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TGF-β-induced SOCS3 expression augments TNF-α-induced osteoclast formation

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Abstract

Osteoclast differentiation is dependent on TGF-β to prime precursors to the osteoclast lineage. The mechanism by which TGF-β enables osteoclast formation is unknown. One possibility is that TGF-β opposes pro-inflammatory JAK/STAT signalling. Recently, we showed that TGF-β-induces SOCS3, an inhibitor of the JAK/STAT pathway, in precursors and enhances SOCS3 in RANKL-induced osteoclasts. We therefore elected to test the role of SOCS3 in the effect of other regulators of osteoclastic differentiation. We found that TNF-α-induced osteoclasts also express SOCS3 and TGF-β strongly up-regulates this. Moreover, TNF-α-induced osteoclast differentiation and total resorbed bone area were enhanced in SOCS3-retrovirally infected precursors, whereas antisense knockdown of SOCS3 suppressed formation and the augmentative effect of TGF-β. Furthermore, SOCS3 overexpression blunted the anti-osteoclastic effect of IFN-β but not IL-10. This suggests that TGF-β-induced expression of SOCS3 may represent a crucial mechanism by which TGF-β antagonizes specific anti-osteoclastic JAK/STAT signals, priming precursors for resorption rather than inflammatory functions.

Keywords: Osteoclast; Lineage-commitment; TGF-β; SOCS3; TNF-α

It is well established that receptor activator of NFκB ligand (RANKL), an osteoblast-derived member of the tumour necrosis factor (TNF) superfamily, induces osteoclast differentiation from mononuclear precursors [1,2]. TNF-α has also been shown to directly induce osteoclast formation [3] and increased TNF-α production may lead to bone loss in ovariectomised mice [4].

The ability of these TNF superfamily members to commit precursors to the osteoclastic lineage is enabled by transforming growth factor-β (TGF-β) [5–7]. The proportion of precursors that form osteoclasts is enhanced by TGF-β, while type II soluble TGF-β receptors abolish RANKL and TNF-α-induced osteoclast formation [5,6]. This suggests that osteoclast differentiation is dependent on TGF-β to prime or maintain non-committed precursors in a RANKL/TNF-α responsive state. The mechanism by which TGF-β facilitates osteoclast formation is not known. One possibility is that the environment in vitro is essentially pro-inflammatory [8], acting to prime precursors for alternative inflammatory roles, and TGF-β which has anti-inflammatory actions opposes this.

Many factors that suppress osteoclast formation signal through the janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway [9]. Following cytokine stimulation JAK/STAT signals are switched off by a group of negative feedback regulators termed SOCS (suppressor of cytokine stimulation) [10]. These STAT-induced factors inhibit subsequent JAK activity and STAT phosphorylation, thereby preventing overstimulation [11,12]. At present 15 SOCS have been described, all of which contain a 40 amino acid sequence at the C terminus termed the SOCS-box [13]. The function of all SOCS isoforms is not known, but SOCS1 and SOCS3 have been shown to inhibit signalling by inflammatory cytokines such as IFN-γ and IFN-β [14,15], and the antagonistic effect of interleukin-10 (IL-10) on inflammatory cytokines is mediated through the upregulation of SOCS3 expression [16]. In light of this,

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it is therefore of great interest that we have recently shown that TGF-β induces the expression of SOCS3 mRNA in non-committed osteoclast precursors and strongly up-regulates SOCS3 expression in RANKL-induced osteoclasts [17]. Furthermore, overexpression of SOCS3 in non-committed precursors using a retroviral vector enhances RANKL-induced osteoclast formation [17].

Therefore, to further examine the role of SOCS expression in osteoclastic differentiation we examined the effect of TGF-β and TNF-α on SOCS expression in osteoclast. We also assessed the role of SOCS in TNF-α-induced osteoclast formation, using SOCS3 expressing retroviruses and specific SOCS3 antisense oligonucleotides (ODN). Furthermore, to determine whether SOCS expression represents a common mechanism by which TGF-β suppresses diverse inhibitory JAK/STAT signals we examined the ability of SOCS3 overexpression to suppress the anti-osteoclastic effect of IFN-β and IL-10.

