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The Role of Jak/STAT Pathways in Osteoclast Differentiation

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Abstract

Osteoclasts are bone-resorbing cells of monocyte/macrophage origin and are culprits of bone destruction associated with osteoporosis, rheumatoid arthritis, and cancer bone metastasis. Recent advances in osteoclast biology revealed central roles of various cytokines in regulating osteoclastogenesis both *in vitro* and *in vivo*. However, exact underlying mechanisms including signaling pathways downstream of receptor ligation are still under pursuit. In the present review, the role of Jak/STAT proteins and their regulators will be discussed in connection with osteoclastogenesis, since growing evidence indicates that a number of cytokines and growth factors utilizing Jak/STAT signaling pathways affect osteoclastogenesis. A better understanding on the role of Jak/STAT pathways in osteoclast differentiation will not only strengthen our knowledge on osteoclast biology but also provide invaluable insights into the development of anti-resorptive strategies for treating bone-lytic diseases.

Key Words: Jak, STAT, Osteoclast, Differentiation

OSTEOCLAST

Osteoclasts are the bone-resorbing cells of monocyte/macrophage origin (Boyle *et al*., 2003). In mice, CD45R$^-$ CD11b^{low} bone marrow cells were identified as osteoclast precursors at least *in vitro* (Jacquin *et al*., 2006). Mizoguchi *et al*. established that cell cycle arrest in osteoclast precursors is prerequisite for osteoclast differentiation (Mizoguchi *et al*., 2009). These cells were identified as c-Fms$^+$ (M-CSF receptor) RANKL$^+$ cells at the site of osteoclastogenesis *in vivo*. Upon stimulation of these osteoclast precursors with macrophage colony-stimulation factor (M-CSF) and receptor activator of nuclear factor kappa B ligand (RANKL), these cells differentiate and finally fuse to form multinuclear functional osteoclasts through a multi-step process. M-CSF supports the survival and proliferation of osteoclast precursors. The critical role of M-CSF in osteoclastogenesis was revealed by the osteopetrotic bone phenotype of *op/op* mice, which lack functional M-CSF leading to the absence of osteoclasts (Yoshida *et al*., 1990). Genetic ablation of RANKL also induced osteopetrosis accompanied by complete loss of osteoclasts (Kong et al., 1999). Ligation of RANKL receptor (RANK) by RANKL recruits TNF receptor associated factors (TRAFs) and stimulates NF-κB, c-Fos, and NFATc1-mediated gene transcription by activating multiple signaling cascades including mitogen-activated protein kinases such as ERK, JNK, and p38 as well as phosphatidylinositol 3-kinase (PI3K)/Akt pathways (Lee and Kim, 2003). The major osteoclast signaling pathway is depicted in Fig. 1. Transcription factors playing critical roles also have been identified by loss of function mutation studies in mice. One of these transcription factors involved in osteoclastogenesis is hematopoietic transcription factor PU.1. Mice deficient in PU.1 developed osteopetrosis due to the loss of osteoclasts (Tondravi *et al*., 1997). Another family of transcription factors affecting osteoclastogenesis is microphthalmia-associated transcription factor (MITF). Mice having mutations in this transcription factor have long been known for their osteopetrotic bone phenotype (Walker, 1975). The importance of NFκB in osteoclastogenesis was suggested by the study by Iotsova *et al*., which reported severe osteopetrosis in NFκB1 (p50) and NFκB2 (p52) double knockout mice (Iotsova *et al*., 1997). Mice deficient in c-Fos also developed osteopetrosis due to a lineage shift in myeloid precursors that blocked osteoclastogenesis while stimulating macrophage differentiation (Grigoriadis *et al*., 1994). In addition to these transcription factors, nuclear factor of activated T cells cytoplasmic 1 (NFATc1) is considered as a mater transcription factor for osteoclast terminal differentiation and function (Takayanagi *et al*., 2002a). Not only NFATc1-deficient cells did not differentiate into osteoclasts in response to RANKL stimulation, overexpression of NFATc1

