*The Plymouth Student Scientist, 2008, **1**, (1), 312-330*

The Effect of Nucleotide-binding Oligomerization Domain (NOD) Proteins on the Secretion of Cytokines from Macrophages

Joanne Aggett

2007

Project Advisor: Andrew Foey, School of Biological Sciences, University of Plymouth, Drake Circus, Plymouth, PL4 8AA

The study of Nucleotide-Binding Oligomerization Domain (NOD) Proteins is a very new area of immunology, with the gene for NOD2 having only been mapped in 2001 (Ogura *et al* 2001). It holds particular significance for those researching autoimmune disorders such as Blau syndrome and Crohns Disease (CD), as it has been found that a mutation in the NOD2 gene is present in a substantial proportion of CD sufferers, and having the mutation increases a person's susceptibility to developing the disease later in life (Economou *et al* 2004). This review will look at the current research surrounding the NOD proteins and what products they form, and how this can be used to further our knowledge of CD and how it can be managed. It will be specifically looking into the effects that NOD proteins in macrophages have on the secretion of various pro- and anti-inflammatory cytokines and how these can be augmented by the interaction of NOD protein signalling pathways with those of other molecules in the cell.

ISSN 1754-2383 [Online]
©University of Plymouth

Macrophages

To understand the effects of NOD proteins in macrophages, it is important to first understand the macrophage and how its subsets can augment effects from many different stimuli.

Macrophages are specialized cells of the immune system capable of phagocytosing exogenous antigens, for example bacterial microorganisms and their cellular components; and endogenous matter, such as apoptotic bodies and cellular debris. Originating from myeloid progenitor cells, macrophages have several stages of development. The first stage of this development leads to the formation of a Granulocyte-monocyte progenitor cell, which can develop further into either a neutrophil or a Monocyte, the precursor to a macrophage (Goldsby *et al* 2003). When activated, macrophages secrete several different molecules known as chemokines or cytokines, depending on their function, including interleukin-1β (IL-1β), IL-10, IL-12, IL-8 and TNF-α. These soluble proteins act as chemical messengers, which serve to regulate immune responses to antigens, and will be discussed in further detail later.

First proposed by Stein *et al* in 1992, it is now widely accepted that there are at least 2 distinct types of macrophage; Classically activated (M1) and Alternatively activated (M2), which work together to regulate the immune response to pathogens. Research is currently ongoing into the possibility of further subsets of M2 macrophages (Mantovani *et al* 2004, Gordon 2006, Mosser 2003). Synthetic chemical stimulation can be used to differentiate monocytes to these different states of activation, as demonstrated in studies by Schwende (1996), whose investigation involved the exposure of human monocytic THP-1 cells to Phorbol-12-myristate-13-acetate (PMA) or 1,25-dihydroxyvitamin D_3 (VD_3) to stimulate differentiation to M1 or M2 macrophages respectively. Mantovani *et al* (2004) have recently published supporting evidence that monocytes can undergo different forms of activation, resulting in polarized macrophages which differ in their response to antigenic stimulation. They propose that this polarization can be induced naturally via stimulation with

Interferon-γ (IFN- γ) or Interleukin-10 (IL-10), which form M1 and M2 macrophages respectively, and that M2 macrophages can also be induced by exposure to IL-13 or IL-4. Conversely, recent theories proposed by S. Gordon (2003) hypothesize that IL-10 acts to deactivate M1 macrophages, and M2 macrophages are only formed when monocytes are exposed to IL-13 or IL-4. Both granulocyte-macrophage-colony stimulating factor (GM-CSF) and M-CSF have also been implicated in the differentiation of monocytes to M1 and M2 macrophages respectively (Verrick *et al* 2004); however more research is required into this area before the exact requirements for the various differentiation pathways can be stated conclusively.

The study by H. Schwende revealed clear differences in the morphology, proliferation and phagocytic ability of M1 and M2 macrophages; however it did not find accurate differences in the cytokines and chemokines that are released. This detail has since been determined and is outlined in Mantovani's study (2004), which reveals variances in the cytokines, chemokines and effecter molecules that are secreted, as well as variances in the membrane cytokine and chemokines receptors that are expressed on the surface of the macrophage. Mantovani *et al* demonstrated that M2 macrophages express decreased levels of pro-inflammatory cytokines compared with M1 type macrophages, which may be explained by the comparatively high expression of scavenger receptors on the M2 cell surface. Scavenger receptors are transmembrane proteins which bind to microorganisms to promote phagocytosis and internalization to lysosomal compartments within the macrophage, and do not involve any signaling cascades (Kaisho and Akira 2006). These opposing types of macrophage are proposed by S. Gordon (2003) to be responsible for the induction of pro- and anti-inflammatory states and as such responsible for immune activation and deactivation.