Materials and methods

Media and reagents. Non-adherent, M-CSF-dependent bone marrow cells were incubated in minimum essential medium with Earle's salts (EMEM), supplemented with 10% fetal bovine serum (FBS), 2 mmol/L glutamine, 100 IU/ml benzylpenicillin, and 100 μg/ml streptomycin (PSG) (all from Imperial Laboratories, Andover, Hants, UK). The Phoenix retroviral packaging cell line [18] was incubated in Dulbecco's minimum essential medium (DMEM), supplemented with 10% FBS and PSG. Incubations were performed at 37 °C in 5% CO_2 and cultures fed every 2–3 days. Recombinant human TGF-β_1 and recombinant murine IFN-β were obtained from R&D systems (Minneapolis, MN). Recombinant human M-CSF was provided by Chiron Corp (Emeryville, CA); recombinant murine IL-10 and recombinant murine TNF-α were from Insight Biotechnology (Wembley, Middlesex, UK). Bovine cortical bone slices were prepared as previously described [19].

Isolation of stroma-depleted non-adherent M-CSF-dependent bone marrow precursors. Male MF-1 mice (4–6 weeks old) were killed by cervical dislocation. Femora were removed and dissected free of adherent soft tissue. The bone ends were cut and the marrow flushed out with a 21-gauge needle. The bone marrow cells were washed twice, resuspended in EMEM, and incubated for 24 h in M-CSF (5 ng/ml) at a density of 3×10^5/ml. After 24 h, non-adherent cells were harvested, washed, and incubated as described below.

Northern blot analysis of SOCS expression. For the assessment of SOCS expression in osteoclasts, bone marrow precursors (3×10^4/ml) were incubated for 5 days with M-CSF (30 ng/ml) with or without TNF-α (30 ng/ml) and TGF-β_1 (0.1 ng/ml). Total RNA was extracted according to an established method [20], and 35 μg total RNA separated on a 1.2% agarose–formaldehyde gel, transferred to a hybond N membrane (Amersham, Amersham, Bucks, UK), and hybridised with specific [^{32}P]-labelled cDNA probes for murine SOCS1 (724 bp), SOCS2 (679 bp), SOCS3 (760 bp), CIS (740 bp), and β-actin (760 bp) prepared by the random primer method (Amersham). After hybridization the membrane was stringently washed and autoradiographed using Hyperfilm (Amersham).

SOCS3-expressing retroviral vector. SOCS3 was transduced using a retroviral vector, pBabe puro, which expresses cDNA inserts constitutively under the control of a retroviral enhancer-promoter [21]. The coding region of mouse SOCS3 (760 base pairs) was PCR amplified from pEF-FLAG-I/mSOCS-3 [22] with a 5′ primer containing a *Bam*HI site and a Kozak sequence, and a 3′ primer containing a *Sal*I site, and cloned into pBabe puro. The resulting plasmid pBabe-SOCS3 was sequenced (Cambridge Biolabs, Cambridge, UK) to confirm sequence integrity. pBabe-SOCS3 and pBabe-empty (control) vectors were then transfected into the Phoenix retroviral packaging cell line [18] using FuGENE-6 (Roche). After 48 h, stably transfected cells were selected by replacing growth medium with fresh medium containing puromycin (2.5 μg/ml). Stably transfected clones were picked 4–7 days later and grown to confluency in 25 cm^2 flasks. Clones producing high viral titers were identified by infecting NIH-3T3 cells with viral supernatant for 16 h. Supernatants of the retrovirus producing cells showed a similar viral titer.

Infection of M-CSF-dependent precursors with control and SOCS3-expressing retroviruses. Non-adherent M-CSF-dependent bone marrow cells (10^6 cells per ml) were added to 24-well plates or 96-well plates containing bone slices and cultured with M-CSF (10 ng/ml) for 48 h. Medium was then removed and replaced with supernatant of pBabe-SOCS3 or pBabe-empty-virus-producing Phoenix cells, together with 8 μg/ml polybrene and M-CSF (10 ng/ml). The cells were then centrifuged at 93*g* to increase infection efficiency and incubated for 16 h. Viral supernatant was then removed, and replaced with EMEM containing M-CSF (30 ng/ml) and TNF-α (30 ng/ml). After incubation for a further 2 days, stably infected cells were selected by addition of puromycin (2.5 μg/ml) for 3 days. Control cultures to which virus had not been added showed no viable cells. Infection rates for both retroviruses were similar (pBabe-SOCS3 50–70% and pBabe-empty 50–65%). Cultures were assessed for osteoclast formation after 6 days and resorption after 12 days as described below.