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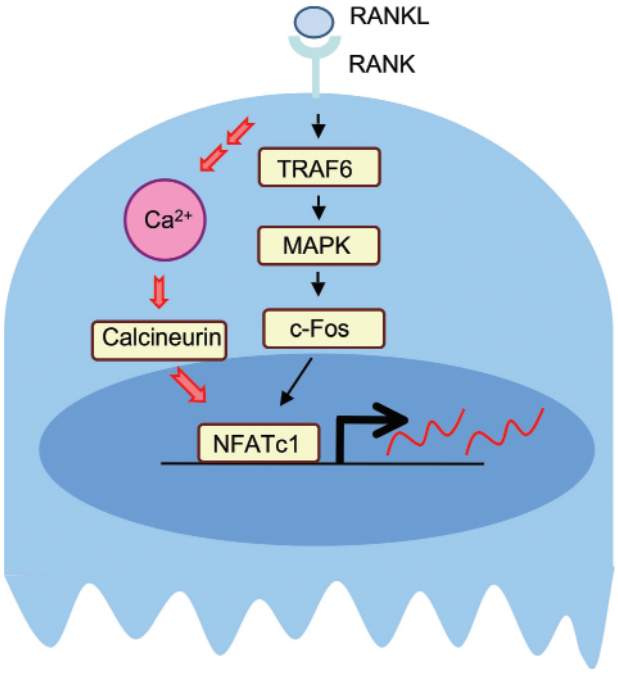

Fig. 1. Osteoclast signaling pathway. Upon RANK ligation by RANKL, TRAF is recruited followed by activation of MAPKs such as ERK, JNK, and P38. Consequently, c-Fos expression and activity is increased resulting in the induction of NFATc1. Alternatively, increased in the intracellular Ca^{2+} concentration upon RANKL stimulation activates Ca^{2+}-dependent phosphatases calcineurin. Dephosphorylation by calcineurin promotes nuclear translocation of NFATc1, supporting the transcription of osteoclastogenic genes.

alone was sufficient to induce osteoclastogenesis in the absence of RANKL, suggesting that NFATc1 is both necessary and sufficient for osteoclast differentiation. Genes induced by these osteoclastogenic transcription factors include proteases, ion pumps, and membrane proteins involved in osteoclast fusion and function. For example, protease cathepsin K, produced upon MITF transactivation (Hu *et al.*, 2007), catabolizes collagen matrices to degrade bone and cartilage. A proton pump vacuolar ATPase (V-ATPase) showed significantly higher expression in mature osteoclasts than in their precursors. Interestingly, deletion of d2 subunit of V-ATPase during early stages of osteoclastogenesis resulted in defective osteoclast fusion (Lee *et al.*, 2006). However, when depleted during later stages of osteoclast differentiation, it was evident that V-ATPase functions as proton pump that acidifies extracellular matrices during bone resorption (Wu *et al.*, 2009). Dendritic cell-specific transmembrane protein (DC-STAMP) was identified as a protein highly expressed in osteoclast compared with its precursor (Yagi *et al.*, 2005) and its expression was greatly induced by NFATc1 (Kim *et al.*, 2008). In DC-STAMP-deficient mice, osteoclast fusion was completely impaired although expression of osteoclast differentiation markers was normal, suggesting that the function of DC-STAMP is cell fusion-specific (Yagi *et al.*, 2005). Upon these concerted actions of multiple genes, mature multinucleated osteoclasts are formed. These osteoclasts undergo structural changes that allow the formation of sealing zone between bone surface and osteoclast basal membrane, and the secretion of acids as well as lytic enzymes into the lacunae leading to the bone resorption (Boyle *et al.*, 2003).

