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TGF-β_1 and IFN-γ Direct Macrophage Activation by TNF-α to Osteoclastic or Cytocidal Phenotype[1]

Simon W. Fox, Karen Fuller, Kathryn E. Bayley, Jenny M. Lean, and Timothy J. Chambers[2]

TNF-related activation-induced cytokine (TRANCE; also called receptor activator of NF-κB ligand (RANKL), osteoclast differentiation factor (ODF), osteoprotegerin ligand (OPGL), and TNFSF11) induces the differentiation of progenitors of the mononuclear phagocyte lineage into osteoclasts in the presence of M-CSF. Surprisingly, in view of its potent ability to induce inflammation and activate macrophage cytocidal function, TNF-α has also been found to induce osteoclast-like cells in vitro under similar conditions. This raises questions concerning both the nature of osteoclasts and the mechanism of lineage choice in mononuclear phagocytes. We found that, as with TRANCE, the macrophage deactivator TGF-β_1 strongly promoted TNF-α-induced osteoclast-like cell formation from immature bone marrow macrophages. This was abolished by IFN-γ. However, TRANCE did not share the ability of TNF-α to activate NO production or heighten respiratory burst potential by macrophages, or induce inflammation on s.c. injection into mice. This suggests that TGF-β_1 promotes osteoclast formation not only by inhibiting cytocidal behavior, but also by actively directing TNF-α activation of precursors toward osteoclasts. The osteoclast appears to be an equivalent, alternative destiny for precursors to that of cytocidal macrophage, and may represent an activated variant of scavenger macrophage. ***The Journal of Immunology,* 2000, 165: 4957–4963.**

The osteoclast is the cell that resorbs bone. Excessive activity by this cell is responsible for the development of postmenopausal osteoporosis and for the destruction of joints and generalized bone loss that accompanies diseases such as rheumatoid arthritis. Although it has been known for some time that the osteoclast derives from the mononuclear phagocyte system and shares some cell surface markers with macrophages (see Ref. 1), it is also distinctly different from any other known mononuclear phagocyte derivative (see Refs. 2 and 3). Osteoclasts lack many of the Ags that are characteristic of macrophages and inflammatory polykaryons, in particular Fc and C3 receptors, and express very high levels of tartrate-resistant acid phosphatase (TRAP)[3] and vitronectin receptor ($\alpha v\beta_3$), and express calcitonin receptors that are absent from macrophages (1–3). Most distinctively, osteoclasts ex vivo excavate bone within hours, but macrophages show no excavation whatsoever, even on extended incubation on bone surfaces (4–6).

It was recently found that osteoclast differentiation is induced in mononuclear phagocyte precursors by TNF-related activation-induced cytokine (TRANCE; also called receptor activator of NF-κB ligand (RANKL), osteoclast differentiation factor (ODF), osteoprotegerin ligand (OPGL), and TNFSF11), which was originally identified as a member of the TNF superfamily that stimulates dendritic cells (7–9). TRANCE is expressed by osteoblastic and bone marrow stromal cells, and soluble recombinant TRANCE, with M-CSF, substitutes for stromal cells in osteoclast formation and activation (10–13). Transgenic experiments have shown that deletion of the gene for TRANCE, or overexpression of OPG, a soluble decoy receptor for TRANCE, is associated with failure of osteoclast formation and osteopetrosis (14–16).

Recently, TNF-α was reported also to be able to induce osteoclastic cells from bone marrow macrophages in vitro (17). This was unexpected because TNF-α plays a major role in host defense. It exerts proinflammatory activities through a range of cell types, including mononuclear phagocytes, in which it is responsible for the activation of bacteriocidal/cytocidal systems (see Refs. 18 and 19). The specialized function of the osteoclast seems quite distinct from that of such macrophages, yet TNF-α can activate both phenotypes from the same precursors.

The observation raises two major questions. First, if TNF-α is capable of activation of macrophages both to cytocidal and osteoclastic phenotypes, does TRANCE share this capacity? The number and diversity of cells and tissues that express TRANCE (20) implies actions beyond those already described in bone and lymphoid biology. TRANCE might be a stromal cell counterpart of the predominantly macrophage-derived TNF-α, as a mediator of host defense. Therefore, we tested the ability of TRANCE to activate macrophages for NO production in vitro and to exert proinflammatory TNF-α-like activity in vivo.