SOCS3 antisense studies. The effect of SOCS3 "knockdown" on osteoclast formation was examined using a phosphorothioate antisense oligonucleotide (ODN) (5′-TCA CTC TGC AGC GAA AAG-3′) specifically targeting nucleotides 320–337 of mouse SOCS3 mRNA (Biognostik Gottingen, Germany). Control ODN contain the reverse complement of the target sequence and have no cross homology to other sequences on the GenBank database. Non-adherent M-CSF-dependent mononuclear cells (1×10^5/ml) were incubated in 96-well plates containing M-CSF (30 ng/ml), TNF-α (30 ng/ml) with or without TGF-β_1 (0.1 ng/ml) in the presence of FuGENE-6 (0.15 μl per well) (Roche), antisense SOCS3 ODN (2 μM) or control ODN (2 μM) for 6 days. Cells were fixed and assessed for osteoclast formation.

Assessment of osteoclast formation and bone resorption. Osteoclast formation was evaluated by quantification of tartrate resistant acid phosphatase-positive cells (TRAP-positive). After incubation, cells were washed, fixed in 10% formalin for 10 min, permeabilised in acetone for 10 min, washed, and stained for acid phosphatase in the presence of 0.05 mol/L sodium tartrate, using naphthol AS-BI phosphate as a substrate. Cells were counterstained with hematoxylin. To assess resorption bone slices were immersed in 10% sodium hypochlorite (British Drug Houses, UK) for 10 minutes to remove cells, washed, and dried. After drying slices were mounted on stubs and sputter coated with gold. The entire surface of each bone slice was then examined in a scanning electron microscope (S90; Cambridge instruments, Cambridge, UK) and the area resorbed per bone slice calculated.

Statistical analysis. Differences between groups were assessed using ANOVA (Statview vs. 5.01). $P < 0.05$ was considered significant.

Results

TGF-β strongly up-regulates SOCS3 mRNA expression in TNF-α-induced osteoclasts

To assess the pattern of TNF-α-induced SOCS expression in osteoclasts, we performed Northern blot

analysis on total RNA extracted from bone marrow precursors incubated in the presence of M-CSF with or without TNF-α and TGF-β. As noted previously, SOCS3 was not detected in precursors treated with M-CSF alone (Fig. 1A) [17]. However, TNF-α-induced osteoclasts expressed low levels of SOCS3 and this was strongly up-regulated in osteoclasts formed in the presence of TGF-β (Fig. 1A). The ability of TGF-β to strongly up-regulate SOCS3 expression in TNF-α-induced osteoclast and rapidly induce sustained SOCS3 expression in non-committed precursors [17] raises the possibility that SOCS3 represents a central mechanism by which TGF-β primes precursors for TNF-α as well as RANKL-induced osteoclast formation. The expression