BONE-DESTRUCTIVE DISEASES AND ANTI-OSTEOCLASTOGENESIS DRUGS

Since bone homeostasis is maintained by bone-formation by osteoblasts and bone-degradation by osteoclasts, many of the skeletal diseases involve unregulated osteoclastogenesis that leads to excessive bone resorption (Arai *et al.*, 1999; Teitelbaum, 2000; Boyle *et al.*, 2003). These include osteoporosis, rheumatoid arthritis, periodontal diseases, cancer metastasis, and multiple myeloma bone diseases. Thus the inhibition of osteoclast differentiation and/or activity is expected to alleviate bone destruction associated with these conditions. Most widely used anti-osteoclastogenic drugs are bisphosphonates (Favus, 2010). This class of drugs has high affinity to bones and is ingested by bone-resorbing osteoclasts (Sato *et al.*, 1991; Masarachia *et al.*, 1996). Once inside the cells, bisphosphonate induces apoptosis of osteoclasts either by acting as ATP analogue (non-nitrogen containing bisphosphonates), or by inhibiting protein prenylation that is important for the survival and activity of osteoclasts (nitrogen containing bisphosphonates) (Frith *et al.*, 1997; van Beek *et al.*, 2003).

Another promising class of drugs targets RANKL-RANK axis. Among them is a humanized anti-RANKL antibody denosumab developed by Amgen, which is approved by FDA in 2010 for use in post-menopausal osteoporosis. This drug mimics endogenous osteoprotegerin (OPG), which binds to RANKL and blocks RANKL-dependent osteoclastogenesis.

In the last decade extensive studies on osteoclast biology as well as on the regulation of osteoclastogenesis by immune and endocrine system greatly advanced our understanding on the process of osteoclastogenesis. Upon this understanding, several drug pipelines are under clinical trials (Yasothan and Kar, 2008), and still more possible target molecules are being suggested through basic and clinical studies. Among the wide variety of mechanisms reported to govern osteoclastogenesis, the present review will focus on the Janus kinase/signal transducers and activators of transcription (Jak/STAT) signaling pathways.

THE JAK/STAT PATHWAY

Jak/STAT signaling pathway is one of the most extensively studied signal transduction cascade in mammals. More than 30 cytokines and receptor ligands have been shown to utilize JAK/STAT pathway to integrate extracellular signal to modulate gene expression and cellular functions (Rawlings *et al.*, 2004; Murray, 2007). These include type I and type II interferons, GP130 family cytokines, interleukins, and growth hormones. There are four Jak family members (Jak1, Jak2, Jak3, and Tyk2) (Stark *et al.*, 1998) and seven STAT family members (STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6) (Darnell, 1997). The importance of Jak/STAT signaling has been underscored by embryonic or perinatal lethality or severe defects in immune system or growth hormone pathways in knockout mice (Shuai and Liu, 2003). Upon ligation, the Jak-binding receptors multimerize allowing trans-phosphorylation of Jaks (Murray, 2007). Subsequently, phosphory-

lated active Jak kinases phosphorylate cytosolic domains of receptors that serve as docking sites for the Src homology 2 (SH2) domains in STATs. Finally, STATs are phosphorylated by Jaks and other proximal kinases, dimerize, and translocate into the nucleus to regulate gene transcription. These Jak/STAT signaling pathways are further fine-tuned by negative regulation by suppressor of cytokine signaling (SOCS) (Hilton, 1999) and protein inhibitor of activated STAT (PIAS) (Shuai, 2000).

