physiology *(GI Liv Physiol 32)*, G490-G499.

Lerch, M.M., Weidenbach, H., Hernandez, C.A., Preclik, G., and Adler, G. (1994). Pancreatic outflow obstruction as the critical event in human gallstone induced pancreatitis. Gut *35*, 1501-1503.

Li, Y. and Owyang, C. (1994). Endogenous cholecystokininstimulates pancreatic exocrine secretion via vagal afferent pathway in rats. Gastroenterology *107*, 525-531.

List, S. and Gluuid, C. (1994). A meta-analysis of HLA-antigen prevalences in alcoholics and alcoholic liver disease. Alcohol & Alcoholism *29*, 757-764.

Liu, Y., Wei, S., H-Y., Ho, A.S., de Waal Malefyt, R., and Moore, K.W. (1994). Expression cloning and characterisation of a human IL-10 receptor. J Immunol *152*, 1821-1826.

Lloyd, A.R., Oppenheim, J.J., Kelvin, D.J., and Taub, D.D. (1996). Chemokines regulate T cell adherence to recombinant adhesion molecules and extracellular matrix proteins. J Immunol *156*, 932-938.

Louis, E., Satsangi, J., Roussomoustakaki, M., Parkes, M., Fanning, G., Welsh, K.I., and Jewell, D.P. (1996). Cytokine gene polymorphisms in inflammatory bowel disease. Gut *39*, 705-710.

Lumeng, L. and Crabb, D.W. (1994). Genetic aspects and risk factors in alcoholism and alcoholic liver disease. Gastroenterology *107*, 572-578.

Mann, D.V., Hershman, M.J., Hittinger, R., and Glazer, G. (1994). Multicentre audit of death from acute pancreatitis. British Journal of Surgery *81*, 890-893.

Mansfield, J.A., Holden, H., Tarlow, J.K., McDowell, T.L., Wilson, A.G., di Giovine, F.S., Holdsworth, C.D., and Duff, G.W. (1994). Novel genetic association between ulcerative colitis and the anti-inflammatory cytokine interleukin-1 receptor antagonist. Gastroenterology *106*, 637-642.

Mantovani, A., Bussolino, F., and Introna, M. (1997). Cytokine regulation of endothelial cell function. Immunology Today *18*, 231-239.

Manus, R.M., Wilson, A.G., Mansfield, J.A., Weir, D.W., Duff, G.W., and Kelleher, D. (1996). TNF2, a polymorphism of the tumour necrosis factor alpha gene promoter, is a component of the celiac disease major histocompatibility complex haplotype. Eur J Immunol *29*, 2113-2118.

Marchant, A., Deviere, J., Baudouin, B., De Groote, D., Vincent, J., and Goldman, M. (1994). Interleukin-10 production during septicaemia. Lancet *343*, 707-708.

Mascher, B., Schmidt, W., Csemok, E., Tatsis, E., Reil, A., Gross, W.L., and Sayfarth, M. (1996). Polymorphisms in the tumour necrosis factor genes and Wegener's granulomatosis. Exp Clin Immunogenet *14*, 226-233.

Matsumoto, M., Takahashi, H., Murayama, K., Higuchi, S., Matsushita, S., Muramatsu, T., Okuyama, K., Yokoyama, A., Nanako, M., and Ishii, H. (1996). Genotypes of alcohol-metabolising enzymes and the risk for alcoholic chronic pancreatits in Japanese alcoholics. Alcoholism Clin Exp Res *20*, 289A-292A.

Matsushita, M., Endo, Y., Nonaka, A., and Fujita, T. (1998). Complement related serine proteases in vertebrates. Current Opinion in Immunology *10*, 29-35.

Maudsley, D.J. (1995). Modulation of MHC expression by viruses. In Modulation of MHC antigen expression and disease. G.E. Blair, C.R. Pringle, and D.J. Maudsley, eds. (Cambridge: Cambridge University Press), pp. 133-149.

Mc Dowell, P.L., Symonds, J.A., Ploski, R., Forre, O., and Duff, G.W. (1995). A genetic association between juvenile rheumatoid arthritis and a novel interleukin-1 alpha polymorphism. Arthritis rheum *38*, 221-228.

McArthur, K.E. (1996). Review article: drug-induced pancreatitis. Aliment Pharmacol Ther *10*, 23-38.

McFadden, D.W. (1991). Organ failure and multiple organ system failure in pancreatitis. Pancreas *6*, S37-S43.

McGuire, W., Hill, A.V.S., Allsopp, C.E.M., Greenwood, B.M., and Kwiatkowski, D. (1994). Variation in the TNF? promoter region associated with susceptibility to cerebral malaria. Nature *371*, 508-511.

McGuire, W., Hill, A.V.S., Allsopp, C.E.M., Greenwood, B.M., and Kwiatkowski, D. (1994). Variation in the TNF? promoter region associated with susceptibility to cerebral malaria. Nature *371*, 508-511.

McKay, C., Curran, F., Sharples, C., and Imrie, C.W. (1997). Lexipafant in severe acute pancreatitis: a prospective, placebo-controlled, randomised trial. British Journal of Surgery *84*,

McKay, C.J., Gallagher, G., Brooks, B., Imrie, C.W., and Baxter, J.N. (1996). Increased monocyte cytokine production in association with systemic complications in acute pancreatitis. British Journal of Surgery *83*, 919-923.

McLaughlin, P.J. and Davies, H.M. (1990). Tumour necrosis factor in normal plasma. Lancet *ii*, 1014-1015.

McManus, R., Moloney, M., Borton, M., Finch, A., Chaun, Y.T., Lawlor, E., Weir, D.G., and Kelleher, D. (1996). Association of celiac disease with microsatellite polymorphisms close to the tumor necrosis factor genes. Hum Immunol *45*, 24-31.

Medich, D.S., Lee, T.K., Melhem, M.F., Rowe, M.I., Schraut, W.H., and Lee, K.K.W. (1993). Pathogenesis of pancreatic sepsis. American Journal of Surgery *165*, 46-52.

Mehrota, P.T., Donelly, R.P., Wong, S., and et al (1998). Production of IL-10 by human NK cells stimulated with IL-2 and/or Il-12. J Immunol *160*, 2637-2344.

Meier, P. (1995). Who gets and what causes pancreatitis. J Lab Clin Med *125*, 298-300.

Meldrum, D.R. (1998). Tumor necrosis factor in the heart. American Journal of Physiology *274*, R577-R595.

Mergener, K. and Baillie, J. (1998). Acute Pancreatitis. British Medical Journal *316*, 44-48.