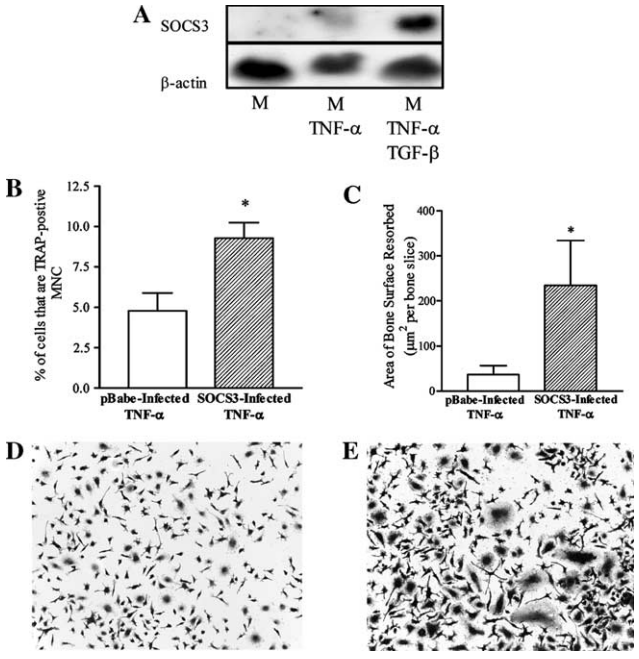

Fig. 1. TGF-β enhances SOCS3 mRNA expression in TNF-α-induced osteoclast and SOCS3 expression augments TNF-α-induced osteoclast formation (A) RNA was prepared from bone marrow precursors incubated for 5 days with M-CSF (30 ng/ml) and combinations of TNF-α (30 ng/ml) and TGF-β_1 (0.1 ng/ml). Total RNA was separated on an agarose–formaldehyde gel, transferred to a hybond N membrane, and hybridised with specific [^{32}P]-labelled cDNA probes for murine SOCS3, or β-actin. After hybridization the membrane was stringently washed and autoradiographed. (B) Non-adherent M-CSF-dependent bone marrow cells were incubated with M-CSF (10 ng/ml) for 2 days, infected with pBabe-SOCS3 or pBabe-empty (control) retroviruses, and incubated with M-CSF (30 ng/ml) and TNF-α (30 ng/ml) for 6 days. Infected cells were selected with puromycin (2.5 μg/ml) between days 2 and 5. Cells were fixed and total cell number and the number of TRAP-positive MNC counted. Values are means ± SEM of three separate experiments $n = 24$. A, $^*P < 0.05$ versus pBabe TNF-α. (C) Non-adherent M-CSF-dependent bone marrow cells were incubated on bone slices, infected, and then incubated as above for 12 days. The extent of bone resorption was then assessed by scanning electron microscopy. Values are means ± SEM of 2 separate experiments $n = 66$. A, $^*P < 0.05$ versus pBabe TNF-α. (D) Photomicrograph of pBabe-infected TNF-α-treated culture; (E) Photomicrograph of SOCS3-infected TNF-α-treated culture.

of SOCS1, SOCS2 or CIS mRNA was not detected in osteoclasts incubated with or without TGF-β (data not shown).

SOCS3 expression augments TNF-α-induced osteoclast formation

Non-adherent M-CSF-dependent mononuclear phagocyte precursors infected with pBabe-empty virus formed strongly TRAP-positive multinuclear cells (MNCs) in the presence of TNF-α within 6 days (Fig. 1B). In pBabe-SOCS3-infected cultures, there was a significant increase in the number of TRAP-positive MNCs that formed in the presence of TNF-α compared to cells infected with pBabe-empty (Fig. 1B). This was not attributable to differences in infection rates or total cell numbers, as cell numbers in SOCS3 and pBabe-empty-infected cultures did not differ significantly (data not shown). Furthermore, in keeping with the increase in osteoclast number and similar to our previous studies [17], SOCS3-infected precursors also resorbed a greater total area of bone surface compared to control cultures (Fig. 1C). This raises the possibility that SOCS3 expression forms part of the mechanism by which TGF-β enhances osteoclast formation by diverse osteoclast-inductive stimuli, preventing inhibitory JAK/STAT signals and thereby priming precursors to the osteoclastic rather than alternative inflammatory lineages.

However, retroviral driven SOCS3 expression elevates levels of SOCS3 beyond those induced by TGF-β. Therefore, to further test the role of SOCS3 in osteoclast formation we examined the effect of "knocking down" SOCS3 using a specific antisense ODN. The number of TRAP-positive cells induced by TNF-α was significantly reduced in anti-SOCS3 ODN-treated cultures compared with controls (Fig. 2), suggesting that TNF-α-induced osteoclast formation is largely dependent on SOCS3 induction by either TNF-α or by TGF-β present in the serum or produced by the precursors themselves. The role of SOCS3 in osteoclast/macrophage lineage switching is further shown by the ability of anti-SOCS3 ODN to blunt the enhancing effect of exogenously administered TGF-β on TNF-α-induced osteoclast formation (Fig. 2).

TGF-β prevents the inhibitory effect of IFN-β and IL-10 on TNF-α-induced osteoclast formation

The culture environment potentially contains numerous inflammatory stimuli that may act to prime mononuclear phagocyte precursors to lineages other than that of the osteoclast. Recently, IFN-β has been suggested to have an important role in limiting osteoclast differentiation, acting as an autocrine negative feedback regulator of osteoclast formation [23]. We found that similar to its suppressive effect on

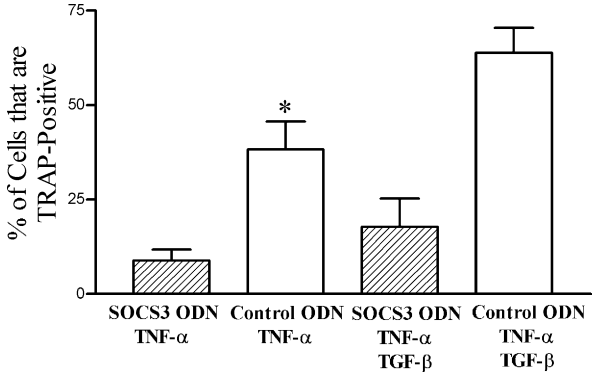

Fig. 2. Antisense SOCS3 oligonucleotides suppress osteoclast formation and blunt the enhancing effect of TGF-β. Non-adherent M-CSF-dependent bone marrow precursors were incubated for 6 days with combinations of M-CSF (30 ng/ml), TNF-α (30 ng/ml), TGF-β_1 (0.1 ng/ml), and either antisense SOCS3 ODN (2 μM) or control ODN (2 μM) with Fugene6. Cells were fixed and the number of TRAP-positive cells quantified. Values are expressed as means ± SEM of two separate experiments $n = 12$. There was no significant difference between antisense SOCS3 ODN cultures incubated with or without TGF-β. *Significantly different versus all other groups $p < 0.05$.