REGULATION OF OSTEOCLAST DIFFERENTIATION BY INTERFERONS

Due to the dramatic expansion in the understanding of molecular mechanisms underlying osteoclastogenesis including regulation by immune system, a variety of cytokines has been shown to have anti-osteoclastogenic effects and suggested as anti-resorptive strategy. Among them, interferon γ produced by T cells was reported to inhibit RANKL-dependent osteoclast differentiation (Takayanagi *et al.*, 2000). Interferon γ rapidly induced proteasome-mediated TRAF6 degradation, resulting in the loss of RANKL-stimulated NFκB and JNK activities. Accordingly, overexpression of TRAF6 in osteoclast precursors reduced the inhibitory effect of interferon γ on osteoclastogenesis. Interferon β added another layer of fine-tuning mechanism by which RANKL-dependent osteoclast differentiation is regulated. Takayanagi *et al.* reported that RANKL stimulation of osteoclast precursors rapidly induced the production of interferon β in a c-Fos dependent mechanism (Takayanagi *et al.*, 2002b). Interestingly, interferon β strongly inhibited RANKL-induced osteoclastogenesis by reducing the expression of c-Fos. Indeed, both interferon β-deficient mice and interferon α/β-receptor-deficient mice showed reduced bone mass with significantly increased osteoclast number. Thus, interferon β comprised an autoregulatory negative feedback loop in which bone homeostasis might be regulated by limiting extensive bone destruction by osteoclasts.

REGULATION OF OSTEOCLAST DIFFERENTIATION BY INTERLEUKINS THAT STIMULATE JAK/STAT PATHWAY

A number of interleukins that utilize JAK/STAT signaling pathways were investigated regarding the role in regulating osteoclast differentiation. Among them, IL-6 is one of the most extensively studied. Duplomb *et al.* showed that IL-6 potently inhibited RANKL-induced osteoclastogenesis of mouse macrophages as well as human monocytes in a STAT3 dependent fashion (Duplomb *et al.*, 2008). IL-6 diverted osteoclast precursors into macrophages in the presence of RANKL in RAW 264.7 cells, mouse bone marrow macrophages, and human peripheral blood monocytes. Yoshitake *et al.* showed that IL-6 also inhibited RANKL-induced NFκB and MAPK signaling pathways in mouse bone marrow macrophages (Yoshitake *et al.*, 2008). On the other hand, IL-6 stimulated the formation of osteoclasts when added to osteoblast/osteoclast co-cultures (Tamura *et al.*, 1993; Richards *et al.*, 2000). This indirect stimulation of osteoclastogenesis might have resulted from the increased production of RANKL by osteoblasts in the presence of IL-6 (Palmqvist *et al.*, 2002). For the augmented osteoclastogenesis by osteoblasts in the presence of IL-6, the activity of STAT3 was crucial since no increase in the RANKL in osteoblasts was observed when dominant-negative form of STAT3 was overexpressed (O'Brien *et al.*, 1999).

IL-4 has been shown to suppress osteoclast differentiation via STAT6 by several independent research groups. Abu-Amer was one of the researchers first described the inhibitory role of IL-4 on osteoclast differentiation (Abu-Amer, 2001). IL-4 significantly reduced RANKL-dependent NFκB activation and completely inhibited osteoclastogenesis in mouse bone marrow macrophages. However, this negative regulation of osteoclastogenesis was not observed when bone marrow macrophages from STAT6-deficient mice were used. Moreno *et al.* showed that IL-4 reduced the expression of RANK mRNA, thereby reducing osteoclastogenesis in human peripheral blood monocytes and mouse bone marrow macrophages (Moreno *et al.*, 2003). These effects were STAT6-dependent, since IL-4 did not reduce RANK expression in STAT6-deficient osteoclast precursors. Yamada *et al.* showed that IL-4 inhibited osteoclastogenesis in mouse bone marrow macrophages while stimulating STAT6 phosphorylation and reducing NFATc1 expression (Yamada *et al.*, 2007). Wei *et al.* reported that IL-4 inhibited RANKL-induced IκB degradation, NFκB activation, and MAPK activation in a STAT6-dependent manner in mouse bone marrow macrophages (Wei *et al.*, 2002). IL-4 also inhibited the bone-resorbing activity of mature osteoclasts by reducing the RANKL-stimulated NFκB and Ca^{2+} responses (Mangashetti *et al.*, 2005). In addition, IL-4 stimulated the production of osteoprotegerin (OPG), a decoy receptor for RANKL, in mouse osteoblasts (Yamada *et al.*, 2007).

