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At the Cutting Edge

Current insights into the role of transforming growth factor-β in bone resorption

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Abstract

Transforming growth factor-β (TGF-β) elicits a variety of effects on cellular proliferation and differentiation. The major repository for TGF-β is bone, where it possesses separate facilitative and suppressive actions on osteoclast differentiation and bone resorption. Without a direct enabling stimulus from TGF-β monocytes cannot form osteoclasts but instead follow macrophage differentiation pathways. This facilitative action depends on an ability to promote a state in which precursors are resistant to anti-osteoclastic inflammatory signals. Following the initiation of resorption TGF-β is released from bone matrix. This acts on osteoblasts to reduce the availability of the osteoclast differentiation factor, RANKL (receptor activator of NFκB ligand) and thereby indirectly limits further osteoclast formation. Thus TGF-β has a fundamental role in the control of bone resorption having actions that first allow monocytes to develop into osteoclasts then subsequently limiting the extent and duration of resorption after its release from the bone matrix.

Keywords: Transforming growth factor-β; Monocytes; Inflammation and bone resorption

Contents

1. Bone remodelling and regulation of osteoclast differentiation

Bone remodelling the process by which the skeleton adapts is dependent on the orchestrated actions of osteoclasts which resorb bone and osteoblasts which synthesise new bone matrix. During this process resorption and formation are temporally and spatially coupled to ensure no loss of skeletal integrity. Osteoclasts are large multinuclear cells generated in response to resorptive stimuli such as increases in circulating parathyroid hormone (PTH). However, while osteoclasts have a central role in repair and adaptation, excessive osteoclast activity underlies the loss of bone seen in several debilitating skeletal diseases. One such disease is osteoporosis which affects a large proportion of the aging population and is characterised by a significant increase in osteoclast formation and bone resorption. Aberrant

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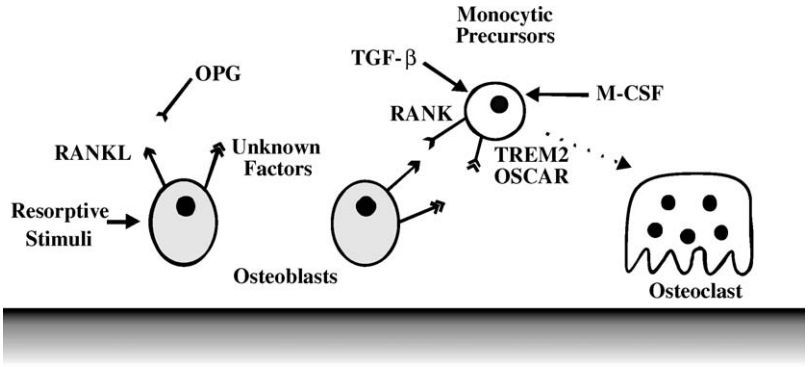

Fig. 1. Regulation of osteoclast formation. Resorptive stimuli activate osteoclast differentiation by increasing osteoblastic RANKL expression and suppressing expression of its soluble decoy receptor OPG. The subsequent activation of RANK in conjunction with binding of unknown osteoblastic factors to TREM2/OSCAR promotes precursors primed by TGF-β and M-CSF to differentiate into osteoclasts.

osteoclast activity is also responsible for the bone pain and increased fracture risk associated with cancers that metastasise to bone, such as multiple myeloma, lung and breast cancer.

Osteoclasts are derived from a pool of non-committed monocytic precursors which also possess the potential to differentiate into macrophages and dendritic cells. The formation of osteoclasts from monocytes is a tightly regulated process that is dependent on an osteoblast expressed member of the tumour necrosis factor (TNF) superfamily termed RANKL (receptor activator of NFκB ligand) (Fig. 1). Resorptive stimuli initiate osteoclast formation by promoting the expression of osteoblastic RANKL, which binds to its receptor RANK (receptor activator of NFκB) on monocytes (Fig. 1) (Kong et al., 1999; Yasuda et al., 1998b). Binding of RANKL to RANK recruits several signalling intermediates including NFκB (nuclear factor-κB) and NFATc1 (nuclear factor of activated T cells), which induce the transcription of genes involved in osteoclast differentiation (see Blair et al., 2005). A further layer of control is provided by osteoprotegerin (OPG), a soluble decoy receptor for RANKL, which suppresses osteoclast differentiation by binding RANKL before it can interact with RANK (Fig. 1) (Simonet et al., 1997; Tsuda et al., 1997; Yasuda et al., 1998a). Osteoclast formation is dependent on a fine balance between the level of RANKL and OPG; with resorptive stimuli increasing RANKL and suppressing OPG expression to initiate osteoclast formation. Alongside RANKL, monocytes require further co-stimulatory signals to undergo osteoclast differentiation. The precise nature of these osteoblast-expressed ligands is yet to be determined, but they are thought to control the activation of RANKL-induced NFATc1 following binding to immunoglobulin-like receptors TREM-2 and OSCAR (Fig. 1) (Koga et al., 2004; Mocsai et al., 2004).