RANKL-induced osteoclast differentiation, IFN-β also inhibits TNF-α-induced osteoclast formation in vitro, and that TGF-β reverses this (Fig. 3). In addition to IFN-β other cytokines, including IL-10, a T-cell-derived cytokine synthesis inhibitory factor, have been shown to suppress osteoclast differentiation in haemopoietic co-culture systems [24]. However, due to the presence of numerous cell types in these systems it is unclear whether IL-10 acts directly on osteoclast precursors or indirectly via another cell type. Therefore, to investigate the cellular mechanism through which IL-10 acts we compared its ability to inhibit TNF-α-induced osteoclast formation in stromal-depleted mononuclear phagocyte

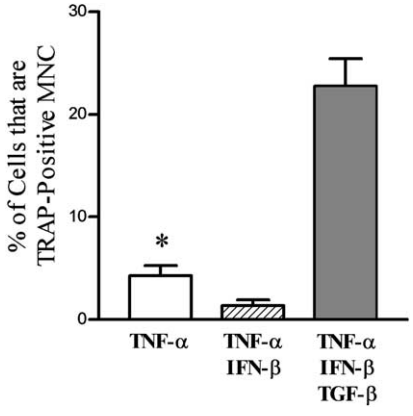

Fig. 3. TGF-β opposes the inhibitory effect of IFN-β on TNF-α-induced osteoclast formation. Non-adherent M-CSF-dependent bone marrow precursors were incubated for 6 days with M-CSF (30 ng/ml), TNF-α (30 ng/ml) with or without IFN-β (0.5 U/ml) and/or TGF-β_1 (1 ng/ml). Cells were fixed and the number of TRAP-positive MNC quantified. Values are expressed as means ± SEM of two separate experiments. $n = 16$. *$p < 0.05$ versus all other groups.

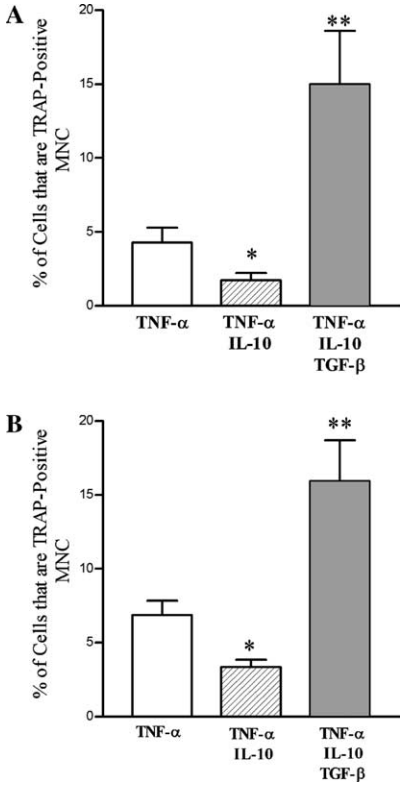

Fig. 4. TGF-β opposes the direct anti-osteoclastic effect of IL-10. Non-adherent M-CSF-dependent bone marrow precursors (A) or RAW 264.7 cells (B) were incubated for 6 days with combinations of M-CSF (30 ng/ml), TNF-α (30 ng/ml), IL-10 (100 ng/ml), and TGF-β (1 ng/ml). Cells were fixed and the number of TRAP-positive MNC counted. Values are expressed as means ± SEM of two separate experiments $n = 12$. *$p < 0.05$ versus TNF-α **$p < 0.05$ versus all other groups.

precursors and the murine monocytic cell line RAW 264.7. We found that IL-10 inhibited the formation of TNF-α-induced TRAP-positive osteoclasts from mononuclear phagocyte precursors and in cultures of RAW 264.7 cells to a similar extent (Figs. 4A and B), suggesting that IL-10 directly inhibits precursor differentiation. This effect was prevented by the co-administration of TGF-β in both cell types, which in keeping with its ability to augment osteoclast differentiation significantly increased the number of TRAP-positive cells compared with precursors incubated with TNF-α alone (Figs. 4A and B).