Messer, G., Spengler, U., Jung, M.C., Honold, G., Blomer, K., Pape, G.R., Reithmuller, G., and Weiss, E.H. (1991). Polymorphic structure of the tumor necrosis factor (TNF) locus: an NcoI polymorphism in the first intron of the human TNF-b gene correlates with a variant amino acid in position 26 and a reduced level of TNF-b production. J Exp Med *173*, 209-219.

Moffatt, M.F. and Cookson, W.O. (1997). Tumour necrosis factor haplotypes and asthma. Hum Mol Genet *6*, 551-554.

Mok, C.C., Lanchbury, J.S., Chan, D.W., Lau, C.S. (1998). Interleukin-10 promoter polymorphisms in Southern Chinese patients with systemic lupus erythematosus. Arthritis & Rheumatism. *4*, 1090-5.

Molero, X., Guarner, F., and Salas, A. (1995). Nitric oxide modulates pancreatic basal secretion and response to cerulein in the rat: effects in acute pancreatitis. Gastroenterology *108*, 1855-1862.

Monaco, J.J. (1992). Not so groovy after all? Curr Biol *2*, 433-435.

Monos, D.S., Kamoun, M., Udalova, I.A., Czansky, E., Cizman, B., Turetskaya, R.L., Smirnova, J.B., Zharkov, V.G., Gasser, D., and Zmijewski, C.M. (1995). Genetic polymorphism of the human tumor necrosis factor region in insulin-dependent diabetes mellitus.Linkage disequilibrium of TNFab microsatellite alleles with HLA haplotypes. Hum Immunol *44*, 70-79.

Moore, K.W., O'Garra, A., de Waal Malefyt, R., Vierra, P., and Mosmann, T.R. (1993). Annu Rev Immunol *11*, 165-190.

Mora, A., Perez-Mateo, M., Viedma, J.A., Carballo, F., Sanchez-Paya, J., and Liras, G. (1997). Activation of cellular immune response in acute pancreatitis. Gut *40*, 794-797.

Moreau, J.A., Zinsmeister, A.R., Melton, L.J., and DiMagno, E.P. (1988). Gallstone pancreatitis and the effect of cholecystectomy: a population-based cohort study. Mayo Clinic Proceedings *63*, 466-473.

Morel, P.A., Dorman, J.S., Todd, J.A., McDevitt, H.O., and Trucco, M. (1988). Aspartic acid at position 57 of the HLA-DQB chain protects against type I diabetes: a family study. Proc. Natl. Acad. Sci. USA *85*, 8111-8115.

Mosmann, T.R., Cherwinski, H., Bond, M.W., Giedlin, M.A., Coffman, R.L., and Locksley, R.M. (1986). Journal of Immunology *136*, 2348-2357.

Mullighan CG, Fanning G, Chapel HM, Welsh KI. (1997). TNF and lymphotoxin-alpha polymorphisms associated with common variable immune deficiency; role in the pathogenesis of granulomatous disease. J Immunol *159*, 6236-41.

Multigener, L., Sarles, H., Lombardo, D., and De Caro, A. (1985). Pancreatic stone protein.II. Implication in stone formation during the course of chronic calcifying pancreatitis. Gastroenterology *89*, 387-391.

Muncy, D.M., Haley-Russell, D., Stanislawka, M., Vakil, A., Rodriguez, L., and Moody, F.G. (1993). Bacterial translocation following acute pancreatitis in rats. Gastroenterology *102*, A490(Abstract)

Murakami, H., Nakao, A., Kishimoto, W., and et al (1995). Detection of O_2^- generation and neutrophil accumalation in rat lungs after acute necrotizing pancreatitis. Surgery *118*, 547-554.

Nadel, S., Newport, M.J., Booy, R., and Levin, M. (1996). Variation in the tumour necrosis factor-a gene promotor region may be associated with death from meningococcal disease. Journal of Infectious Diseases *174*, 878-880.

Nadel, S., Newport, M.J., Booy, R., and Levin, M. (1996). Variation in the tumour necrosis factor-a gene promotor region may be associated with death from meningococcal disease. Journal of Infectious Diseases *174*, 878-880.

Nanako, M., Knowlton, A.A., Dibbs, Z., and Mann, D.L. (1998). Tumor necrosis factor-alpha confers resistance to hypoxic injury in the adult mammalian cardiac myocyte. Circulation *14*, 1392-1400.

Napolitano, L.M., Buzdon, M.M., Shi, H.J., and Bass, B.L. (1997). Intestinal epithelial cell regulation of macrophage and lymphocyte interleukin-10 expression. Arch Surg *132*, 1271-1276.

Nedaspasov, S.A., Udalova, I.A., Kuprash, D.V., and Turetskaya, R.L. (1991). DNA sequence polymorphism at the human tumor necrosis factor (TNF) locus. J Immunol *147*, 1053-1059.

Neiderau, C., Neiderau, M., Borchard, F., and et al (1992). Effects of antioxidants and free radical scavengers in three different models of acute pancrreatitis. Pancreas *7*, 486-496.

Neiderau, C., Neiderau, M., Luthen, R., Strohmeyer, G., Ferrell, L.D., and Grendell, J.H. (1990). Pancreatic exocrine secretion in acute experimental pancreatitis. Gastroenterology *99*, 1120-1127.

Newton, C.R., Graham, A., Heptinstall, L.E., Powell, S.J., Summers, C., Kalsheker, N., Smith, J.C., and Markham, A.F. (1989). Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS). Nucleic Acids Research *17*, 2503-2516.

Nicklin, M.J.H., Weith, A., and Duff, G.W. (1994). a physical map of the region encompassing the human interleukin 1 and IL-1 receptor antagonist genes. Genomics *19*, 382-384.

Nishizuka, Y. (1984). Turnover of inositol phospholipids and signal transduction. Science *225*, 1365-1370.

Nonaka, A., Manabe, T., and Tobe, T. (1991). Effect of a new synthetic ascorbic acid derivative as a free radical scavenger on the development of acute pancreatitis in mice. Gut *32*, 528-532.

Nonaka, A., Manabe, T., Tamura, K., and et al (1989). Changes in lipid peroxide and oxygen radical scavengers in Cerulein induced pancreatitis. Digestion *43*, 41-46.

Norman, J.G., Fink, G.W., and Franz, M.G. (1995a). Acute pancreatitis induces intrapancreatic tumor necrosis factor gene expression. Arch Surg *130*, 966-970.

Norman, J.G., Fink, G.W., Franz, M.G., Guffey, J., Carter, G., Davison, B., and Glaccum,

M. (1996a). Active Interleukin-1 receptor required for maximal progression of acute pancreatitis. Annals of Surgery *223*, 163-169.

Norman, J.G., Fink, G.W., Messina, J., Carter, G., and Franz, M.G. (1996b). Timing of tumor necrosis factor antagonism is critical in determining outcome in murine lethal acute pancreatitis. Surgery *120*, 515-521.