Prior to activation of osteoclast differentiation by RANKL additional signals are necessary to commit precursors to the osteoclast lineage. This notion arises from several observations; first, only a proportion of precursors become osteoclasts when incubated with RANKL in vitro and these precursors become increasingly resistant to osteoclast formation with time, favouring differentiation towards macrophage lineages (Fuller et al., 2000). Second, osteoclasts are rarely seen outside of bone. This is somewhat surprising since monocytes express RANK and RANKL is expressed by extra-osseous cells, such as fibroblasts (Quinn et al., 2000). Even administration of RANKL in doses several orders of magnitude greater than that needed to stimulate osteoclast differentiation in vitro is not sufficient to promote osteoclast formation outside of bone (Fox et al., 2000a). This suggests that further factors present in the bone environment are required for osteoclast formation, or alternatively anti-osteoclastic signals are present outside of the skeleton which commit precursors to other lineages.

2. Transforming growth factor-β

TGF-β is a member of the TGF/activin sub-group of the TGF superfamily and has a critical role in cellular differentiation. Members of this family influence a wide range of cellular events by activating specific receptors on target cells, which generally consist of two type I and two type II serine/threonine kinase subunits. Binding of TGF-β to the extracellular domain of its receptor brings these subunits into close proximity, leading to the phosphorylation of multiple serine and threonine residues on the type I proteins. This allows binding and subsequent activation of SMAD2, which in conjunction with SMAD4, transmits the signal to the nucleus (Fig. 2) (Shi and Massague, 2003). In addition to this classical SMAD pathway, TGF-β also activates further signalling cascades through its ability to phosphorylate TAK1 (TGF-activated kinase-1), which in turn activates MAPKK6 (mitogen activated protein kinase kinase), MAPKK7 and NFκB (Fig. 2) (Shibuya et al., 1996; Ninomiya-Tsuji et al., 1999). Unsurprisingly for a cytokine that recruits such a wide range of signalling intermediates, it has been implicated in the pathogenesis of several disorders including Parkinson's disease (Sanchez-Capelo et al., 2003) and tumour growth in breast cancer (Guise and Chirgwin, 2003).

3. TGF-β and bone resorption

The major repository for TGF-β is bone and many bone cells express TGF-β receptors (Zheng et al., 1994) and produce TGF-$\beta_{1,2}$ and $_3$ including; osteoblasts, fibroblasts and osteoclasts (Bonewald, 1995). In light of this, it is not surprising that