SOCS3 expression blunts the anti-osteoclastic effect of IFN-β but not IL-10

The ability of SOCS3 to suppress a wide range of cytokines that signal via separate JAK/STAT pathways raises the possibility that SOCS3 expression may represent a common mechanism by which TGF-β suppresses inflammatory signals. Therefore, we examined the effect of SOCS3 overexpression on the inhibitory action of IFN-β and IL-10. Binding of IFN-β to its receptor leads to the activation of STAT 1 and STAT 2

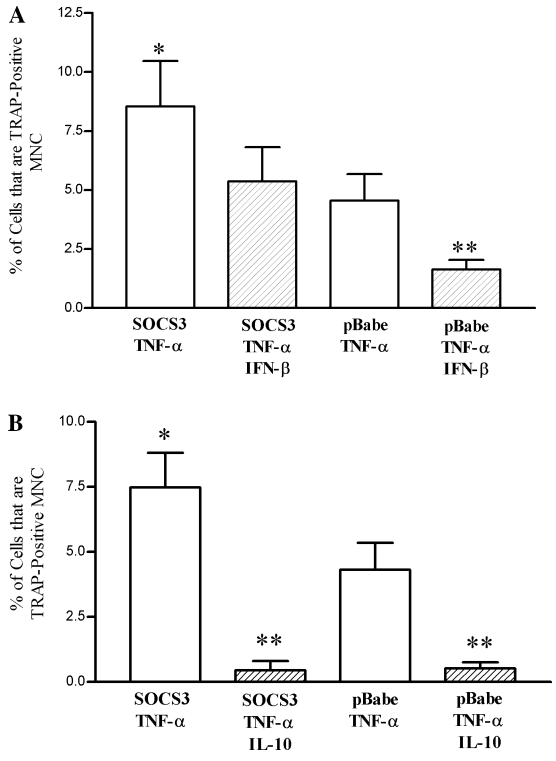

Fig. 5. SOCS3 expression opposes the anti-osteoclastic effect of IFN-β but not IL-10 Non-adherent M-CSF-dependent bone marrow cells were incubated in M-CSF (10 ng/ml) for 2 days. Cells were then infected with SOCS3 or pBabe-empty retroviruses, and incubated with M-CSF (30 ng/ml), TNF-α (30 ng/ml) with or without IFN-β (0.5 U/ml) or IL-10 (100 ng/ml) for 6 days. Cells were fixed and total cell number and the number of TRAP-positive cells counted. Values are means ± SEM of two separate experiments $n = 16$. *$p < 0.05$ versus pBabe TNF-α, **$p < 0.05$ versus SOCS3 TNF-α or pBabe TNF-α. There was no significant difference in the number of TRAP-positive cells formed in SOCS3-infected cells incubated with or without IFN-β.

that form part of the IFN-stimulated gene factor-3 signalling complex [25]. In contrast, binding of IL-10 to its receptor activates JAK1 and Tyk 2, which in turn phosphorylate STAT3 (see [26]).

We found that SOCS3 expression markedly blunted the anti-osteoclastic effect of IFN-β, there being no significant difference in the number of TRAP-positive cells induced by TNF-α in SOCS3-infected cultures incubated with or without IFN-β (Fig. 5A). This suggests that one mechanism by which TGF-β may augment osteoclast formation is via suppression of the anti-osteoclastic action of IFN-β. However, similar to recent studies showing that SOCS3 expression in macrophages prevents IL-6 but not IL-10-induced STAT3 activation [27], SOCS3 overexpression was unable to prevent the inhibitory effect of IL-10 on osteoclast differentiation (Fig. 5B). The ability of SOCS3 to blunt IFN-β but not IL-10 signalling may be related to differences in affinity of SOCS3 for IFN-β and IL-10 receptors. Interestingly, SOCS3 has been shown to have a low affinity for phosphorylated tyrosine motifs of the IL-10 receptor in macrophages [27], suggesting that SOCS3 would be unable to suppress STAT phosphorylation by this receptor.