Norman, J.G., Franz, M.G., Fink, G.W., Messina, J., Fabri, P.J., Gower, W.R., and Carey, L.C. (1995b). Decreased mortality of severe acute pancreatitis after proximal cytokine blockade. Annals of Surgery *221*, 625-634.

Norman, J.G., Franz, M.G., Messina, J., Riker, A., Fabri, P.J., Rosemurgy, A.S., and Gower, W.R. (1995c). Interleukin-1 receptor antagonist decreases severity of experimental acute pancreatitis. Surgery *117*, 648-655.

Norman, J.G., Yang, J., Fink, G.W., Carter, G., Ku, G., Denham, W., and Livingston, D. (1997). Severity and mortality of experimental pancreatitis are dependent on Interleukin-1 converting enzyme (ICE). Journal of Interferon and Cytokine Research *17*, 113-118.

O'Donovan, D.A., Kelly, C.J., Abdih, H., Bouchier-Hayes, D., Watson, R.W.G., Redmond, H.P., Burke, P.E., and Bouchier-Hayes, D.A. (1995). Role of nitric oxide in lung injury associated with experimental acute pancreatitis. British Journal of Surgery *82*, 1122-1126.

Oettinger, M.A., Schatz, D.G., Gorka, C., and Baltimore, D. (1990). RAG-1 and RAG-2, adjacent genes that synergistically activate V(D)J recombination. Science *248*, 1517-1523.

O'Farrell, A.M., Liu, Y., Moore, K.W., and Mui, A.L. (1998). IL-10 inhibits macrophage activation and proliferation by distinct signalling pathways. EMBO Journal *17*, 1006-1018.

O'Garra, A., Chang, R., Hastings, R., Houghton, G., and Howard, M. (1992). B1 cells are the main source of B cell derived IL-10. Eur J Immunol *22*, 711-717.

Onozaki, K., Matsushima, K., Aggarwal, B.B., and Oppenheim, J.J. (1985). Interleukin-1 as a cytocidal factor for several tumor cell lines. J Immunol *135*, 3962-3968.

Opie, E.L. (1901). The etiology of acute haemorrhagic pancreatitis. John Hopkins Hospital Bulletin *12*, 182-188.

Palade, G. (1975). Intracellular aspects of the process of protein synthesis. Science *189*, 347-358.

Palmer, R.M.J., Ashton, D.S., and Moncada, S. (1988). Vascular endothelial cells synthesize nitric oxide from L-arginine. Nature *333*, 664-646.

Panes, J. and Granger, D.N. (1998). Leukocyte-endothelial cell interactions: Molecular mechanisms and implications in gastrointestinal disease. Gastroenterology *114*, 1066-1090.

Parenti, D.M., Steinberg, W., and Kang, P. (1996). Infectious causes of acute pancreatitis. Pancreas *13*, 356-371.

Parham, P. (1990). Antigen processing. Transporters of delight. Nature *348*, 674-675.

Parham, P., Lomen, C.E., Lawlor, D.A., Ways, J.P., Holmes, N., Coppin, H.L., Salter, R.D., Wan, A.M., and Ennis, P.D. (1988). nature of polymorphism of HLA-A, -B, and -C molecules. Proc. Natl. Acad. Sci. USA *85*, 4005-4009.

Parillo, J.E. (1993). Pathogenetic mechanisms of septic shock. New England Journal of Medicine *328*, 1471-1477.

Pennica, D., Nedwin, G.E., Hayflick, J.S., Seeburg, P.H., Derynck, R., Palladino, M.A., Kohr, W.J., Aggarwal, B.B., and Goedel, D.V. (1984). Human tumor necrosis factor: precursorstructure, expression and homology to lymphotoxin. Nature *312*, 724-729.

Petrone, W.F., English, D.K., Wong, K., and McCord, J.M. (1980). Free radicals and inflammation: Superoxide-dependent activation of a neutrophil chemotactic factor in plasma. Proc. Natl. Acad. Sci. USA *77*, 1159-1163.

Pezzilli, R., Billi, P., Beltrandi, E., Casedei Maldini, M., and Mancini, R. (1997a). Impaired lymphocyte proliferation in human acute pancreatitis. Digestion *58*, 431-436.

Pezzilli, R., Billi, P., Miniero, R., and Bakarat, B. (1997b). Serum Interleukin-10 in human acute pancreatitis. Digestive Diseases Science *42*, 1469-1472.

Platanias, L.C. and Vogelzang, N.J. (1990). Interleukin-1:Biology,pathophysiology,and clinical prospects. American Journal of Medicine *89*, 621-629.

Platzer, C., Meisel, C.H., Vogt, K., Platzer, K., and Volk, H.D. (1995). Up-regulation of monocytic IL-10 by tumor necrosis factor-alpha and cAMP elevating drugs. Int Immunol *7*, 517-523.

Plevy, S.E., Targan, S.R., Yang, H., Fernandez, D., Rotter, J.I., and Toyoda, H. (1996). Tumor Necrosis Factor Microsatellites define a Crohn's Disease-Associated haplotype on Chromasome 6. Gastroenterology *110*, 1053-1060.

Pober, J.S., Lapierre, L.A., Stolpen, A.H., and et al (1987). Activation of cultured human endothelial cells by recombinant lymphotoxin: comparison with tumor necrosis factor and Interleukin -1 species. J Immunol *138*, 3319-3324.

Poicot, F., Briant, L., Jongeneel, C.V., Molvig, J., Worsaae, H., Abbal, M., Thomsen, M., Nerup, J., and Cambon-Thomsen, A. (1993). Association of tumour necrosis factor (TNF) and class II major histocompatibility complex alleles with the secretion of TNF-? and TNF-? by human mononuclear cells : a possible link to insulin-dependent diabetes mellitus. Eur J Immunol *23*, 224-231.

Poicot, F., Briant, L., Jongeneel, C.V., Molvig, J., Worsaae, H., Abbal, M., Thomsen, M., Nerup, J., and Cambon-Thomsen, A. (1993). Association of tumour necrosis factor (TNF) and class II major histocompatibility complex alleles with the secretion of TNF-? and TNF-? by human mononuclear cells : a possible link to insulin-dependent diabetes mellitus. Eur J Immunol *23*, 224-231.

Powis, S.H., Mockridge, I., Kelly, A., Kerr, L.A., Glynne, R., Gileadi, U., Beck, S., and Trowsdale, J. (1992). Polymorphism in a second ABC transporter gene located within the class II region of the human major histocompatibility complex. Proc. Natl. Acad. Sci. USA *89*, 1463-1467.