Cytokines: Role in immunity

Cytokines play a very important role in the control and regulation of the immune system. As discussed above, some play a role in the differentiation and activation of macrophages, which in turn have an impact on the immune response to an invasive pathogen. However, this ability to active cells of the immune system is not limited to the activation of macrophages. A wide range of cytokines are capable of activating and suppressing leukocytes of the innate and adaptive immune response, such as antigen-presenting cells, B-cells and T-cells, all of which are vital for an effective response to injury or pathogenic challenge. Regulation of these cytokines is essential for normal immune function, and their uncontrolled expression can lead to the development of immunopathology, such as CD or cancer. Inappropriate secretion of cytokines and expression of cytokine receptors can also be responsible for the development of allergies and some immunodeficiencies, resulting from an imbalance in the levels of T-helper 1 (T_h1)/ T_h2 cells, which promote a cell mediated or humoral response respectively (Viola, Contento and Molon, 2006).

There are four families of cytokines; the hematopoietin family, the interferon family, the chemokine family and the tumour necrosis family. The hematopoietin family encompasses interleukins such as IL-1β and IL-12 which increase inflammation (pro-inflammatory) along with IL-10 and IL-4 which act to decrease inflammation (anti-inflammatory). These cytokines can exert an effect on surrounding cells to modulate the immune response; for example, IL-12 stimulates the differentiation of T_h1 cells and induces the production of IFN-γ by NK cells, which play a pivotal role in the initial immune response to pathogens and are involved in the destruction of cancerous cells. Although most of the hematopoietin family are interleukins, interleukins are not confined solely to the hematopoietin family. IL-8 is a member of the chemokine family, and acts as a chemo-attractant, drawing cells of the immune system toward the area of infection or injury. It has a vital role in directing the immune response to the appropriate area, and its secretion from macrophages has been linked to NOD2

modulated NF-κB activity. Most pro-inflammatory cytokines are secreted as a result of NF-κB dependant gene transcription, while anti-inflammatory cytokines such as IL-10 use a different mechanism (Goldsby *et al* 2003).

NOD proteins: structure and function

NOD1 and NOD2 (also termed CARD4 and CARD15 respectively) are members if the Nod-like receptor family, a group of intracellular proteins which detect microbial motifs. NOD proteins are Pattern Recognition Receptors (PRR), which recognise Pathogen Associated Molecular Patterns (PAMP), highly conserved regions within microbial components (Chamaillard *et al*., 2003). Both NOD1 and NOD2 recognise distinct components of peptidoglycan (PGN), which is present in all bacterial cell walls, with the exception of the *Mycoplasma* genus, making them very effective intracellular surveillance systems (Philpott and Girardin 2004). NOD1 is present in the cytosol of a wide range of cells, while NOD2 is present in the cytosol of myeloid cells, such as neutrophils, dendritic cells and, most notably, the macrophage. NOD2 is also present in astrocytes, osteoblasts and the paneth cells of the small intestine, which form part of the epithelium, but neither NOD proteins are present in cells of the adaptive immune system such as B and T cells (Leung *et al* 2006). The expression of NOD2 can be up-regulated upon treatment with pro-inflammatory stimuli such as IFN-γ or TNFα (Viala, Sansonetti and Philpott 2004).

NOD proteins contain a Nucleotide binding site (NBS), leucine rich repeats (LRR) and at least one caspase-activating and recruitment domain (CARD) (Athman and Philpott 2004). As shown in figure 1, NOD1 contains one of these CARD domains while NOD2 contains two. Research by Staskawicz *et al* (2001) indicates that binding of the appropriate ligand occurs at the LRR region of the NOD protein; however this theory is based on similarities to the structure of TLRs and R-proteins in plants, both of which have an LRR binding site, and there is no direct experimental evidence to support the LRR as a binding site in NOD proteins as yet.

Although both NOD1 and NOD2 detect components of PGN and are structurally very similar, the exact motifs they require differ. The minimal naturally occurring PGN structure required for detection by NOD proteins is shown is figure 1, and consists of a sugar backbone connected to a peptide containing L-Ala-γ-D-Glu (shown in the red and green boxes) which forms the molecule muramyl dipeptide (MDP).