The second question posed by the ability of TNF-α to induce osteoclastic cells in vitro is: Why are these cells not a common feature of inflamed tissues? It seems most likely that additional signals are present in vivo that are important in determining the direction of macrophagic activation. For example, although IFN-γ primes macrophages for cytocidal activation by TNF-α, it has been suggested that TGF-β_1 deactivates macrophages in the subsequent healing phase (21–23; see Refs. 24 and 25 for reviews). We found that TGF-β_1 substantially increased the proportion of precursors that formed osteoclastic cells in culture in response to TNF-α. This suggests a model in which the osteoclast is an alternative and equivalent destiny for precursors to that of the cytocidal macrophage.

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[3] Abbreviations used in this paper: TRAP, tartrate-resistant acid phosphatase; TRANCE, TNF-related activation-induced cytokine; ODF, osteoclast differentiation factor; OPG, osteoprotegerin; OPGL, OPG ligand.

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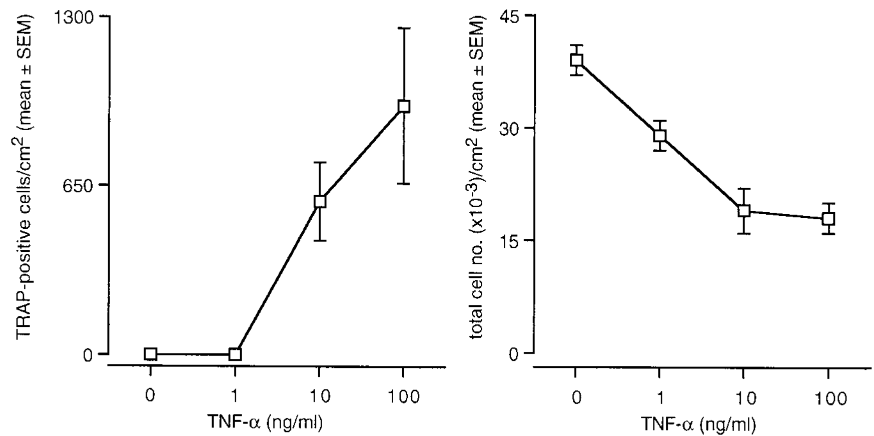

FIGURE 1. Effect of TNF-α on TRAP-positive cell production by bone marrow macrophages. Nonadherent bone marrow cells were incubated in M-CSF for 3 days, followed by 6 days of incubation in M-CSF with serial concentrations of TNF-α. $n = 8$ cultures per variable. The panel on the left shows the number of TRAP-positive cells/cm^2; that on the right shows total number of cells/cm^2 present on the coverslips (total of TRAP-positive and TRAP-negative cells).

Materials and Methods

Media and reagents

Cells were incubated in MEM with Earle's salts supplemented with 10% FBS, 2 mM glutamine, 100 IU/ml benzylpenicillin, and 100 μg/ml streptomycin (all from Imperial Laboratories, Andover, Hants, U.K.). Recombinant human M-CSF was provided by Genetics Institute (Cambridge, MA); soluble recombinant human TRANCE was obtained from Insight Biotechnology (Wembley, Middlesex, U.K.); and purified human TGFβ_1, recombinant murine IFN-γ and TNF-α, and pan-specific TGF-β-neutralizing Ab were purchased from R&D Systems (Abingdon, Oxon, U.K.). All other reagents were obtained from Sigma (Poole, Dorset, U.K.) unless otherwise stated. All incubations were performed at 37°C in a humidified atmosphere of 5% CO_2 in air.

Isolation and culture of bone marrow precursors

Bone marrow cells were isolated from 5- to 8-wk-old MF1 mice as described previously (26). Mice were killed by cervical dislocation. Femora and tibiae were aseptically removed and dissected free of adherent soft tissue. The bone ends were cut, and the marrow cavity was flushed out into a petri dish by slowly injecting medium 199 (Imperial) at one end of the bone using a sterile 21-gauge needle. The bone marrow suspension was carefully agitated with a plastic Pasteur pipette to obtain a single-cell suspension. The bone marrow cells were washed twice, resuspended in MEM containing 10% FBS, and incubated for 24 h in M-CSF (5 ng/ml) at a density of 3×10^5 cells/ml in a 75-cm^2 flask (Helena Biosciences, Sunderland, Tyne & Weir, U.K.). After 24 h, nonadherent cells were harvested, washed, and resuspended (3×10^5/ml) in MEM-FBS. This suspension was added (100 μl/well) to the wells of 96-well plates (Helena Biosciences) containing a 6-mm Thermanox coverslip (Life Technologies, Paisley, U.K.). To each of these wells an additional 100 μl of medium containing M-CSF (60 ng/ml) with/without cytokines was added. Cultures were fed every 2–3 days by replacing 100 μl of culture medium with an equal quantity of fresh medium and reagents. Absence of contaminating stromal cells was confirmed in cultures in which M-CSF was omitted. Such cultures showed no cell growth. Cultures were assessed for TRAP or NO production as described below.