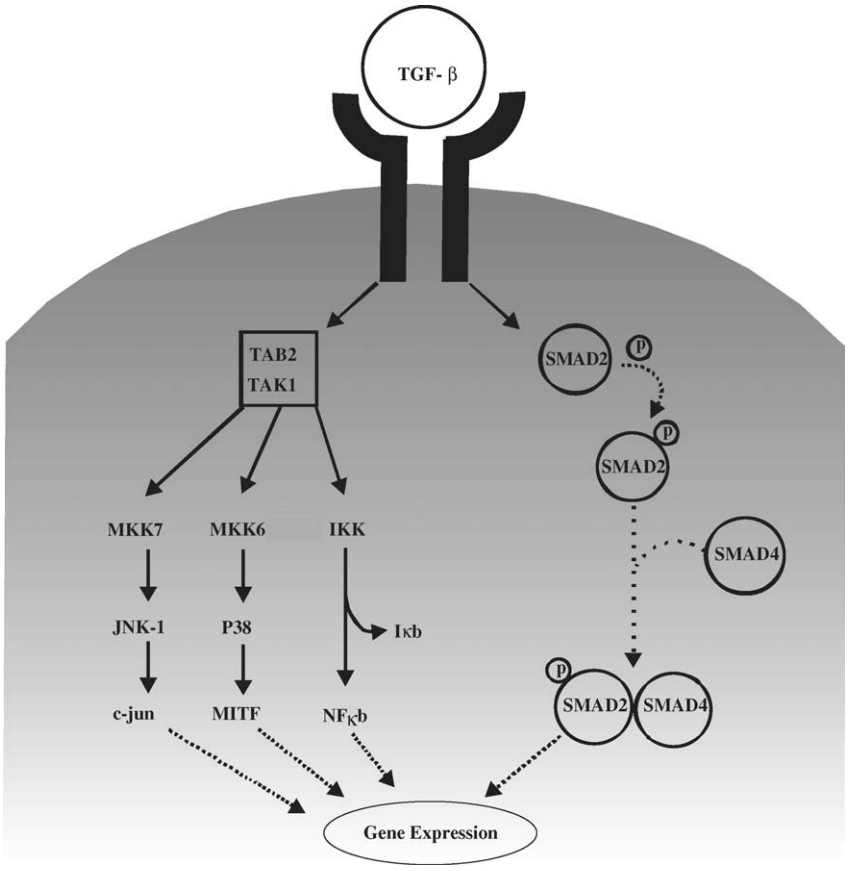

Fig. 2. Diagram of the major TGF-β activated signalling pathways.

TGF-β is considered an important regulator of osteoblast and osteoclast activity (Centrella et al., 1991) and is also suggested to couple bone formation to resorption during the remodelling cycle (Erlebacher et al., 1998; Martin and Sims, 2005). However, until recently its precise role in resorption was unclear since initial studies on osteoclast formation using mixed populations of osteoblasts and osteoclasts produced conflicting results. For instance, TGF-β had both positive and negative actions on osteoclast formation in co-cultures of osteoblasts and monocytes (Chenu et al., 1988; Hattersley and Chambers, 1991; Hughes et al., 1996; Shinar and Rodan, 1990), ex vivo mouse organ cultures (Dieudonné et al., 1991) and in vivo (Geiser et al., 1998; Erlebacher and Derynck, 1996; Filvaroff et al., 1999; Rosier et al., 1998). The divergent action of TGF-β in these studies stems from the tight coupling between bone formation and resorption making it difficult to attribute any change in osteoclast number to a primary effect on osteoclasts themselves rather than as a secondary consequence of a change in osteoblast activity. However, with the discovery of RANKL came the ability to generate osteoclasts free from the confounding influence of osteoblasts, permitting study of the direct effect of TGF-β on osteoclast formation and resorption. It is now apparent that TGF-β has a fundamental role in the control of bone resorption. Without the priming and augmentative stimulus provided by TGF-β, osteoclast formation will not occur. In addition, TGF-β has a separate osteoblast mediated action that limits the extent and duration of resorption preventing excessive bone loss.

4. TGF-β primes monocytes for osteoclast formation

Monocytes can follow several distinct differentiation pathways, lineage commitment being determined by the nature of the cytokine that the precursor first encounters (Erwig et al., 1998) (Fig. 3) Once precursors have committed to a certain lineage, they are thereafter unresponsive to other cytokines and so can not divert their route of differentiation from their intended pathway. Commitment to macrophage lineages is dependent on inflammatory cytokines such as IFN-γ (interferon-γ) (Fox et al., 2000a). Whereas the TGF-β produced by osteoblasts or stromal cells commits monocytes to the osteoclast lineage enabling resorption to proceed in the presence of RANKL or TNF-α

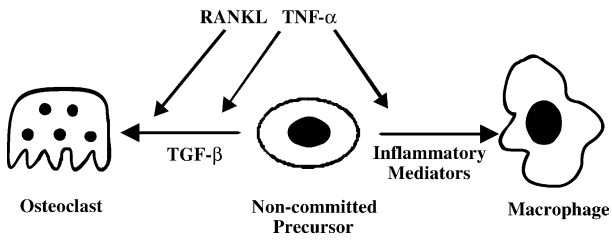

Fig. 3. Regulation of monocytic lineage switching. TGF-β is needed to prime non-committed precursors for RANKL/TNF-α-induced osteoclast formation, whereas inflammatory cytokines (IFN-γ, IFN-β, IL-10, IL-4, etc.) commit the same precursor for macrophage formation in the presence of TNF-α. Once a precursor has been primed to a specific lineage alternative cytokine inputs have no effect on its ultimate fate.