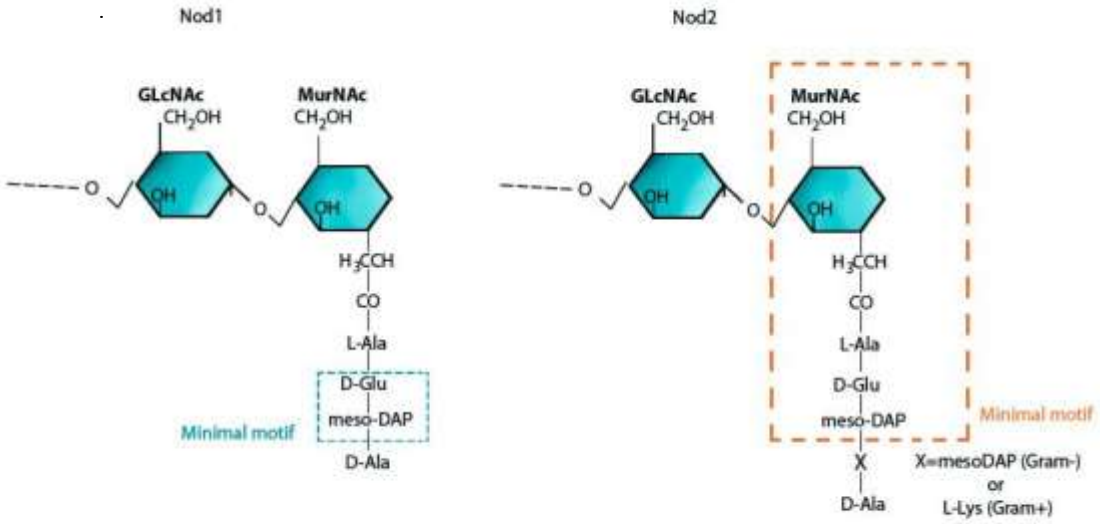

Figure 1: Showing the minimal structural requirements of PGN for detection by NOD1 and NOD2 (Adapted from Girardin,*et al* 2003)

This minimal structure is the same for both NOD1 and NOD2, however NOD1 requires *meso*-DAP to be in the terminal position of the peptide (outlined in blue) forming the molecule GM-triDAP, in order to detect the molecule as a pathogen, while NOD2 detects MDP with either *meso*-DAP or L-Lys in the terminal position. This allows detection of both Gram negative and Gram positive bacteria respectively (Girardin *et al* 2003a). The mechanism used to internalise the MDP into the cell is currently unclear, but research indicates that when the PGN of the bacterial cell wall is broken down in the phagolysosome the MDP is exposed, which is then detected by NOD2 within the cell, causing a signal cascade which culminates with the release of pro and anti-inflammatory cytokines, including IL-1β, IL-8, IL-10, TNF-α and IL-6 (Strober, W *et al* 2006).

Abbott *et al* (2004) were the first to describe the signalling pathway downstream of NOD2, and the mechanism by which it activates NF-κB; however, although there is speculation as to the pathway by which NOD1 activates NF-κB, it has yet to be elucidated. An overview of the current known processes is shown in figure 2. It has been determined that NOD2 recruits the CARD containing serine/threonine kinase RICK (Receptor-activating serine/threonine kinase) though CARD-CARD interactions, which then mediates K63-linked polyubiquitylation of IκB-kinase-γ (IKKγ), an inhibitor of NF-κB. The subsequent phosphorylation of IKKβ allows the translocation of transcriptional components (p50 and p65) to the nucleus. This initiates the transcription and translation of pro-inflammatory cytokine genes.