The ability of TGF-β but not SOCS3 to blunt the anti-osteoclastic action of IL-10 indicates that TGF-β must activate additional mechanisms that account for its suppressive effect on IL-10. These mechanisms could also further contribute to its facilitative action on osteoclast lineage commitment. At present, the nature of these additional pathways is unknown but could involve the induction of different inhibitors of JAK/STAT activation or novel SOCS. On the other hand, TGF-β may suppress the anti-osteoclastic action of IL-10 downstream of STAT3 activation. The nature of the molecular mechanism by which IL-10 suppresses osteoclast formation is unknown, but interestingly IL-10 and TGF-β have been shown to have opposing actions on AP-1 activation [28,29], which is essential for osteoclast formation.

Whatever the nature and relevance of these additional pathways, the above data suggest that SOCS3 expression in part accounts for the augmentative effect of TGF-β on TNF-α as well as RANKL-induced osteoclast formation, priming precursors to the osteoclast lineage by suppressing specific anti-osteoclastic JAK/STAT signals. Greater understanding of the pathways by which TGF-β facilitates osteoclast formation could lead to the development of novel strategies to combat the deregulated resorption that occurs in diseases such as osteoporosis and inflammatory osteolysis.

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References

[1] H. Yasuda, N. Shima, N. Nakagawa, K. Yamaguchi, M. Kinosaki, S.-I. Mochizuki, A. Tomoyasu, K. Yano, M. Goto, A. Murakami, E. Tsuda, T. Morinaga, K. Higashio, N. Udagawa, N. Takahashi, T. Suda, Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and identical to TRANCE-RANKL, Proc. Natl. Acad. Sci. USA 95 (1998) 3597–3602.

[2] T.J. Chambers, Regulation of the differentiation and function of osteoclasts, J. Pathol. 192 (2000) 4–13.

[3] K. Kobayashi, N. Takahashi, E. Jimi, N. Udagawa, M. Takami, S. Kotake, N. Nakagawa, M. Kinosaki, K. Yamaguchi, N. Shima, H. Yasuda, T. Morinaga, K. Higashio, T.J. Martin, T. Suda, Tumor necrosis factor α stimulates osteoclastic differentiation by a mechanism independent of the ODF/RANKL-RANK interaction, J. Exp. Med. 191 (2000) 275–285.

[4] C. Roggia, Y. Gao, S. Cenci, M.N. Weitzmann, G. Toraldo, G. Isaia, R. Pacifici, Up-regulation of TNF-producing T cells in the bone marrow: a key mechanism by which estrogen deficiency induces bone loss in vivo, Proc. Natl. Acad. Sci. USA 98 (2001) 13960–13965.