Direct addition of IL-7 to mouse bone marrow cells significantly suppressed osteoclast formation in the presence of M-CSF and RANKL suggesting a negative role of this interleukin on osteoclastogenesis, which was further supported by enhanced osteoclast differentiation from IL-7-deficient bone marrow cells (Lee *et al.*, 2003). However, Weitzmann *et al.* reported a dramatic increase in the osteoclast differentiation from human peripheral blood mononuclear cells by IL-7 (Weitzmann *et al.*, 2000). In this case, the stimulatory effect of IL-7 was mediated via up-regulation of RANKL expression in T cells.

IL-15 stimulated the formation of osteoclasts from rat whole bone marrow cells as well as stroma-depleted marrow cells following vitamin D_3 addition (Ogata *et al.*, 1999). In support of the pro-osteoclastogenic role of IL-15, recent report by Djaafar *et al.* showed significantly increased bone mass in IL-15 receptor-deficient mice (Djaafar *et al.*, 2010). Interestingly, IL-15 receptor-deficient T cells showed impaired expression of RANKL and failed to support wild type osteoclasts, while IL-15 receptor-deficient osteoclast precursors did not form mature osteoclasts even in the presence of wild type T cell. These results suggested the existence of both direct and indirect inhibitory mechanisms on osteoclastogenesis by IL-15.

IL-12 has been known to induce Jak2- and Tyk2-dependent STAT phosphorylation (Zou *et al.*, 1997). IL-12 inhibited osteoclastogenesis from murine spleen cells as well as osteoblast/splenocyte co-cultures (Horwood *et al.*, 2001). These inhibitory effects almost completely disappeared when T cells were removed from the spleen cell cultures, suggesting that T cells mediated the IL-12-dependent suppression of osteoclastogenesis. However, contradictory results were obtained when Nagata *et al.* treated mouse bone marrow macrophages with IL-12 (Nagata *et al.*, 2003). Although IL-12 had no effect on the

differentiation of osteoclast from bone marrow macrophages stimulated by M-CSF and RANKL, IL-12 potently inhibited osteoclastogenesis when mouse whole bone marrow cells were used as osteoclast precursors. In addition, this inhibitory effect of IL-12 appeared to be mediated by interferon γ, since the addition of anti-interferon γ antibody blunted the suppression of osteoclastogenesis by IL-12. In contrast to the results by Horwood *et al.* (2001). IL-12 still potently blocked the formation of osteoclastogenesis from bone marrow cells even after T cell depletion or when bone marrow cells from T cell-deficient nude mice were used. On the other hand, B cell depletion slightly reduced the inhibitory effect of IL-12 on osteoclast differentiation, suggesting an involvement of B cells.

IL-23 is a heterodimeric cytokine sharing a subunit of IL-12 (Lankford and Frucht, 2003). Both inhibitory and stimulatory roles of IL-23 on osteoclastogenesis through T cells were reported. Quinn *et al.* reported that IL-23 inhibited osteoclast formation from mouse spleen cells in $CD4^+$ T- and γδT cell-dependent mechanism (Quinn *et al.*, 2008). Mice lacking a subunit of IL-23 (IL-23p19 deficiency) represented reduced bone mass phenotype, supporting the role of IL-23 in limiting bone resorption in resting conditions. In contrast, Ju *et al.* reported that IL-23 stimulated the expression of RANKL on $CD4^+$ T cells from arthritic IL-1 receptor antagonist (IL-1Ra)-deficient mice, enhancing osteoclastogenesis (Ju *et al.*, 2008). However, these authors could not observed the IL-23-dependent augmentation of osteoclast differentiation when wild type bone marrow cells were used, suggesting that the inflammatory condition affected the effects of IL-23 on osteoclastogenesis.