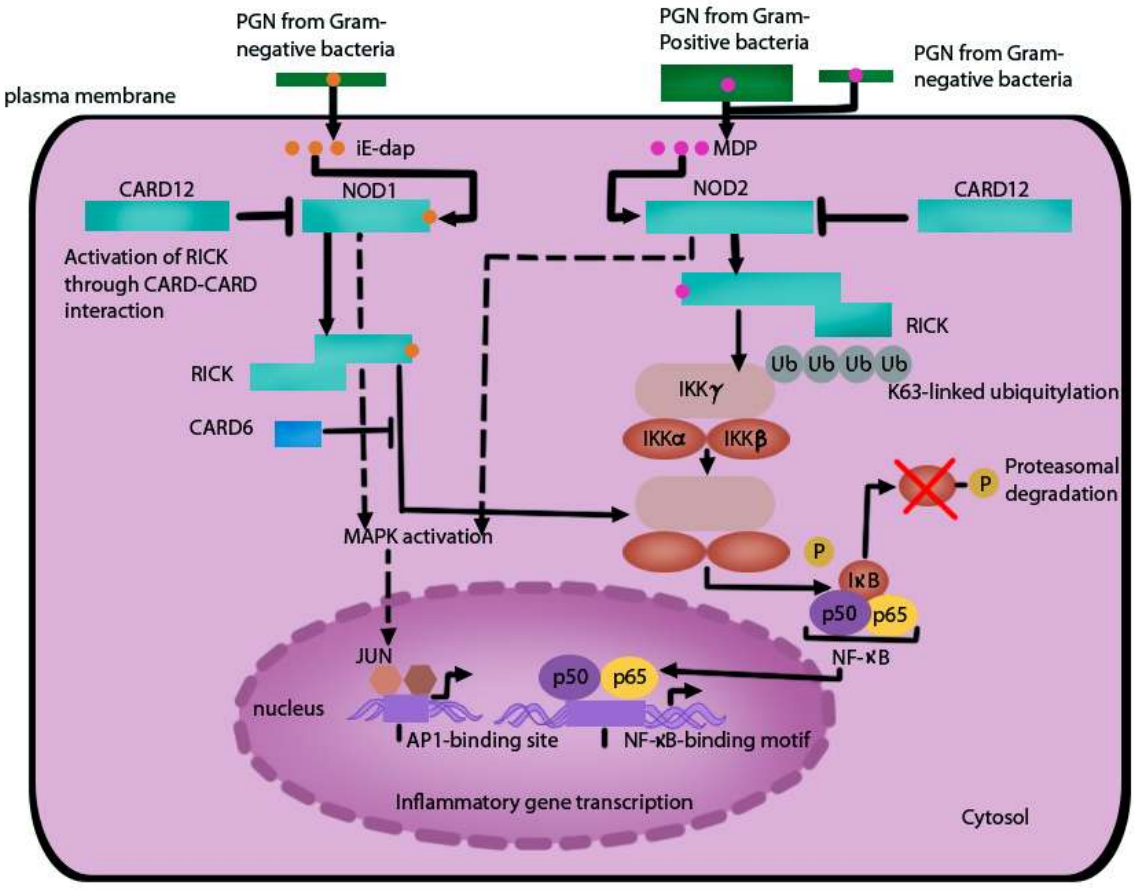

Figure 2: Showing the possible pathways downstream of NOD1 and NOD2 which lead to the transcription of inflammatory genes (Adapted from Stober *et al* 2006).

It has been theorized that NOD2 may play a role in the suppression of IL-12 in normally functioning cells, based on studies Watanabe *et al* (2004), who found that stimulation of cells expressing mutant NOD2 led to an increase in IL-12 expression compared with cells expressing wild-type NOD2. This is supported by data from Mannon *et al* (2004), who found treatment of CD patients with antibodies against IL-12 was effective at reducing inflammation of the gut. Yoo *et al* (2002) showed that NOD1 is able to associate with pro-caspase 1 via CARD-CARD interactions, possibly converting the pro-caspase into a caspase which can cleave Pro-IL-1β and Pro-IL-18 into the mature forms IL-1β and IL-18. Association with pro-caspase is also thought to occur with NOD2 (Damiano *et al* 2004), which lends weight to the argument that NOD2 is involved in up-regulation of the expression of pro-inflammatory cytokines.

In 2001, Girardin *et al* showed that stimulation of NOD1 led to the activation of JUN N-terminal Kinase (JNK), which binds to Activator Protein-1 (AP-1) in the nucleus, leading to the transcription of pro-inflammatory genes. Following this, Pauleau and Murray found in 2003 that NOD1 and NOD2 exert an activating effect on the mitogen-activated protein kinase (MAPK) pathway. Their experiments showed that activation of NOD2 led to the activation of p38MAPK and extracellular-signal-regulated kinase (ERK), and a mutation of NOD2 eliminated this activating ability. These pathways can promote cell growth (ERK) or apotosis (p38MAPK), and thus a balance between the two must be maintained in order to prevent inflammation or uncontrolled apoptotic cell death (Li *et al* 2003).

Toll-Like Receptors: Where do they fit in?