[5] S.W. Fox, K. Fuller, K.E. Bayley, J.M. Lean, T.J. Chambers, TGF-β_1 and IFN-γ direct macrophage activation by TNF-α to osteoclastic or cytocidal phenotype, J. Immunol. 165 (2000) 4957–4963.
[6] K. Fuller, J.M. Lean, K.E. Bayley, M.R. Wani, T.J. Chambers, A role for TGF-β_1 in osteoclast differentiation and survival, J. Cell Sci. 113 (2000) 2445–2453.
[7] T. Kaneda, T. Nojima, M. Nakagawa, A. Ogasawara, H. Kaneko, T. Sato, H. Mano, M. Kumegawa, Y. Hakeda, Endogenous production of TGF-beta is essential for osteoclastogenesis induced by a combination of receptor activator of NF-kappa B ligand and macrophage-colony-stimulating factor, J. Immunol. 165 (2000) 4254–4263.
[8] J. Thyberg, Differentiated properties and proliferation of arterial smooth muscle cells in culture, Int. Rev. Cytol. 169 (1996) 183–265.
[9] C. Schindler, J.E. Darnell, Transcriptional responses to polypeptide ligands: the Jak-Stat pathway, Ann. Rev. Biochem. 64 (1995) 621–651.
[10] R. Starr, T.A. Willson, E.M. Viney, L.J. Murray, J.R. Rayner, B.J. Jenkins, T.J. Gonda, W.S. Alexander, D. Metcalf, N.A. Nicola, D.J. Hilton, A family of cytokine-inducible inhibitors of signalling, Nature 387 (1997) 917–921.
[11] T. Naka, M. Fujimoto, T. Kishimoto, Negative regulation of cytokine signaling: STAT-induced STAT inhibitor, TIBS 24 (1999) 394–398.
[12] T. Naka, M. Narazaki, M. Hirata, T. Matsumoto, S. Minamoto, a. Aono, N. Nishimoto, T. Kajita, T. Taga, K. Yoshizaki, S. Akira, T. Kishimoto, Structure and function of a new STAT-induced STAT inhibitor, Nature 387 (1997) 924–929.
[13] D.J. Hilton, R.T. Richardson, W.S. Alexander, E.M. Viney, T.A. Willson, N.S. Spriggs, R. Starr, S.E. Nicholson, D. Metcalf, N.A. Nicola, Twenty proteins containing a C-terminal SOCS box form five structural classes, Proc. Natl. Acad. Sci. USA 95 (1998) 114–119.
[14] M.M. Song, K. Shuai, The suppressor of cytokine signaling (SOCS) 1 and SOCS3 but not SOCS2 proteins inhibit interferon-mediated antiviral and antiproliferative activities, J. Biol. Chem. 273 (1998) 35056–35062.
[15] A. Crespo, M.B. Filla, W.J. Murphy, Low responsiveness to IFN-gamma, after pretreatment of mouse macrophages with lipopolysaccarides, develops via diverse regulatory pathways, Eur. J. Immunol. 32 (2002) 710–719.
[16] S. Ito, P. Ansari, M. Sakatsume, H. Dickensheets, N. Vazquez, R.P. Donnelly, A.C. Larner, D.S. Finbloom, Interleukin-10 inhibits expression of both interferon alpha- and interferon gamma-induced genes by suppressing tyrosine phosphorylation of STAT1, Blood 93 (1999) 1456–1463.
[17] S.W. Fox, S.J. Haque, A.C. Lovibond, T.J. Chambers, The Possible role of TGF-beta-induced suppressors of cytokine signaling expression in osteoclast/macrophage lineage commitment in vitro, J. Immunol. 170 (2003) 3679–3687.
[18] T.M. Kinsella, G.P. Nolan, Episomal vectors rapidly and stably produce high-titer recombinant retrovirus, Hum Gene Ther. 7 (1996) 1405–1413.
[19] T.J. Chambers, P.M. McSheehy, B.M. Thomson, K. Fuller, The effect of calcium-regulating hormones and prostaglandins on bone resorption by osteoclasts disaggregated from neonatal rabbit bones, Endocrinology 116 (1985) 234–239.
[20] P. Chomczynski, N. Sacchi, Single-step method of RNA isolation by acid guanidinium–thiocynate–phenol–chloroform extraction, Anal. Biochem. 162 (1987) 156–159.
[21] J.P. Morgenstern, H. Land, Advanced mammalian gene transfer: high titre retroviral vectors with multiple drug selection markers and a complementary helper-free packaging cell line, Nucleic Acids Res. 18 (1990) 3587–3596.
[22] S.J. Haque, P.C. Harbor, B.R. Williams, Identification of critical residues required for suppressor of cytokine signaling-specific regulation of interleukin-4 signaling, J. Biol Chem. 275 (2000) 26500–26506.
[23] H. Takayanagi, S. Kim, K. Matsuo, H. Suzuki, T. Suzuki, K. Sato, T. Yokochi, H. Oda, K. Nakamura, N. Ida, E.F. Wagner, T. Taniguchi, RANKL maintains bone homeostasis through c-Fos-dependent induction of interferon-beta, Nature 416 (2002) 744–749.
[24] J.M. Owens, A.C. Gallagher, T.J. Chambers, IL-10 modulates formation of osteoclasts in murine hemopoietic cultures, J. Immunol. 157 (1996) 936–940.
[25] G.R. Stark, I.M. Kerr, B.R. Williams, R.H. Silverman, R.D. Schreiber, How cells respond to interferons, Ann. Rev. Biochem. 67 (1998) 227–264.
[26] H. Fickenscher, S. Hor, H. Kupers, A. Knappe, S. Wittmann, H. Sticht, The interleukin-10 family of cytokines, Trends Immunol. 23 (2002) 89–96.
[27] C. Niemand, A. Nimmesgern, S. Haan, P. Fischer, F. Schaper, R. Rossaint, P.C. Heinrich, G. Muller-Newen, Activation of STAT3 by IL-6 and IL-10 in primary human macrophages is differentially modulated by suppressor of cytokine signaling 3, J. Immunol. 170 (2003) 3263–3272.
[28] W.H. Dokter, S.B. Koopmans, E. Vellenga, Effects of IL-10 and IL-4 on LPS-induced transcription factors (AP-1, NF-IL6 and NF-kappa B) which are involved in IL-6 regulation, Leukemia 10 (1996) 1308–1316.
[29] Y. Zhang, X.H. Feng, R. Derynck, Smad 3 and Smad4 cooperate with c-jun/c-fos to mediate TGF-β induced transcription, Nature 394 (1998) 909–913.