IL-3 directly inhibited osteoclast differentiation from human peripheral blood monocytes (Gupta *et al.*, 2010). The addition of IL-3 greatly suppressed the expressions of c-Fms, PU.1, and c-Fos. Interestingly, IL-3 not only inhibited osteoclastogenesis, but also diverted osteoclast precursors into dendritic cell-like phenotype. IL-3 also suppressed osteoclastogenesis from mouse bone marrow macrophages (Khapli *et al.*, 2003). In this setting, IL-3 inhibited IκB phosphorylation, NFκB nuclear translocation, and c-Fos transcription upon RANKL stimulation of osteoclast precursors. In contrast to the results in human cells, IL-3 increased the expression of macrophage markers such as Mac-1 and F4/80.

IL-10 suppressed RANKL-dependent osteoclast differentiation from RAW264.7 macrophages as well as mouse bone marrow cells by reducing the expression of c-Fos, c-Jun, and NFATc1 (Mohamed *et al.*, 2007). Similar inhibitory role of IL-10 on osteoclastogenesis was reported when mouse osteoblast/bone marrow cell co-culture system was used (Hong *et al.*, 2000).

Both leukemia inhibitory factor (LIF) and oncostatin M (OSM) stimulated osteoclast formation by stimulating RANKL expression in mouse calvariae (Palmqvist *et al.*, 2002). Similarly, IL-11 induced RANKL expression in cultured mouse calvarial bones and stimulated bone resorption (Ahlen *et al.*, 2002). The effect of various interleukins on osteoclastogenesis is summarized in Table 1.

ROLE OF JAK FAMILY PROTEINS

In spite of the vast amount of evidence suggesting that interferons and interleukins that stimulate Jak/STAT pathways affect the differentiation of osteoclasts and bone homeostasis, little is known on the role of Jak family kinases in these processes. We previously showed that the expression of Jak1 significantly decreased during RANKL-induced osteoclast differentiation of mouse bone marrow macrophages, while other

Table 1. Effect of selective interleukins on osteoclastogenesis

Interleukin	Cell	Effect	Reference
IL-6	Mouse BMM, Human Monocytes	Inhibition	Duplomb *et al.*, 2008
	Mouse BMM	Inhibition	Yoshitake *et al.*, 2008
	OB/OC co-culture	Stimulation	Tamura *et al.*, 1993; Richards *et al.*, 2000
IL-4	Mouse BMM	Inhibition	Abu-Amer, 2001
	Human PBMC, Mouse BMM	Inhibition	Moreno *et al.*, 2003
	Mouse BMM	Inhibition	Yamada *et al.*, 2007
	Mouse BMM	Inhibition	Wei *et al.*, 2002
IL-7	Mouse BMM	Inhibition	Lee *et al.*, 2003
	Human PBMC	Stimulation	Weitzmann *et al.*, 2000
IL-15	Rat bone marrow	Stimulation	Ogata *et al.*, 1999
IL-12	Mouse splenocyte	Inhibition	Horwood *et al.*, 2001
	Mouse bone marrow	Inhibition	Nagata *et al.*., 2003
IL-23	Mouse splenocyte	Inhibition	Quinn *et al.*, 2008
IL-3	Human PBMC	Inhibition	Gupta *et al.*, 2010
	Mouse BMM	Inhibition	Khapli *et al.*, 2003
IL-10	RAW264.7, Mouse bone marrow	Inhibition	Mohamed *et al.*, 2007
	Mouse OB/bone marrow co-culture	Inhibition	Hong *et al.*, 2000
LIF	Mouse calvaria	Stimulation	Palmqvist *et al.*, 2002
OSM	Mouse calvaria	Stimulation	Palmqvist *et al.*, 2002
IL-11	Mouse calvaria	Stimulation	Ahlen *et al.*, 2002