NOD proteins do not exert an appreciable response when stimulated alone. It has been found that simultaneous stimulation of Toll-like receptors is required to obtain measurable levels of cytokine secretion (Yang *et al* 2001). Toll-like receptors (TLRs), so called due to their resemblance to the antifungal Toll receptor in the *Drosphasila* fruit fly, are critical to the response of the innate

immune system to microbial infection. First discovered by Rock *et al* in 1998, there are now descriptions of 10 distinct TLRs. As shown in figure 3, TLRs I, 2, 4, 5 and 6 reside on the outer surface of the surface of the cell membrane, while TLRs 3, 7, 8 and 9 reside on the inner surface of endosomes within the cell (kaisho and Akira 2006). The exact location and function of TLR 10 has yet to be elucidated. TLRs are PRRs, which directly detect PAMPs in microbial components which act as ligands, causing a signal cascade within the cell, usually leading to the secretion of cytokines which ultimately leads to T_h1 differentiation. This has led to research into the possibility of blocking or augmenting the function of TLRs in order to change the T_h1/ T_h2 balance, which would open the possibility of managing immune disorders such as allergy, autoimmune disease and cancer (Kaisho and Akira 2006).

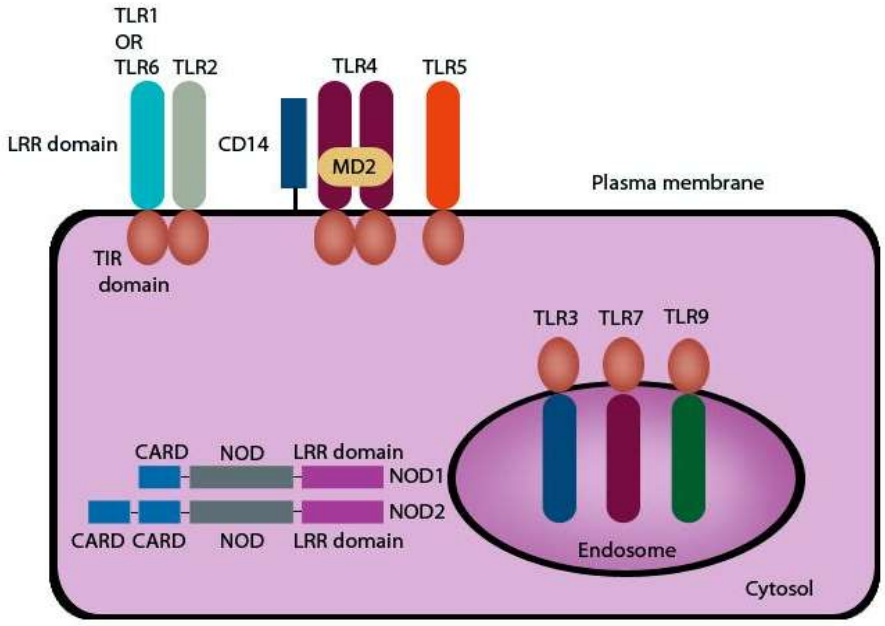

Figure 3: Showing the basic structure and position of the TLRs and NOD proteins within the cell (Adapted from Strober *et al* 2006).

Athman and Philpott (2004) state that TLRs are usually found in high proportion in areas of the body that are sterile under normal conditions, and their expression is down-regulated in areas that regularly encounter pathogenic

challenge, such as the gut, to avoid constitutive activation which would lead to chronic inflammation. This down-regulation could be responsible for the up-regulated presence of NOD proteins in the intestinal tract in order to maintain a defence against invasive bacteria.

TLRs and NOD2: Possible interactions

It is important to note that TLRs 2 and 4 use a signalling pathway dependant on a MyD88 adaptor molecule, a distinctly different pathway to that used by either of the NOD proteins (Girardin, Hugot and Sansonetti 2003)

Yang *et al* (2001) used the human monocytic THP-1 cell line to show that exposure to MDP alone gives no appreciable cytokine response, which led to further investigation into the synergistic effects of LTA or LPS when used in conjunction with MDP, to simultaneously activate NOD2 and TLR2 or 4 respectively. The study revealed an increased secretion of IL-8, IL-1β and IL-6 when the cells were exposed to both LPS and MDP compared with controls, and a similar result was found when cells were exposed to both LTA and MDP. This indicates that there is an augmentation in the signalling pathways, which leads to an increase in transcription of NF-κB. Evidence supporting these results was obtained during similar experiments conducted by Netea *et al* (2005a) using human peripheral mononuclear blood cells and human TLR2 transfected Chinese hamster ovary fibroblasts. *In vitro* and *in vivo* studies by Watanabe *et al* (2004) have also indicated the possibility of cross-talk between TLR2 and NOD2, which is currently hypothesized to occur due to NOD2 sequestering the RICK molecule which would otherwise be involved in the downstream signaling pathway of TLR2; however this theory is contradicted by studies showing that there is no modulation of TLR4 signaling by NOD2, despite there being evidence to show that TLR4 can also signal via the RICK molecule (Strober *et al* 2006).