Premack, B.A. and Schall, T.J. (1996). Chemokine receptors: Gateways to inflammation and infection. Nature Medicine *2*, 1174-1178.

Pretolani, M. and Goldman, M. (1997). IL-10: a potential therapy for allergic inflammation? Immunology Today *18*, 277-283.

Prins, J.B., Neisler, C.U., Winterford, C.M., and et al (1997). Tumor necrosis factor ? induces apoptosis of human adipose cells. Diabetes *46*, 1939-1944.

Pujol-Borrell, R., Todd, I., Doshi, M., and et al (1987). HLA class II induction in human islet cells by interferon-gamma plus tumour necrosis factor or lymphotoxin. Nature *326*, 304-306.

Pujol-Borrell R, Todd I, Londei M, Foulis AK, Feldmann M, Bottazo GF (1986). Innapropriate major histocompatibility complex class II expression by thyroid follicular cells in thyroid autoimmune disease and by pancreatic beta cells in type I diabetes. Mol Biol Med *3*, 159-65.

Puolakkainen, P., Valtonen, V., Paananen, A., and Schroder, T. (1987). C-reactive protein

(CRP) and serum phospholipase A_2 in the assesment of severity of acute pancreatitis. Gut *28*, 764-771.

Radi, R., Beckman, J.S., Bush, K.M., and Freeman, B.A. (1991). Peroxynitrite oxidation of sulfhydryls: the cytotoxic potential of superoxide and nitric oxide. J Biol Chem *266*, 244-250.

Ragoussis, J. (1995). Organization of the MHC. In Modulation of MHC expression and disease. G.E. Blair, C.R. Pringle, and D.J. Maudsley, eds. (Cambridge: Cambridge University Press), pp. 27-42.

Ranson, J.H.C., Rifkind, K.M., Roses, D.F., Fink, S.D., Eng, K., and Spencer, F.C. (1974). Prognostic signs and the role of operative management in acute pancreatitis. Surgery, Gynaecology, Obstetrics *139*, 69-81.(Abstract)

Reed, T., Page, W.F., Viken, R.J., and Christian, J.C. (1996). Genetic predisposition to organ-specific endpoints of alcoholism. Alcoholism Clin Exp Res *20*, 1528-1533.

Riche, F., Panis, Y., Laisne, M., Briard, C., Cholley, B., Bernard-Poenaru, O., Graulet, A., Gueris, J., and Valleur, P. (1996). High tumor nrcrosis factor serum level is associated with increased survival in patients with abdominal septic shock: a prospective study in 59 patients. Surgery *120*, 801-807.

Rinderknecht, H. (1988). Fatal pancreatitis, a consequence of excessive leukocyte stimulation? International Journal of Pancreatology *3*, 105-112.

Rinderknecht, H. (1993). Pancreatic secretory enzymes. In The pancreas: Biology, Pathobiology, and disease. V.L.W. Go, E.P. DiMagnio, J.D. Gardner, E. Lebenthal, H.A. Reber, and G.A. Scheele, eds. (New York: Raven Press), pp. 219-251.

Rinderknecht, H. (1994). Genetic determinants of mortality in acute necrotizing pancreatitis. International Journal of Pancreatology *16*, 11-15.

Rink, L. and Kirchner, H. (1996). Recent progress in the tumor necrosis factor-a field. Allergy and Immunology *111*, 199-209.

Rongione, A.J., Kusske, A.M., and Kwan, K. (1997). Interleukin-10 reduces the severity of acute pancreatitis in rats. Gastroenterology *112*, 960-967.

Ros, E., Navarro, S., Garcia-Puges, A., and Valderrama, R. (1991). Occult microlithiasis in 'idiopathic' acute pancreatitis: prevention of relapses by cholecystectomy or ursodeoxycholic acid therapy. Gastroenterology *101*, 1701-1709.

Rosai, J. (1998). Pancreas and ampullary region. In Ackerman's Surgical Pathology. J. Rosai, ed. (St Louis: Mosby), pp. 969-970.

Rothe, J., Lesslauer, W., Lotscher, H., Lang, Y., Koebel, P., Kontgen, F., Althage, A., Zinkernagel, R., Steinmetz, M., and Bluethmann, H. (1993). Mice lacking the tumour necrosis factor receptor 1 are resistant to TNF-mediated toxicity but highly susceptible to infection by Listeria monocytogenes. Nature *364*, 798-802.

Rousset, F., Garcia, E., Defrance, T., Peronne, C., Vezzio, N., Hsu, D., Kastelein, R., Moore, K.W., and Banchereau, J. (1992). IL-10 is a potent growth and differentiation factor for activated human B lymphocytes. Proc. Natl. Acad. Sci. USA *89*, 1890-1893.

Roussomoustakaki, M., Satsangi, J., Welsh, K.I., Louis, E., Fanning, G., Targan, S.R., Landers, C., and Jewell, D.P. (1997). Genetic markers may predict disease behavior in patients with Ulcerative colitis. Gastroenterology *112*, 1845-1853.

Rudlaweit, M., Tikly, M., Khamashta, M., Gibson, K., Klinke, J., Hughes, G., and Wordsworth, P. (1996). Interethnic differences in the association of tumor necrosis factor

promoter polymorphisms with systemic lupus erythematosus. J Rheumatol *23*, 1725-1728.

Rudlaweit, M., Tikly, M., Khamashta, M., Gibson, K., Klinke, J., Hughes, G., and Wordsworth, P. (1996). Interethnic differences in the association of tumor necrosis factor promoter polymorphisms with systemic lupus erythematosus. J Rheumatol *23*, 1725-1728.

Runkel, N. (1996). Bacterial translocation in acute pancreatitis. Digestive Surgery *13*, 269-272.

Runkel, N., Moody, F.G., Smith, G.S., Rodriguez, L., Chen, Y., LaRocco, M.T., and Miller, T.A. (1993). Alterations in rat intestinal transit by morphine promote bacterial translocation. Digestive Diseases and Sciences *38*, 1530-1536.

Runkel, N., Moody, F.G., Smith, G.S., Rodriguez, L., LaRocco, M.T., and Miller, T.A. (1991). The role of the gut in the development of sepsis in acute pancreatitis. Journal of Surgical Research *51*, 18-23.

Runkel, N., Rodriguez, L., and Moody, F.G. (1995). Mechanisms of sepsis in acute pancreatitis in opossums. American Journal of Surgery *169*, 227-232.

Runkel, N., Rodriguez, L., LaRocco, M.T., and Moody, F.G. (1990). Mechanisms of pancreatic infection in acute pancreatitis in opposums. Curr Surg *47*, 460-462.

Ryan, J.W., Moffat, J.G., and Thompson, A.G. (1964). Role of bradykinin in the development of acute pancreatitis. Nature *204*, 1212-1213.