(Fig. 3). Evidence for this essential role in osteoclast differentiation was provided by studies which showed that RANKL was unable to induce osteoclast formation in cultures that lacked TGF-β (Kaneda et al., 2000; Fuller et al., 2000; Fox et al., 2000a). Furthermore, TGF-β enables RANKL-induced osteoclast differentiation in pure populations of monocytes suggesting that this action is a direct effect on precursors themselves. TGF-β is also essential for and enhances osteoclast formation elicited by pathological activators of resorption, such as TNF-α (tumour necrosis factor-α) (Fox et al., 2000a; Lovibond et al., 2003). TGF-β, therefore has a pivotal role in monocyte-lineage switching acting to directly prime or maintain precursors on the bone surface in a state where they are responsive to subsequent osteoclast-inductive stimuli (Fig. 4A). In addition to priming precursors for osteoclast formation TGF-β also augments RANKL and TNF-α induced osteoclast formation, increasing the number of multinuclear osteoclasts that form in monocytic cell lines, osteoblast-free bone marrow precursors and human peripheral blood mononuclear precursors (Sells Galvin et al., 1999; Fuller et al., 2000; Kaneda et al., 2000; Quinn et al., 2001; Koseki et al., 2002; Massey et al., 2001; Wahab et al., 1997; Wildemann et al., 2005). Thus TGF-β has a critical role in the initial stages of osteoclast formation and without this important enabling stimulus both physiological and pathological activation of bone resorption cannot occur.

5. TGF-β enables osteoclast formation by opposing inflammatory stimuli

Classically TGF-β is thought to act as a macrophage deactivator and it is likely that this ability to resolve inflammation underlies its facilitative role in osteoclast formation (Fox et al., 2000a; Lovibond et al., 2003; Karsdal et al., 2003). During the initial stage of an inflammatory response, IFN-γ and other cytokines promote macrophage formation to help clear the pathogenic challenge and as a consequence of this inflammatory mediators such as IFN-γ and IFN-β are also potent inhibitors of osteoclast formation (Fox et al., 2000b; Takayanagi et al., 2002). As the inflammatory episode progresses, increased levels of TGF-β suppress signalling by inflammatory cytokines leading to a reduction in cytotoxic macrophage number and generating an environment conducive for tissue repair (Schmidt-Weber and Blaser, 2004). Several lines of evidence suggest that the enabling action of TGF-β is dependent on this ability to antagonise inflammatory signals, priming precursors for resorption rather than inflammation (Fox et al., 2000a; Fox et al., 2003; Lovibond et al., 2003). First, pre-incubation with TGF-β for 24 h is sufficient to prevent IFN-γ or IFN-β from committing precursors to macrophage lineages (Fox and Lovibond, 2004; Koseki et al., 2002). Similarly, concurrent incubation with TGF-β suppresses the anti-osteoclastic effect of IFN-γ and IL-10 (Fox and Chambers, 2000; Lovibond et al., 2003) and prevents