There is no evidence at present to suggest any interaction between NOD2 with TLRs 3, 5, 6, 7, 8 or 9 and NOD proteins, or interaction of NOD1 with any TLRs.

NOD2: Related Immunopathology

The first description of a mutation in the NOD2 gene linked to Crohns disease (CD) was made in 2001 (Hugot *et al*), and since then there have been more than 60 variations described, three of which have since been identified as responsible for 82% of total NOD2 mutations (R702W, G908R and 3020insC) (Girardin, Hugot and Sansonetti 2003). The 3020insC mutation has since been shown to be the most consistent mutation amongst differing population groups (Economou *et al* 2004) and effects the C-terminal third of the NOD2 protein within the LRR region, which is thought to be the binding region for MDP. The product of the mutated gene forms a truncated version of NOD2, missing the final 33 amino acids which would encode the 10^{th} leucine repeat (Leung *et al* 2006). It has been observed that this mutation leads to a decrease in transcription of NF-κB in response to MDP and as such a decrease in pro-inflammatory cytokine expression (Li *et al* 2004); however CD sufferers maintain increased levels of pro-inflammatory cytokines, which results in the chronic inflammation of the gut that is characteristic of CD. This has lead to debate over whether there is a loss or gain of function caused by the 3020insC mutation under physiological conditions.

Supporting the idea of mutation causing a loss of function, Siegmund (2002) proposed that one of the main pathogenic mechanisms of CD is an imbalance in the T_h1/ T_h2 polarisation, which is known to lead to allergy and autoimmunity. This idea is supported by Watanabe *et al* (2004), who propose that mutations in the NOD2 gene leads to disease by allowing an increase in IL-12 secretion, causing an excessive T_h1 response, shifting the T_h1/ T_h2 balance, tending the immune system toward a more pro-inflammatory response. The gain of function argument put forward by Maeda *et al* (2005) suggests that the mutation causes the NOD2 to promote processing of Pro-IL-1β, leading to increased secretion of

mature IL-1β. This argument is supported by evidence from investigations using inhibitors of IL-1β signalling which showed attenuation in chemically stimulated inflammation in mice (Maeda *et al* 2005).

Studies by Leung *et al* (2006) have investigated the possibility of down-regulation of NOD2 activity by the presence of alternatively spliced NOD2 transcript variants, a mechanism found to be used by TLRs, which are unresponsive to MDP, but do not antagonise the activity of wild-type NOD2. This method of alternative splicing is employed in TLR4, in which soluble splice variants form part of a feedback loop, which prevents uncontrolled TLR4 activity. The study also investigated the possibility of restoring mutant NOD2 function by the transfection of a "stand-alone" LRR domain into the effected cells, as it has been shown possible to restore the function of plant R proteins, with similar mutations, by the transfection of missing LRR domain. The investigation revealed that the mutant NOD2 proteins did not have the ability to co-express the LRR domain in conjunction with the truncated NOD2 gene product.

Concluding Remarks

Although their function is still not fully understood, it seems that there are several factors which combine to determine the effect NOD proteins have on the secretion of cytokines from macrophages. The type of macrophage is important as it ultimately determines whether the immune response to the invading pathogen is to be pro- or anti-inflammatory by its interaction with other cells of the immune system. The type of TLR ligand is also important, as current research indicates that there is only interaction between the signalling pathways of NOD2 with TLRs 2 and 4, and as such only lipid ligands would be expected to produce a synergistic or suppressive effect in conjunction with NOD2.

There are several lines of research still underway to determine the exact mechanisms by which NOD proteins function, for example in 2005 Barnich *et al* showed that NOD2 was capable of associating with the cell surface membrane of intestinal epithelial cells, and proposed that this membrane targeting was

necessary for NF-κB activation via MDP stimulation, as the cell membrane association was not observed in cells expressing the NOD2 3020insC mutant. This would support a loss of function hypothesis; however this data has yet to be substantiated by independent studies. Studies by Netea *et al* (2005b) have indicated that there is cross-talk between the NOD1 and NOD2 signalling pathways, having observed a decreased response to the NOD1 ligand GM-triDAP and gram-negative PGN when the cell is homozygous for the mutant 3020insC NOD2 gene. This effect was only observed when this specific mutation is present, and did not occur when any other NOD2 gene mutation is induced; indicating that a decreased NOD1 function may also contribute to the severity of CD, however again this data has not yet been confirmed by independent studies.