Saghizadeh, M., Ong, J.M., Garvey, W.T., Henry, R.R., and Kern, P.A. (1996). The expression of TNF? by human muscle. Journal of Clinical Investigation *97*, 1111-1116.

Saito, I., Hashimoto, S., Saluja, A., Steer, M.L., and Meldolosi, J. (1987). Intracellular transport of pancreatic zymogens during caerulein supramaximal stimulation. American Journal of Physiology *(GI Liv Physiol 16)*, G508-G516.

Saluja, A., Sadmitsu, H., Saluja, M., Powers, R.E., Meldolosi, J., and Steer, M.L. (1987). Subcellular redistribution of lysosomal enzymes during caerulein induced pancreatitis. American Journal of Physiology *253*, G508-G516.

Saluja, A., Saluja, M., Villa, A., Leli, U., Rutledge, P., Meldolosi, J., and Steer, M.L. (1989). Pancreatic duct obstruction in rabbits causes digestive zymogen and lysosomal enzyme colocalisation. Journal of Clinical Investigation *84*, 1260-1266.

Sandoval, D., Gukovskaya, A.S., Reavey, P., Gukovsky, S., Sisk, A., Braquet, P., Pandol, S.J., and Poucell-Hatton, S. (1996). The role of neutrophils and platelet activating factor in mediating experimental pancreatitis. Gastroenterology *111*, 1081-1091.

Santamaria, P., Gehrz, R.C., Bryan, M.K., and Barbosa, J.J. (1989). Involvement of class II MHC molecules in the LPS-induction of IL-1/TNF secretions by human monocytes. Journal of Immunology *143*, 913-922.

Sarner, M. and Cotton, P.B. (1984). Classification of pancreatitis. Gut *25*, 756-759.

Satiani, B. and Stone, H.H. (1979). Predictability of present outcome and future recurrence in acute pancreatitis. Archives of Surgery *114*, 711-716.

Sato, M., Ishikawa, O., Abe, M., and Miyachi, Y. (1998). Opposite effects of tumour necrosis factor-alpha on type I and II collagengene expression by human dermal fibroblasts in monolayer and three-dimensional cultures. Br J Dermatol *138*, 118-121.

Savolainen, V.T., Pajarinen, J., Perola, M., Pentilla, A., and Karhunen, P.J. (1997). Polymorphism in the cytochrome P450 2E1 gene and risk of alcoholic liver disease. J Hepatol *26*, 55-61.

Schilling, P.J., Murray, J.L., and Markowitz, A.B. (1992). Novel tumor necrosis factor

toxic effects. Pulmonary haemorrhage and severe hepatic dysfunction. Cancer *69*, 256-260.

Schmidt, H.W. and Walter, U. (1994). NO at work. Cell *78*, 919-925.

Schmiegel, W., Burchert, M., Kalthoff, H., Roeder, C., Butzow, G., Grimm, H., Kremer, B., Soehenra, N., Schreiber, H., Thiele, H., and Greten, H. (1990). Immunohistochemical characterization and quantitative distribution of pancreatic stone protein in sera and pancreatic secretions in pancreatic disorders. Gastroenterology *99*, 1421-1430.

Schoenberg, M.H., Birk, D., and Beger, H.G. (1995). Oxidative stress in acute and chronic pancreatitis. Am J Clin Nutr *62*, 1306-1314.

Schoenberg, M.H., Buchler, M., and Younes, M. (1994). Effect of antioxidant treatment in rats with acute hemorrhagic pancreatitis. Dig Dis Sci *39*, 1034-1040.

Schoenberg, M.H., Buchler, M., Gaspar, M., and et al (1990). Oxygen free radicals in acute pancreatitis of the rat. Gut *31*, 1138-1143.

Schreiber, S., Heinig, T., Thiele, H., and Raedler, A. (1995). Immunoregulatory role of interleukin-10 in patients with inflammatory bowel disease. Gastroenterology *108*, 1434-1444.

Schwaeble, W., Schaefer, M.K.H., Petry, F., Fink, T., Knebel, D., Weihe, E., and Loos, M. (1995). Follicular dendritic cells, interdigitating cells, and cells of the monocyte-macrophage lineage are the C1q-producing sources in the spleen. Journal of Immunology *155*, 4971-4978.

Seitzer, U., Swider, C., Stuber, F., Suchnicki, K., Lange, A., Richter, E., Zabel, P., Muller-Quernheim, J., Flad, H.G., and Gerdes, J. (1997). Tumor necrosis factor-alpha gene polymorphism in sarcoidosis. Cytokine *9*, 789-790.

Selby, P., Hobbs, S., Viner, C., Jackson, E., Jones, A., Newell, D., Calvert, A.H., McElwain, T., Fearon, K.H.C., Humphreys, J., and Shiga, T. (1987). Tumour necrosis factor in man: Clinical and biological observations. British Journal of Cancer *56*, 803-808.

Senninger, N., Moody, F.G., Coelho, J.C.U., and Van Buren, D.V. (1986). The role of biliary obstruction in the pathogenesis of acute pancreatitis in the opossum. Surgery 688-693.

Sherlock, S. and Dooley, J. (1993). Alcohol and the liver. In Diseases of the liver and biliary system. S. Sherlock and J. Dooley, eds. (Oxford: Blackwell Scientific Publications), pp. 370-389.

Singer, M.V., Gyr, K., and Sarles, H. (1985). Revised classification of pancreatitis. Gastroenterology *89*, 683-685.

Singh, M. and Simsek, H. (1990). Ethanol and the pancreas: Current status. Gastroenterology *98*, 1051-1062.

Situnayake, R.D., Crump, B.J., and Thurnam, D.I. (1990). Lipid peroxidation and hepatic antioxidants in alcoholic liver disease. Gut *31*, 1311-1316.

Slater, T.F. (1984). Free radical mechanism in tissue injury. Biochem J *221*, 1-15.

Solling, H. and Fest, W. (1986). Synthesis of platelet activating factor in exocrine glands and it's control by secretagogues. J Biol Chem *261*, 1316-1322.

Soudah, H.C., Lu, Y., Hasler, W., L., and Owyang, C. (1992). Cholecystokinin at physiological levels evokes pancreatic exocrine secretion via a cholinergic pathway. American Journal of Physiology *263*, G102-G107.

Spies, T., Bresnahan, M., Bahram, S., Arnold, D., Blanck, G., Mellins, E., Pious, D., and DeMars, R. (1990). A gene in the human major histocompatibility complex class II region controlling the class I antigen presentation pathway. Nature *348*, 744-747.

Steer, M.L. and Meldolosi, J. (1987). The cell biology of experimental pancreatitis. N Engl J Med *316*, 144-150.