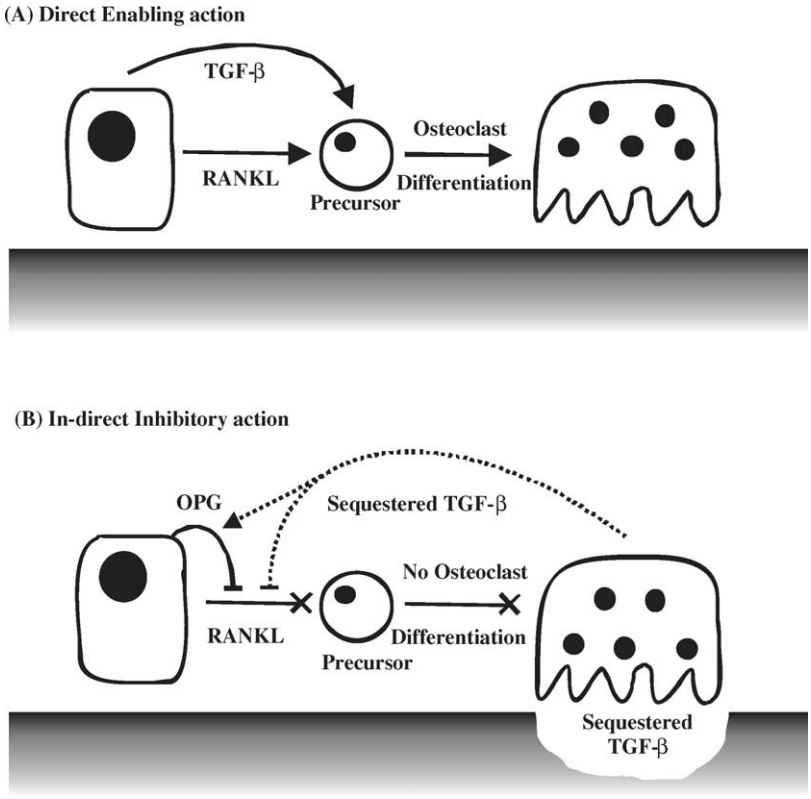

Fig. 4. (A) Osteoblast-derived TGF-β acts directly on osteoclast precursors to prime them for RANKL-induced osteoclast formation. (B) The subsequent release of sequestered TGF-β from the matrix during resorption indirectly limits further osteoclast formation by modifying osteoblastic RANKL/OPG production, decreasing RANKL and increasing the expression of its soluble decoy receptor OPG. The shift in the RANKL/OPG axis in favour of OPG reduces the osteoclast inductive stimuli leading to a severe reduction in the rate of osteoclast formation.

the resistance to osteoclast formation that occurs in response to the inflammatory stimulus provided by the culture environment (Fuller et al., 2000). This hypothesis is further strengthened by studies indicating that immunosuppressants, like TGF-β, enhance RANKL-induced osteoclast formation (Takuma et al., 2003; Shui et al., 2002) and the suggestion that TGF-β only augments osteoclast formation from human monocytes in the presence of inflammatory T cells (Massey et al., 2001). This body of evidence indicates strongly that the enabling action of TGF-β is dependent on its ability to prevent inflammatory cytokines from committing monocytes to macrophage lineages thereby maintaining precursors in a state from which they can form osteoclasts after activation by RANKL/TNF-α.

6. Mechanism of enabling action

While it is clear that TGF-β directly enables osteoclast formation by preventing inflammatory cytokines committing precursors for cytotoxic functions, the precise nature of this interaction is less certain. One possibility is that lineage switching is dependent on an antagonistic crosstalk between TGF-β and inflammatory cytokine signalling pathways. In this model, TGF-β-induced signals inhibit activation of the JAK/STAT signalling pathway (Janus kinase/signal transducer and activator of transcription) through which the majority of inflammatory cytokines transmit signals to the nucleus. The JAK/STAT pathway is regulated by several processes, among which negative feedback regulation by the suppressors of cytokine signalling (SOCS), is particularly important. SOCS are a family of STAT-induced factors which inhibit STAT activation and thereby switch off subsequent JAK/STAT signalling to prevent over-stimulation. Interestingly, osteoclasts have been shown to express several SOCS isoforms including SOCS3 and SOCS1 (Hayashi et al., 2002) and mounting evidence indicates that SOCS3 may mediate the antagonistic effect of TGF-β on specific inflammatory cytokines, preventing STAT phosphorylation and thereby inhibiting the transcription of anti-osteoclastic genes (Fig. 5). Evidence for this comes from a series of studies