Previous research has revealed many challenges to the investigation of the function of NOD proteins in humans. Murine macrophages are widely used a model for human cells, but some reports indicate that murine macrophages react differently compared with human THP-1 cells with identical treatments (Pauleau and Murray 2003). The transfection of NOD2 into other cell types can be unreliable, as they contain the apparatus for distinctly different signaling pathways to that found in the human macrophage. Human peripheral blood mononuclear cells have also been used, which include several cell types that do not have the NOD2 protein, but may have other methods of detecting MDP. Research now needs to be focused on the use of human macrophages, such as the human monocytic cell line THP-1 in order to reduce the error introduced into experiments by the use of alternative cell types.

Further studies required

Determination of the role NOD1 and NOD2 play in the activation or suppression of the MAPK pathways could reveal a role for NOD proteins in cell growth or apoptosis, and the clarification of the cytokines which are directly affected by NOD proteins would greatly increase understanding of the role of

NOD proteins in normal immune function and in disease states. The theory that NOD2 is able to affect the function of IL-1β Converting Enzyme (ICE, Caspase-1) may also link to increased apoptosis of macrophages, as high levels of IL-1β secretion are usually observed only when the macrophage is induced to undergo apoptosis (Hogquist *et al* 1991). Further study of the interaction of NOD2 with ICE could lead to possible treatments targeting ICE and the production of the pro-inflammatory cytokines IL-1β and IL-18 to reduce the inflammation characteristic of IBD. Investigation into the use of antibiotics to determine the contribution of commensual bacteria as a stimulus for the initiation and continuation of IBD would be of great interest, and could be studied in conjunction with NOD2 gene variants to determine any relationship between bacterial challenge and mutated NOD2 function.

References

Abbott, D.W *et al* (2004) The Crohn's disease protein, NOD2, requires RIP2 in order to induce ubiquitinylation of a novel site on NEMO *Current biology* 14, 2217-2227

Athman, R and Philpott, D (2004) Innate immunity via Toll-Like receptors and NOD proteins *Current opinion in Microbiology* 7, 25-32

Barnich, N *et al* (2005) Membrane recruitment of NOD2 in intestinal epithelial cells is essential for nuclear factor-{kappa}B activation in muramyl dipeptide recognition; *Journal of Cell Biology* 170, 21-26

Carneiro, L. A; Travassos, L. H; and Philpott, D. J (2004) Innate immune recognition of microbes through Nod1 and Nod2: implications for disease: *Microbes and Infection* 6(6), 609-616

Chamillard *et al* (2003) Nods, Nalps and Naip: intracellular regulators of bacterial-induced inflammation *Cellular Microbiology* 5(9), 581-592

Eckmann, L and Karin M (2005) NOD2 and Crohn's disease: Loss or Gain of function? *Immunity* 22, 661-667

Economou, M *et al* (2004) Differential effects of NOD2 variants on Crohns Disease risk and phenotype in diverse populations; a metaanalysis *American Journal of Gastroenterology* 99, 2393-24904.

Girardin, S. E *et al* (2001) CARD4/Nod1 mediates NF-κB and JNK activation by invasive Shigella flexneri *EMBO reports* 2, 736-742

Girardin, S. E *et al* (2003) Nod2 Is a General Sensor of Peptidoglycan through Muramyl Dipeptide (MDP) Detection *Journal of Biological Chemistry* 278(11), 8869-8872

Girardin, S. E, Hugot, J. P and. Sansonetti, P. J (2003) Lessons from Nod2 studies: towards a link between Crohn's disease and bacterial sensing *Trends in Immunology* 24(12), 652-658

Goldsby, R. A *et al* (2003) *Immunology* 5th Edition: USA: W.H Freeman and Company.

Gordon, S (2003) Alternative activation of macrophages *Nature reviews Immunology* 3, 23-35

Hogquist, K. A *et al* (1991) Interleukin 1 is processed and released during apoptosis *Procedures of the National Academy of Science U.S.A* 88, 8485-8489
Hugot *et al* (2001) Association of NOD2 Luecine-rich repeat variants with susceptibility to Crohns Disease *Nature* 411, 599-603

Inohara, N *et al* (2003) Host recognition of bacterial muramyl dipeptide mediated through NOD2: implications for Crohn's disease *Journal of Biological Chemistry* 278(8), 5509-5512

Kaisho T and Akira S (2006) Toll-like receptor function and signalling; *Journal of Allergy and Clinical Immunology* 117(5), 979-987

Kobayashi, K S *et al* (2005) Nod2-Dependent Regulation of Innate and Adaptive Immunity in the Intestinal Tract *Science* 307(5710) 731-734