Steer, M.L., Rutledge, P.L., Powers, R.E., and et al (1991). The role of oxygen derived free radicals in two models of experimental acute pancreatitis:Effects of catalase, superoxide dismutase, dimethylsulfoxide, and allopurinol. Klin Wochenschr *69*, 1012-1017.

Steinberg, W.M. and Schlesselmann, S.E. (1987). Treatment of acute pancreatitis: Comparison of animal and human studies. Gastroenterology *93*, 1420-1427.

Stephens, K.E., Ishizaka, A., Larrick, J.W., and Raffin, T.A. (1988). Tumor necrosis factor causes increased pulmonary permeability and edema. American Review of Respiratory Diseases *137*, 1364-1370.

Stevens, A. and Lowe, J.S. (1992). Digestive Tract. In Histology. London: (Gower), pp. 169-170.

Streb, H., Irvine, R.F., Berridge, M.J., and Schulz, I. (1983). Release of Ca^{2+} from a nonmitochondrial intracellular store in pancreatic acinar cells by inositol-1,4,5-triphosphate. Nature *306*, 67-68.

Stuber, F., Petersen, M., Bokelmann, F., and Schade, U. (1996a). A genomic polymorphism within the tumor necrosis factor locus influences plasma tumor necrosis factor-a concentrations and outcome of patients with severe sepsis. Crit Care Med *24*, 381-384.

Stuber, F., Udalova, I.A., Book, M., Drutskaya, L.N., Kuprash, D.V., Turetskaya, R.L., Schade, U., and Nedaspasov, S.A. (1996b). -308 tumor necrosis factor (TNF) polymorphism is not associated with survival in severe sepsis and is unrelated to lipopolysaccharide inducibility of the human TNF promoter. Journal of Inflammation *46*, 42-50.

Sullivan, K.E., Wooten, C., Schmeckpeper, B.J., Goldmann, D., and Petri, M.A. (1997). A promoter polymorphism of tumor necrosis factor alpha associated with systemic lupus erythematosus in African-Americans. Arthritis rheum *40*, 2207-2211.

Sullivan, K.E., Wooten, C., Schmeckpeper, B.J., Goldmann, D., and Petri, M.A. (1997). A promoter polymorphism of tumor necrosis factor alpha associated with systemic lupus erythematosus in African-Americans. Arthritis rheum *40*, 2207-2211.

Suzuki, K., Uchida, A., Yasuhiko, M., and Fukunaga, T. (1994). A study on ADH2 genotyping by PCR-RFLP and SSCP analyses with description of allele and genotype frequencies in Japanese, Finn, and Lapp populations. Alcohol & Alcoholism *29*, 21-27.

Sweiry, J.H. and Mann, G.E. (1996). Role of oxidative stress in the pathogenesis of acute pancreatitis. Scand J Gastroenterol *S219*, 10-15.

Takahashi, A., Nanako, M., Mashiko, S., and Inaba, H. (1990). The first observation of O_2^- generation in in situ lungs of rats treated with drugs to induce experimental acute respiratory distress syndrome. FEBS Lett *261*, 369-372.

Talvik, R., Liigant, A., Sissak, H.M., and O'Konnel-Bronina, N. (1977). Respiratory failure in acute pancreatitis. Intensive Care Med *3*, 97-98.

Tan, J.C., Braun, S., Rong, H., and et al (1995). Characterisation of recombinant extracellular domain of human interleukin-10 receptor. J Biol Chem *270*, 12906-12911.

Tanaka, F., Shiratori, Y., Yokosuka, O., Imazeki, F., Tsukada, Y., and Omata, M. (1996).

high incidence of ADH2*1/ALDH2*1 genes among Japanese alcohol dependents and patients with alcoholic liver disease. Hepatology *23*, 234-239.

Tanaka, K., Murata, A., Uda, K., Toda, H., Kato, T., Hayashida, H., Matsuura, N., and Mori, T. (1995). Interleukin-1 receptor antagonist modifies the changes in vital organs induced by acute necritizing pancreatitis. Critical Care Medicine *23*, 901-908.

Tarlow, J.K., Blakemore, A.I.F., Lennard, A., Solari, R., Hughes, H.N., Steinkassrer, A., and Duff, G.W. (1993). Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86-bp tandem repeat. Hum Genet *91*, 403-404.

Thompson, S.R., Hendry, W.S., McFarlane, G.A., and Davidson, A.I. (1987). Epidemiology and outcome of acute pancreatitis. British Journal of Surgery *74*, 398-401.

Thomson, B.M., Mundy, G.R., and Chambers, T.J. (1987). Tumor necrosis factors ? and ? induce osteoblastic cells to stimulate osteoclastic bone resorption. J Immunol *138*, 775-779.

Tiwari, J.L. and Terasaki, P.I. (1985). Data and Statistical Analysis. In HLA and Disease Associations. J.L. Tiwari and P.I. Terasaki, eds. (New York: Springer Verlag), pp. 18-27.

Todd, J.A., Bell, J.I., and McDevitt, H.O. (1987). HLA-DQB gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. Nature *329*, 599-604.

Tomaszwska, R., Dembiniski, A., Warzecha, Z., Banas, B., Konturek, S.J., and Stachura, J. (1992). Platelet activating factor inhibitor (TCV-309) reduces caerulin and PAF induced pancreatitis; a morphologic and functional study in the rat. J Physiol Pharmacol *4*, 345-352.

Toyama, M.T., Patel, A.G., Nguyen, T., Ashley, S.W., and Reber, H.A. (1997). Effect of ethanol on pancreatic interstitial pH and blood flow in cats with chrionic pancreatitis. Annals of Surgery *225*, 223-228.

Tracey, K.J., Beutler, B., Lowry, S.F., Merryweather, J., Wolpe, S., Milsark, I.W., Hariri, R.J., Fahey III, T., Zentella, A., Albert, J.D., Shires, G.T., and Cerami, A. (1986). Shock and tissue injury induced by recombinant human cachectin. Science *234*, 470-474.

Tracey, K.J., Fong, Y., and Hesse, D.J. (1987). Ant-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. Nature *330*, 662-664.

Tracey, K.J., Vlassara, H., and Cerami, A. (1989). Cachectin/Tumour necrosis factor. Lancet 1122-1125.

Trapnell, J.E. and Duncan, E.H.L. (1975). Patterns of incidence in acute pancreatitis. British Medical Journal *2*, 179-183.

Trinchieri, G. (1997). Cytokines acting on or secreted by macrophages during intracellular infection. Current Opinion in Immunology *9*, 17-23.