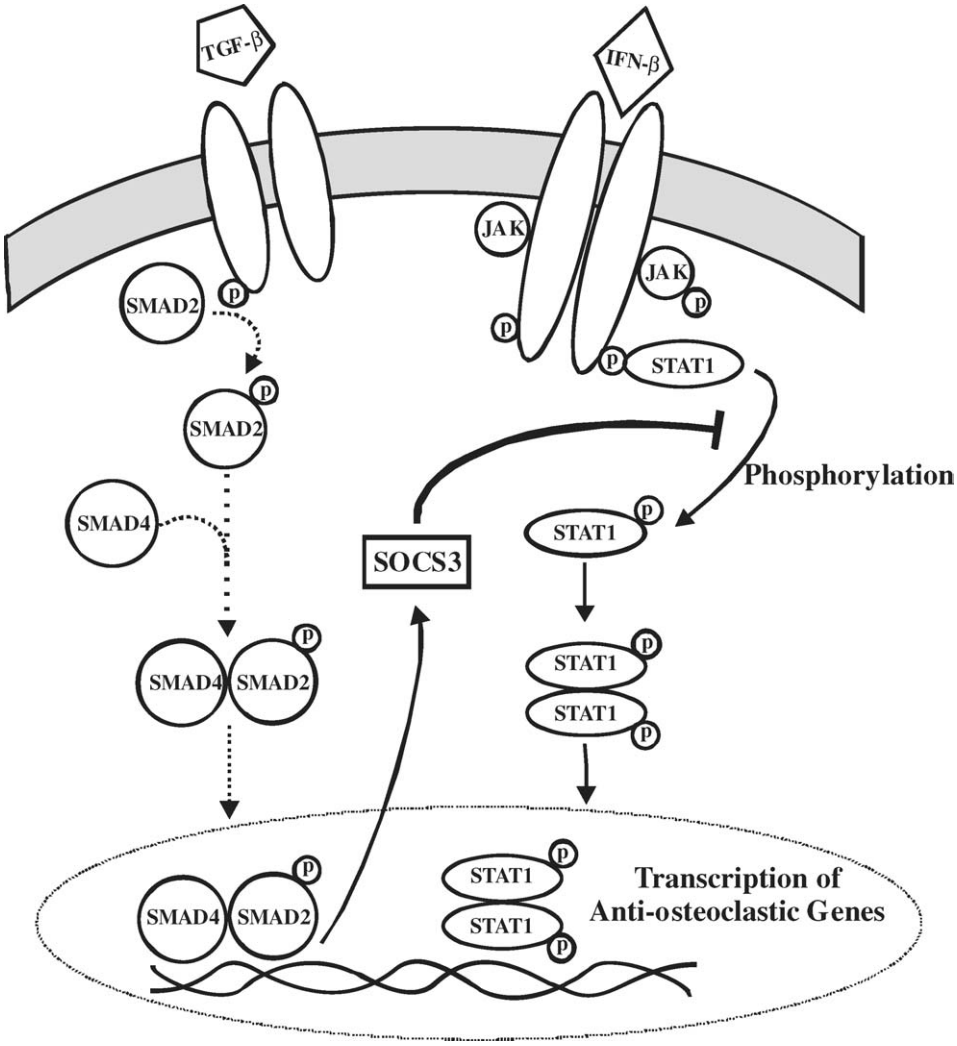

Fig. 5. Antagonistic interaction of TGF-β and IFN-β signalling pathways. TGF-β enables osteoclast formation by suppressing the anti-osteoclastic action of inflammatory cytokines such as IFN-β. This is mediated through an antagonistic interaction between TGF-β and IFN-β signalling pathways. TGF-β rapidly induces the expression of SOCS3, a negative feedback regulator of the JAK/STAT signalling pathway, through which the majority of inflammatory cytokines signal. SOCS3 inhibits IFN-β-induced phosphorylation of STAT1, thereby preventing transcription of anti-osteoclastic genes and priming precursors for osteoclast formation.