Isolation and culture of resident peritoneal macrophages

MF1 mice (5–8 wk old) were anesthetized and exsanguinated. Three milliliters of Dulbecco's PBS was injected i.p., agitated, and removed. The suspension was centrifuged at $1200 \times g$ at 4°C for 10 min. Cells were resuspended (10^6/ml) in MEM-FBS and plated at 10^5/well in 96-well plates. After incubation for 30 min, cultures were washed and cultured as described for bone marrow cells.

TRAP cytochemistry

Osteoclast formation was evaluated by quantification of TRAP-positive cell number using a modification of the method of Burstone (27). After incubation, coverslips or bone slices were washed in PBS, fixed in 10% Formalin for 10 min, and stained for acid phosphatase in the presence of 0.05 M sodium tartrate (Sigma). The substrate used was naphthol AS-BI phosphate (Sigma). The preparations were then counterstained (hematoxylin), and cells were counted using an eyepiece graticule.

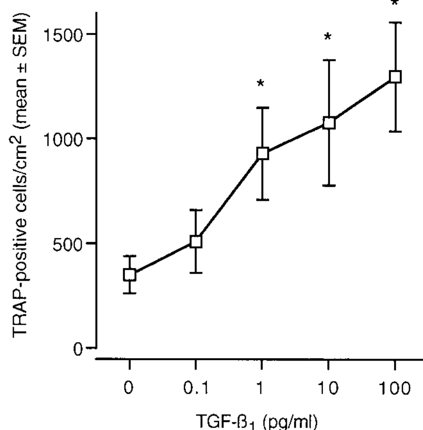

FIGURE 2. Effect of TGF-β_1 on TRAP-positive cell production by bone marrow macrophages. Nonadherent bone marrow cells were incubated in M-CSF for 3 days, followed by 6 days of incubation in M-CSF with TNF-α (50 ng/ml) and serial concentrations of TGF-β_1. $n = 12$ cultures per variable. $p < 0.05$ vs TNF-α alone.

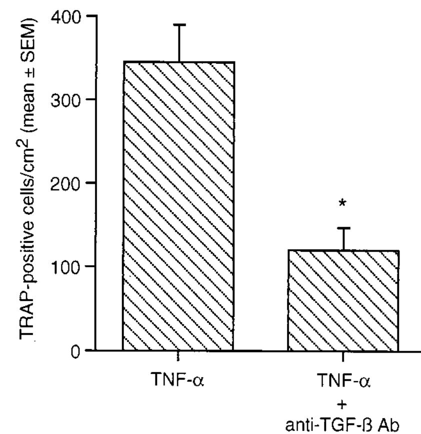

FIGURE 3. Effect of neutralizing Abs to TGF-β on response of bone marrow macrophages to TNF-α. Nonadherent bone marrow cells were incubated in M-CSF for 3 days, followed by 6 days of incubation with M-CSF along with TNF-α (10 ng/ml) alone or with anti-TGF-β Abs (20 μg/ml). $n = 18$ cultures per variable. Total number of cells/cm^2: TNF-α, 41,000 ± 4, 800; TNF-α + anti-TGF-β, 39,000 ± 2, 900. *, $p < 0.05$ vs TNF-α.

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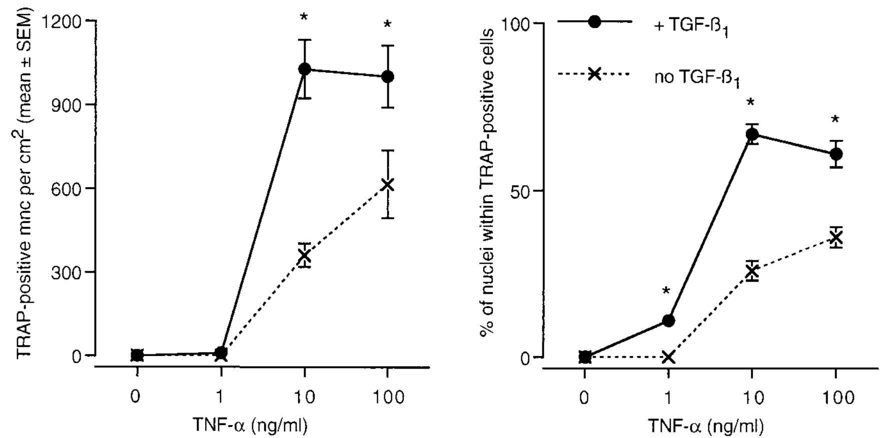

FIGURE 4. Effect of TNF-α and TGF-β_1 on TRAP-positive cell production in cultures of nonadherent M-CSF-dependent bone marrow cells. Bone marrow cells were incubated for 24 h in M-CSF, and nonadherent cells were transferred to new culture vessels and incubated for 6 days with M-CSF in combination with TNF-α with (●) or without (x) TGF-β_1 (0.1 ng/ml). TRAP-positive multinuclear cell = TRAP-positive cell with >2 nuclei; TRAP-positive cells include mono- and multinuclear cells that are TRAP positive. $n = 12$ cultures per variable. *, $p < 0.05$ vs control (no TGF-β_1).