Trowsdale, J. and Campbell, R.D. (1998). The 12th International MHC map. In Genetic diversity of HLA and functional and medical implication. D. Charron, ed. (Paris: EDK), pp. 8-12.

Tsukahara, Y., Horita, Y., Anan, K., Morisaki, T., Tanaka, M., and Torisu, M. (1996). Role of nitric oxide derived from alveolar macrophages in the early phase of acute pancreatitis. Journal of Surgical Research *66*, 43-50.

Tsutsumi, M., Takada, A., and Wang, J. (1994). Genetic polymorphisms of cytochrome P4502E1 related to the development of alcoholic liver disease. Gastroenterology *107*, 1430-1435.

Turner, D.M., Williams, D.M., Sankaran, D., Lazarus, M., Sinnott, P.J., and Hutchinson, I.V. (1997). An investigation of polymorphism in the Interleukin-10 gene promoter. European Journal of Immunogenetics *24*, 1-8.

Udalova, I.A., Nedaspasov, S.A., Webb, G.C., Chaplin, D.D., and Turetskaya, R.L. (1993). Highly informative typing of the human TNF locus using six adjacent polymorphic markers. Genomics *16*, 180-186.

Udalova, I.A., Nedaspasov, S.A., Webb, G.C., Chaplin, D.D., and Turetskaya, R.L. (1993). Highly informative typing of the human TNF locus using six adjacent polymorphic markers. Genomics *16*, 180-186.

Uhrberg, M., Valiante, N.M., Shum, B.P., Shilling, H.G., Lienert-Weidenbach, K., Corliss, B., Tyan, D., Lanier, L.L., and Parham, P. (1997). Human diversity in killer cell inhibitory receptor genes. Immunity 753-763.

Van der Poll, T., Jansen, P.M., Montegut, W.J., and et al (1997). Effects of Interleukin-10 on systemic inflammatory responses during sublethal primate endotoxaemia. J Immunol *158*, 1971-1975.

Van der Poll, T., Van Deventer, S.J.H., Hack, C.E., Wolbink, G.J., Aarden, L.A., Buller, H.R., and ten Cate, J.W. (1992). Effects on leukocytes following injection of tumor necrosis factor into healthy humans. Blood *79*, 693-698.

Van Deventer, S.J.H., Hart, M., Van der Poll, T., Hack, C.E., and Aarden, L.A. (1993). Endotoxin and TNF-induced IL-8 release in humans. Journal of Infectious Diseases *167*, 461-464.

van Dissel, J.T., van Langevelde, P., Westendorp, R.G.J., Kwappenberg, K., and Frolich, M. (1998). Anti-inflammatory cytokine profile and mortality in febrile patients. Lancet *351*, 950-953.

Van Laethem, J., Marchant, A., Delvaux, A., and et al (1995). Interleukin-10 prevents necrosis in murine experimental acte pancreatitis. Gastroenterology *108*, 1917-1922.

Vejans, G.M.G.M., Brinkman, B.M.N., Van Doornik, C.E.M., and Kijlstra, A. (1994). Polymorphism of tumour necrosis factor-alpha (TNF-?) at position-308 in relation to ankylosing spondylitis. Clin Exp Immunol *97*, 45-47.

Vejans, G.M.G.M., Brinkman, B.M.N., Van Doornik, C.E.M., and Kijlstra, A. (1994). Polymorphism of tumour necrosis factor-alpha (TNF-?) at position-308 in relation to ankylosing spondylitis. Clin Exp Immunol *97*, 45-47.

Venable, M.E., Zimmermann, G.A., McIntyre, T.M., and Prescott, S.M. (1993). Platelet activating factor: a phospholipid autocoid with diverse actions. Journal of Lipid Research *34*, 691-702.

Vinasco, J., Beraun, Y., Nieto, A., Fraile, A., Mataran, L., Pareja, E., and Martin, J. (1997). Polymorphism at the TNf loci in rheumatoid arthritis. Tissue Antigens *49*, 74-78.

Vinasco, J., Beraun, Y., Nieto, A., Fraile, A., Mataran, L., Pareja, E., and Martin, J. (1997). Polymorphism at the TNf loci in rheumatoid arthritis. Tissue Antigens *49*, 74-78.

Wang, N., O'Brien, L., and Brindley, D.N. (1998). Effects of cell-permeable ceramides and tumor necrosis factor-a on insulin signaling and glucose uptake in 3T3-L1 adipocytes. Diabetes *47*, 24-31.

Wang, X.D., Deng, X.M., Haraldsen, R., and et al (1995). Antioxidant and calcium channnel blockers counteract endothelial barrier injury induced by acute pancreatitis in rats. Scand J Gastroenterol *30*, 1129-1136.

Ward, J.B., Peterson, O.H., Jenkins, S.A., and Sutton, R. (1995). Is an elevated concentration of acinar cytosolic free calcium the trigger for acute pancreatitis. Lancet *346*, 1016-1019.

Watanebe, O., Baccino, F.M., Steer, M.L., and Meldolosi, J. (1984). Supramaximal caerulein stimulation of the ultrastructure of rat pancreatic acinar cell: early morphological changes during the development of experimental pancreatitis. American Journal of Physiology *246*, G457-G467.

Weber, J.L. and May, P.E. (1989). Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet *44*, 388-386.

Wedgwood, K.R., Adler, G., Kern, H., and Reber, H.A. (1986). Effects of oral agents on pancreatic duct permeability: a model of acute alcoholic pancreatitis. Dig Dis Sci *31*, 1081-1088.

Weidenbach, H., Lersch, M.M., Gross, T.M., and et al (1995). Vasoactive mediators and the progression from oedematous to necrotising experimental acute pancreatitis. Gut *37*, 434-440.

Weiser, M.R., Gibbs, S.A.L., Moore, F.D., and Hechtman, H.B. (1996). Complement inhibition by soluble complement receptor type I fails to moderate cerulein-induced pancreatitis in the rat. International Journal of Pancreatology *19*, 129-134.

Weissensteiner, T. and Lanchbury, J.S. (1997). TNFB polymorphisms characterize three lineages of TNF region microsatellite haplotypes. Immunogenetics *47*, 6-16.

Weissensteiner, T. and Lanchbury, J.S. (1997). TNFB polymorphisms characterize three lineages of TNF region microsatellite haplotypes. Immunogenetics *47*, 6-16.

Werner, J., Laposata, M., Fernandez-del-castillo, C., Saghir, M., Iozzo, R.V., Lewandrowski, K.B., and Warshaw, A.L. (1997a). Pancreatic injury in rats induced by fatty acid ethyl ester, a nonoxidative metabolite of alcohol. Gastroenterology *113*, 286-294.