showing that TGF-β rapidly induces a sustained expression of SOCS3 mRNA in osteoclasts and monocytes and like TGF-β, SOCS3 expression is essential for RANKL and TNF-α-induced osteoclast formation (Lovibond et al., 2003; Fox et al., 2003). Similarly, monocytes isolated from SOCS3-deficient mice are more susceptible to the anti-osteoclastic action of IL-6 and express lower levels of TRAF-6 and IκB, key components of the RANKL signalling pathway (Ohishi et al., 2005). While reduced SOCS3 levels lead to a decrease in osteoclast formation, elevated levels are associated with increases in osteoclast formation and resorption. Retroviral driven SOCS3 expression in monocytes replaces the need for TGF-β in osteoclast formation (Fox et al., 2003; Lovibond et al., 2003) and prevents the anti-osteoclastic effect of IFN-β (Lovibond et al., 2003). Thus, TGF-β-induced SOCS3 expression may in part account for the enabling action of TGF-β, antagonising inflammatory JAK/STAT signalling and thereby priming precursors for bone resorption rather than inflammation (Fig. 5). However, SOCS3 is unable to mediate all of the facilitative actions of TGF-β as SOCS3 over-expression does not prevent the anti-osteoclastic effect of IL-10 or IFN-γ (Lovibond et al., 2003). It is possible that other SOCS isoforms may mediate the action of TGF-β on these cytokines and in keeping with this SOCS1 has been shown to prevent the actions of IFN-γ on monocyte switching (Ohishi et al., 2005). However, although SOCS1 is found in mature osteoclasts, TGF-β has no discernable effect on its expression suggesting that other regulatory factors antagonise these inflammatory cytokines. In addition to suppressing anti-osteoclastic signals, TGF-β may be involved in modulating the behaviour of one or more signalling components on which RANKL-induced osteoclast formation is dependent. By either enhancing or enabling the expression of one of these factors, TGF-β could facilitate, or at the very least enhance RANKL-induced osteoclast formation. In keeping with this, TGF-β has been shown to enhance JunB and p38 MAPK, critical components of the RANKL signalling pathway (Koseki et al., 2002; Karsdal et al., 2001).

As discussed earlier, TGF-β not only enables osteoclast formation but also significantly increases the number of osteoclasts that subsequently form. This may arise as a secondary consequence of the larger pool of precursors available, or alternatively it could be mediated through an entirely separate augmentative mechanism. Most evidence is consistent with separate enabling and augmentative actions. For example, pre-treatment with TGF-β is sufficient to prime precursors for resorption but does not enhance osteoclast formation beyond that of controls; a significant increase in osteoclast formation is only seen when TGF-β is present throughout the culture period (Fox and Lovibond, 2004). Several groups have shown direct effects that could mediate this enhancement of osteoclast formation. For instance in the studies of Chin, TGF-β increased αV integrin expression in pre-osteoclasts, and the increase in this important cell adhesion molecule was associated with a significant increase in RANKL-induced precursor fusion and osteoclast formation (Chin et al., 2003). Further investigations suggest that TGF-β could augment osteoclast formation through an ability to enhance RANK expression on osteoclast precursors (Fuller et al., 2000; Yan et al., 2001). However, not all studies note a change in RANK expression in monocytes (Fuller et al., 2000; Kaneda et al., 2000; Chin et al., 2003) and some observe a decrease in longer-term cultures (Karsdal et al., 2003). Alternatively, this increase may relate to its ability to promote key components of the RANKL signalling pathway (Koseki et al., 2002; Karsdal et al., 2001).

7. Indirect osteoblast mediated suppression of osteoclast formation

The role of TGF-β in bone resorption is not restricted to its direct enabling effect on osteoclast formation. It has a further action to limit osteoclast number and thereby prevent excessive resorption (Fig. 4B). Unlike the enabling action, this effect is mediated indirectly through the regulation of osteoblastic RANKL/OPG expression (Murakami et al., 1998; Thirunavukkarasu et al., 2001; Quinn et al., 2001; Takai et al., 1998). As described earlier, osteoclast differentiation is dependent on a balance between RANKL and its soluble decoy receptor OPG, with resorptive stimuli increasing RANKL expression while down-regulating OPG production (Blair et al., 2005). TGF-β reduces the osteoblastic stimulus for resorption, rapidly increasing OPG production (Murakami et al., 1998; Thirunavukkarasu et al., 2001; Takai et al., 1998) whilst at the same time inhibiting RANKL expression (Fig. 4B) (Quinn et al., 2001). Altering the RANKL/OPG ratio in this manner dampens the stimulus provided by osteoblasts leading to a reduced rate of osteoclast differentiation. Furthermore, since RANKL is a critical survival signal for mature osteoclasts, a decrease in RANK activation will initiate apoptosis of mature osteoclasts. As a consequence of this indirect action and unlike its direct effect in pure monocytic populations (Dieudonné et al., 1991; Fuller et al., 2000; Kaneda et al., 2000; Karsdal et al., 2001; Quinn et al., 2001) the addition of TGF-β to co-cultures of monocytes and osteoblasts inhibits osteoclast formation and resorption (Chenu et al., 1988; Dieudonné et al., 1991; Hattersley and Chambers, 1991; Murakami et al., 1998; Quinn et al., 2001; Sells Galvin et al., 1999).