Leung, E *et al* (2007) [Accessed on-line 13/11/2006] Splicing of NOD2 (CARD15) RNA transcripts; *Molecular Immunology* 44(4), 284-294

Li, J *et al* (2004) Regulation of IL-8 and IL-1ß expression in Crohn's disease associated *NOD2/CARD15* mutations *Human molecular genetics* 13(16), 1715-1725

Li, S. P *et al* (2003) p38 mitogen-activated protein kinase pathway suppresses cell survival by inducing dephosphorylation of Mitogen-activated Protein/Extracellular Signal-regulated Kinase Kinase1,2 *Cancer Research* 63*,* 3473-3477*.*

Maeda, S *et al* (2005) NOD2 mutation in Crohn's disease potentiates NF-κB activity and IL-1β processing *Science* 307, 734-738

Mannon, P.J *et al* (2004) Anti-interluekin-12 for active Crohns disease *New England Journal of Medicine* 531, 2069-2079

Mantovani, A *et al* (2004) The chemokine system in diverse forms of macrophage activation and polarization *Trends in immunology* 25(12), 677-686

Netea, M. G *et al* (2005a) Nucleotide-binding Oligomerization Domain-2 modulates specific TLR pathways for the induction of cytokine release *the Journal of Immunology* 174, 6518-6523

Netea, M. G *et al* (2005b): The Frameshift Mutation in Nod2 Results in Unresponsiveness Not Only to Nod2- but Also Nod1-activating Peptidoglycan Agonists; *Journal of. Biological Chemistry* 280, 35859-35867

Ogura, Y e*t al* (2001) A frameshift mutation in *NOD2* associated with susceptibility to Crohn's disease *Nature* 411, 603-606

Pan, Q *et al* (2006) NF-{kappa}B-Inducing Kinase Regulates Selected Gene Expression in the Nod2 Signaling Pathway; *Infection and Immunity* 74, 2121-2127

Pauleau, A and Murray, P (2003) Role of NOD2 in the response of Macrophages to Toll-like receptor Agonists *Molecular and Cellular Biology* 23(21), 7531-7539

Philpott, D.J and Girardin, S (2004) The role of Toll-like receptors and Nod proteins in bacterial infection *Molecular Immunology* 41(11), 1099-1108

Rock, F. L. *et al*. (1998) A family of human receptors structurally related to Drosophila Toll. *Procedures of the National Academy of Science U.S.A* 95, 588-593

Schwende, H *et al* (1996) Differences in the state of differentiation of THP-1 cells induced by phorbol ester and 1,25-dihydroxyvitamin D_3 *Journal of Leukocyte Biology* 59, 555-561

Siegmund, B (2002) Interleukin-1β converting Enzyme (Caspase-1) in intestinal inflammation *Biochemical pharmacology* 64, 1-8

Stein, M *et al* (1992) Interleukin 4 potently enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation. *Journal of experimental medicine* 176, 287-292

Strober, W *et al* (2006) Signaling pathways and molecular interactions of NOD1 and NOD2: *Nature Reviews Immunology* 6, 9-20

Staskawicz *et al* (2001) Common and contrasting themes of plant and animal diseases. *Science* 292, 2285–2289.

Takeuchi, O and Akira, S (2001) Toll-like receptors: their physiological role and signal transduction system *International Immunopharmacology* 1, 625-635

Travassos, LH *et al* (2004): Toll-like receptor 2-dependent bacterial sensing does not occur via peptidoglycan recognition: *EMBO Reports* 5(10), 1000–1006

Verrick F.A *et al* (2004) Human IL-23 producing type 1 macrophages promote but IL-10-producing type 2 macrophages subvert immunity to (myco)bacteria. *Procedures of the National Academy of Science U.S.A* 101, 4560-4565

Viala, J. Sansonetti, P and Philpott, D (2004) Nods and 'intracellular' innate immunity *C.R. Biologies* 327, 551-555

Viola, A; Contento, R and Molon, B (2006) T cells and their partners: the chemokine dating agency *Trends in immunology* 27(9), 421-427

Watanabe, T *et al* (2004) NOD2 is a negative regulator of Toll-like receptor 2-mediated T helper type 1 responses *Nature Immunology* 5, 800-808

Yang, S et al (2001) Synergistic Effect of Muramyldipeptide with Lipopolysaccharide or Lipoteichoic Acid To Induce Inflammatory Cytokines in Human Monocytic Cells in Culture *Infection and Immunity* 69(4), 2045-20

All Pictures adapted from source by Robert Gregory.