Jak family members such as Jak2 and Jak3 did not change in protein level (Lee *et al.*, 2008). The reduction of Jak1 expression was mediated by ubiquitination followed by proteasome-dependent degradation. The reduction in Jak1 expression during osteoclast differentiation was associated with reduced susceptibility to the inhibition by interferon β. Although interferon β potently suppressed osteoclastogenesis in osteoclast precursors when Jak1 protein is in normal levels, interferon β lost its inhibitory effects especially in the later phase of differentiation, when Jak1 expression was significantly diminished. In addition, the Jak1-dependent inhibition of osteoclastogenesis was mediated by STAT3. Compared with STAT1 and STAT5, only the phosphorylation of STAT3 was impaired in Jak1 knocked down cells as well as in RANKL-primed osteoclast precursors in which Jak1 expression was decreased. In addition, interferon β did not inhibit osteoclast differentiation when STAT3 was knocked down.

Possible role of Jak2 in osteoclast survival was suggested since the incubation of osteoclasts derived from mouse bone marrow macrophages with Jak2 inhibitor AG490 resulted in the inhibition of osteoclast apoptosis upon RANKL removal (Kwak *et al.*, 2008). However, since the specificity of AG490 for Jak2 over other kinases are doubted (Osherov *et al.*, 1993; Kleinberger-Doron *et al.*, 1998), the role of Jak2 in this process requires further corroboration by alterative methods such as overexpression of dominant negative form or gene silencing.

ROLE OF STAT FAMILY PROTEINS

The role of STAT1 in osteoclastogenesis was highlighted by initial descriptions that STAT1 mediated the inhibition of osteoclast differentiation by interferon γ (Takayanagi *et al.*, 2000) and interferon β (Hayashi *et al.*, 2002). STAT1-deficient osteoclast precursors were protected from the inhibitory effects of interferon γ. Interferon β failed to stimulate STAT1 phosphorylation and DNA binding activity in RANKL-primed osteoclast precursors that were resistant to the inhibition by this type I interferon. However, discrepant data were reported regarding the involvement of STAT1 in these processes. Huang *et al.* published that the incubation of osteoclast precursors with RANKL rendered resistance to interferon γ irrespective of STAT1 phosphorylation (Huang *et al.*, 2003). In their experiments, interferon γ still potently induced STAT1 phosphorylation in RANKL-primed osteoclast precursors that were clearly protected from the inhibitory effect of interferon γ on osteoclastogenesis. Similarly, we observed comparable phosphorylation of STAT1 in bone marrow macrophages upon interferon β stimulation both before and after RANKL priming for 2 days (Lee *et al.*, 2008). Indeed the interferon β inhibition of osteoclastogenesis was mediated by STAT3 in these experiments. These discrepancies suggest the possibility that there might be multiple STAT signaling pathways governing the interferon-dependent regulation of osteoclastogenesis. In addition, STAT1 is a negative regulator of osteoblast differentiation by retaining runx2 in cytoplasm through physical interaction, which is independent of STAT1 transcription activity (Kim *et al.*, 2003).

The role of STAT3 in osteoclastogenesis is emphasized by the osteoporotic bone phenotype of mice deficient in STAT3 in hematopoietic cells (Zhang *et al.*, 2005). In these mice, osteoclastogenesis was dramatically increased with enhanced c-Fos expression in osteoclast precursors, suggesting an inhibitory role of STAT3 in osteoclast differentiation.

STAT6 has been associated with the IL-4-dependent inhibition of osteoclastogenesis (Abu-Amer, 2001; Wei *et al.*, 2002; Moreno *et al.*, 2003). In addition, constitutive active STAT6 potently inhibited RANKL-stimulated NFκB and JNK activities as well as osteoclast differentiation (Hirayama *et al.*, 2005). Besides, STAT6 was also involved in the production of OPG in IL-4-stimulated osteoblasts, further suppressing osteoclastogenesis (Palmqvist *et al.*, 2006; Yamada *et al.*, 2007).