Werner, J., Rivera, J., and Fernandez-del-castillo, C. (1997b). Differing roles of nitric oxide in the pathogenesis of acute edematous versus necrotising pancreatitis. Surgery *121*, 23-30.

Westendorp, R.G.J., Langermans, J.A.M., Huizinga, T.W.J., and et al (1996). Genetic influence on cytokine production and fatal meningococcal disease. Lancet *349*, 170-173.

Westendorp, R.G.J., Langermans, J.A.M., Huizinga, T.W.J., and et al (1996). Genetic influence on cytokine production and fatal meningococcal disease. Lancet *349*, 170-173.

Whitcomb, D.C., Gorry, M.C., Preston, R.A., Furey, W., Sossenheimer, M.J., Ulrich, C.D., Martin, S.P., Gates, L.K., Amann, S.T., Toskes, P.P., Liddle, R., McGrath, K., Uomo, G., Post, J.C., and Ehrlich, G.D. (1996a). Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. Nature Gen *14*, 141-145.

Whitcomb, D.C., Preston, R.A., Aston, C.E., Sossenheimer, M.J., Barua, P.S., Zhang, Y., Wong-Chong, A., White, G.J., Wood, P.G., Gates, L.K., Ulrich, C.D., Martin, S.P., Post, J.C., and Ehrlich, G.D. (1996b). A gene for herdeditary pancreatitis maps to chromosome 7q35. Gastroenterology *110*, 1975-1980.

Widdison, A.L. and Cunningham, S. (1996). Immune function early in acute pancreatitis. Br J Surg *83*, 633-636.

Widdison, A.L., Karanjia, N.D., Alvarez, C., and Reber, H.A. (1992). Influence of levimasole on pancreatic infection in acute pancreatitis. American Journal of Surgery *163*, 100-104.

Widdison, A.L., Karanjia, N.D., and Reber, H.A. (1994). Routes of spread of pathogens into the pancreas in a feline model of acute pancreatitis. Gut *35*, 1306-1310.

Wilson, A.G., de Vries, N., Poicot, F., di Giovine, F.S., van der Putte, L.B.A., and Duff, G.W. (1993). An allelic polymorphism within the human tumour necrosis factor ? promoter

region is strongly associated with HLA A1, B8, and DR3 alleles. Journal of Experimental Medicine *177*, 557-560.

Wilson, A.G., de Vries, N., Poicot, F., di Giovine, F.S., van der Putte, L.B.A., and Duff, G.W. (1993). An allelic polymorphism within the human tumour necrosis factor ? promoter region is strongly associated with HLA A1, B8, and DR3 alleles. Journal of Experimental Medicine *177*, 557-560.

Wilson, A.G., de Vries, N., van der Putte, L.B., and Duff, G.W. (1995). Atumour necrosis factor polymorphism is not associated with rheumatoid arthritis. Ann Rheum dis *54*, 601-603.

Wilson, A.G., Symons, J.A., McDowell, T.L., McDevitt, H.O., and Duff, G.W. (1997). Effects of a polymorphism in the human tumor necrosis factor ? promoter on transcriptional activation. Proc. Natl. Acad. Sci. USA *94*, 3195-3199.

Wilson, C. and Imrie, C.W. (1990). Changing patterns of incidence and mortality from acute pancreatitis in Scotland, 1961-1985. British Journal of Surgery *77*, 731-734.

Wilson, C., Imrie, C.W., and Carter, D.C. (1988). Fatal acute pancreatitis. Gut *29*, 782-788.

Wilson, J.S., Bernstein, L., McDonald, C., Tait, A., McNeil, D., and Pirola, R.C. (1985). Diet and drinking habits in relation to the development of alcoholic pancreatitis. Gut *26*, 882-887.

Wilson, J.S., Gossat, D., Tait, A., Rouse, S., Jeng Juan, X., and Pirola, R.C. (1984). Evidence for an inherited predisposition to alcoholic pancreatitis; a controlled HLA typing study. Dig Dis Sci *29*, 727-730.

Wilson, J.S., Korsten, M.A., Leo, M.A., and Lieber, C.S. (1988). Combined effects of protein deficiency and chronic ethanol consumption on rat pancreas. Digestive Diseases and Sciences *33*, 1250-1259.

Wisner, J. and Renner, I.G. (1988). Allopurinol attenuates caerulein induced acute pancreatitis in the rat. Gut *29*, 926-929.

Wisner, J., Green, D., Ferrell, L., and et al (1988). Evidence for a role of oxygen free radicals in the pathogenesis of caerulein induced acute pancreatitis in rats. Gut *29*, 1516-1523.

Wolf, E., Spencer, K.M., and Cudworth, A.G. (1983). The genetic susceptibility to type I (insulin-dependent) diabetes: analysis of the HLA-DR association. Diabetologia *24*, 224-230.

Wucherpfennig, K.W. and Strominger, J.L. (1995). Molecular mimicry in T cell mediated autoimmunity: viral peptides activate human T cell clones specific for myelin basic protein. Cell *80*, 695-705.

Yamaguchi, H., Weidenbach, H., Luhrs, H., Lerch, M.M., Dickneite, G., and Adler, G. (1997). Combined treatment with C1 esterase inhibitor and antithrombin III improves survival in severe acute experimental pancreatitis. Gut *40*, 531-535.

Yamaguchi, M., Maezawa, Y., Toda, G., Suzuki, H., and Sakurai, S. (1995). Association of a restriction fragment length polymorphism in the alcohol dehydrogenase 2 gene with Japanese alcoholic cirrhosis. J Hepatol *23*, 519-523.

Yamanaka, K., Saluja, A., Brown, G.E., Yamaguchi, Y., Hofbauer, B., and Steer, M.L. (1997). Protective effects of prostaqglandin E1 on acute lung injury of caerulein induced acute pancreatitis in rats. Am J Physiol *272*, G23-G30.

Yotsumoto, F., Manabe, T., and Ohshio, G. (1993). Bradykinin involvement in the

aggravation of acute pancreatitis in rats. Digestion *54*, 224-230.

Yotsumoto, L., Manabe, T., Kyogoku, T., Hirano, T., Ohshio, G., Yamamoto, M., Imamura, T., and Yoshitomi, S. (1994). Platelet activating factor involvement in the aggrevation of acute pancreatitis in rabbits. Digestion *55*, 260-267.

Zemmour, J., Koller, B.H., Ennis, P.D., Geraghty, D.E., Lawlor, D.A., Orr, H.T., and Parham, P. (1990). HLA-AR, an inactivated antigen-presenting locus related to HLA-A. J Immunol *144*, 3619-3629.

Zhou, W., McCollum, M.O., Levine, B.A., and et al (1992). Role of platelet activating factor in pancreatitis associated lung injury in the rat. American Journal of Pathology *140*, 971-979.