At first it would appear to be of little physiological value for TGF-β to both stimulate and inhibit resorption. Moreover, as both osteoblasts and monocytes are normally present together on the bone surface (Jilka, 2003) and osteoblasts themselves produce TGF-β (Robey et al., 1987) it could be argued that no osteoclast formation should ever occur due to the negative effect of TGF-β on RANKL expression. However, this is patently not the case and intriguing work by Karst suggests that the effect of TGF-β on the RANKL/OPG axis is more complicated than was first appreciated; low levels of TGF-β increase RANKL production, whereas the higher concentrations seen after TGF-β is released from the matrix during resorption, inhibit RANKL and up-regulate OPG (Karst et al., 2004). In light of this, a system can be envisaged whereby the relatively low concentrations of TGF-β available prior to the start of resorption directly prime precursors for osteoclast formation (Fig. 4A), as well as increasing RANKL expression and therefore osteoclast differentiation. Once these precursors develop into resorptive osteoclasts the process of matrix degradation will release and activate latent

TGF-β stored within bone therefore elevating the concentration of TGF-β within the local environment (Dallas et al., 2002). In order to prevent excessive resorption, TGF-β then acts on osteoblasts to shift the RANKL/OPG axis in favour of OPG, thereby limiting further osteoclast differentiation and inducing apoptosis in resorptive osteoclasts (Fig. 4B). The TGF-β released from the matrix may also act as a local coupling factor, linking formation to resorption through its ability to promote osteoblast differentiation and matrix production (Erlebacher et al., 1998).

8. Conclusion and future directions

TGF-β has a fundamental role in the control of bone resorption and monocytic lineage switching having multiple effects throughout the remodelling cycle which ensure that precursors on the bone surface are not only responsive to resorptive stimuli but also subsequently regulate the extent of resorption following osteoclast differentiation. TGF-β directly enables osteoclast formation by antagonising inflammatory signalling, promoting a state in which precursors are resistant to anti-osteoclastic inflammatory stimuli and able to form osteoclasts. In contrast, TGF-β indirectly suppresses osteoclast formation thorough its ability to limit osteoblastic RANKL availability. Disruption of these processes can have a detrimental effect on bone physiology leading to aberrant osteoclast activity and bone structure.

While the cellular mechanisms by which TGF-β exerts its actions are gradually becoming clearer, there are still fundamental questions that remain to be answered. One of the major questions that remain is whether TGF-β is merely maintaining precursors in a non-committed state or does its enabling action represent an active step towards osteoclast differentiation. There is evidence to support both of these alternative actions, although it is uncertain which of them represents the true mechanism. Consistent with the hypothesis that TGF-β maintains precursors in a non-committed state, TGF-β only enhances osteoclast formation in the presence of inflammatory stimuli in human monocytic cultures (Massey et al., 2001). On the other hand, precursors that have been cultured with TGF-β are resistant to subsequent exposure to anti-osteoclastic cytokines (Fox and Lovibond, 2004), suggesting that lineage commitment and therefore differentiation has occurred. However, whether this commitment constitutes an active step in the osteoclast pathway is presently unclear, since the majority of studies show that TGF-β alone is unable to induce osteoclast formation. Although, it has recently been noted that TGF-β promotes the formation of small resorptive osteoclasts in the absence of other osteoclast inductive stimuli (Itonaga et al., 2004). Additional studies must also be performed to determine the precise mechanism by which TGF-β antagonises the wide range of inflammatory mediators that promote the formation of macrophages and thereby allow osteoclast formation to proceed. These questions are not only important for bone remodelling but are also relevant to trauma and chronic inflammation where the interaction of inflammatory cytokines and TGF-β impact on wound healing, scar formation and the resolution of inflammation.

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