ROLE OF STAT-REGULATING PROTEINS

Suppressor of cytokine signaling (SOCS) family members are STAT-induced STAT inhibitors (Alexander, 2002). SOCS proteins either bind to phosphorylated Jaks or activated receptors to block Jak activities. It was reported that RANKL induced the expression of SOCS1 and SOCS3 in bone marrow macrophages (Hayashi *et al.*, 2002). These STAT inhibitors were suggested to play crucial roles in the protection of RANKL-primed osteoclast precursors from the inhibition of osteoclastogenesis by endogenous interferon β. Indeed, retrovirus-mediated overexpression of SOCS3 in mouse bone marrow macrophages resulted in significantly enhanced RANKL-dependent osteoclast differentiation (Fox *et al.*, 2003). Furthermore, SOCS3 overexpression rendered formation of mature osteoclasts in the presence of interferon β that otherwise suppressed osteoclastogenesis completely. However, Ohishi *et al.* reported that the overexpression of SOCS1, not that of SOCS3, rescued osteoclastogenesis from mouse bone marrow macrophages in the presence of interferon γ and interferon β (Ohishi *et al.*, 2005). These authors further showed that SOCS3-deficient osteoclast precursors were hypersensitive to the inhibitory effect of IL-6 on osteoclastogenesis, while exhibited no difference to that by both type I and type II interferons.

Protein inhibitor of activated STAT (PIAS) family members block STAT-dependent transcription either by directly inhibiting DNA-binding activity or acting as co-repressors (Chung *et al.*, 1997; Liu *et al.*, 1998; Liu *et al.*, 2001; Arora *et al.*, 2003). Among the four PIAS family members (PIAS1, PIAS3, PIASX, PIASY), only PIAS3 has been associated with osteoclastogenesis until present. Hikata *et al.* showed that PIAS-transgenic mice exhibited osteopetrotic bone phenotype with impaired osteoclast differentiation, suggesting that PIAS3 acted as a negative regulator of osteoclastogenesis (Hikata *et al.*, 2009). Overexpression of PIAS3 in RAW264.7 macrophages significantly reduced osteoclastogenesis and suppressed the expression of c-Fos upon RANKL stimulation, supporting that PIAS3 directly inhibited osteoclastogenesis from precursor cells. Kim *et al.* reported that PIAS3 recruited a transcription co-repressor HDAC1 to NFATc1 promoter, thereby reducing NFATc1 expression and osteoclast differentiation upon RANKL stimulation of mouse bone marrow macrophages (Kim *et al.*, 2007). However, although PIAS3 has been reported to directly bind STAT3 (Chung *et al.*, 1997), the role of STAT3 in the PIAS3-mediated inhibition of RANKL-dependent osteoclastogenesis has not been tested in these studies and needs to be unveiled.

CONCLUSION

Growing evidence indicates that Jak/STAT pathways are playing central roles in the regulation of immune responses. During the last decade, enormous data were gathered on the genetic, biochemical, and cell biological aspects on the regulation of osteoclast differentiation and function both *in vivo* and *in vitro*. Current understanding on the osteoclast biology suggests that immune system, endocrine system, and bone are closely related. Especially, the role of cytokines on osteoclastogenesis is under active investigation. This review focused on the studies regarding the role of Jak/STAT signaling pathways on osteoclastogenesis performed mostly in last 10 years. However, in spite of extensive research, our molecular understanding on the exact nature and the role of each component of Jak/STAT pathway is limited. Furthermore, the information on the role of cytokine/receptor/Jak/STAT combination in the context of osteoclastogenesis is lacking. Recent advances in genomic and proteomic methodology will definitely be of great help to decipher the quandary. In addition, the application of cell-specific or conditional transgenic technology will also substantially advance our knowledge on the role of Jak/STAT pathways on osteoclastogenesis *in vivo*, which was hindered by lethality of mice generated by systemic knock out technology.

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