Quantification of NO_2^- release

The accumulation of NO_2^- in the culture supernatants of bone marrow cells was quantified in 96-well plates using the Greiss reagent (Promega, Madison, WI) as described by Ding et al. (28). Fifty-microliter aliquots of the culture supernatants were dispensed into 96-well plates and mixed with 50 μl of 1% (w/v) sulfanilamide, incubated for 10 min at room temperature, before adding 50 μl of 0.1% (w/v) naphthyl-ethylenediamine hydrochloride and 2.5% (v/v) concentrated H_3PO_4. A standard curve consisting of 0.1–5.0 nmol of Na NO_2/100 μl was prepared in culture medium. After incubation at ambient room temperature for 10 min, absorbance was quantified at 550 nM in a micro-ELISA reader (Titertek Multiscan Plus; Life Sciences, Basingstoke, Hampshire, U.K.). Concentrations of NO_2^- were interpolated from the $NaNO_2$ standard curve.

Quantification of respiratory burst

The respiratory burst of adherent bone marrow-derived cells was assessed by the stimulus-induced reduction of cytochrome *c*. Bone marrow cells were isolated and incubated as above for 3 days in 24-well plates (Helena Biosciences) (6×10^5 cells/ml) in M-CSF (30 ng/ml) with or without TNF-α (100 ng/ml) or TRANCE (100 ng/ml). Culture medium was then removed and replaced with 1 ml of Krebs-Ringer phosphate buffer (121 mM NaCl, 5 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 3.1 mM NaH_2PO_4, 12.5 mM Na_2HPO_4, and 11 mM dextrose, pH 7.3) containing 80 μM ferricytochrome *c* and 10^{-6} M PMA. The cells were incubated for 90 min at 37°C, the supernatants were collected, and the absorbance was read at 550 nm. The amount of cytochrome *c* reduced was calculated by using a differential molar extinction coefficient of 2.1×10^4 M^{-1} cm^{-1} (29), and results were expressed as nanomoles of O_2^- produced per 10^6 cells. Cultures were fixed in Formalin after incubation, and cells were counted using an eyepiece graticule. Cultures were set up in triplicate.

Mouse footpad injections

Assessment of the ability of TNF-α and TRANCE to induce inflammation was performed as described by Sharpe et al. (30). Then, 0.05 ml of PBS containing 0.1% BSA and either 50–100 ng of TNF-α, or TNF-α heat treated (90°C for 1 h), or TRANCE (50–500 ng) was injected s.c. into the left hind footpad of 5- to 8-wk-old MF1 mice. An identical amount of diluent, not containing the test substance, was injected into the right hind footpad. At time intervals (6–48 h), animals were killed and footpad tissue was prepared for light microscopy. For this, tissue was fixed in 10% buffered Formalin for 24 h and routinely processed. Paraffin-embedded tissue sections were stained with hematoxylin and eosin. The sections were examined "blind" for inflammatory cells.

Statistical analysis

Differences between groups were analyzed with the unpaired Student's *t* test using StatView (Abacus Concepts, Berkeley, CA). $p < 0.05$ was considered to be significant.

Results

Similar to previous findings (17), when TNF-α was added to M-CSF-dependent precursors that had been incubated in M-CSF for 3 days (bone marrow macrophages), there was a dose-dependent production of strongly TRAP-positive cells (Fig. 1). The proportion of bone marrow macrophages that became TRAP-positive was quite small (see Fig. 1). As previously noted (17), the majority of these TRAP-positive cells were mononuclear, but 3–5% were multinucleated (contained three or more nuclei).

TGF-β_1 caused a dose-dependent increase in the number of such TRAP-positive cells in these cultures (Fig. 2). No TRAP-positive cells were observed in control cultures incubated with either M-CSF alone or with M-CSF and TGF-β_1 (0.1 ng/ml) without TNF-α.

Neutralizing Abs to TGF-β substantially reduced the number of TRAP-positive cells formed in cultures of bone marrow macrophages incubated with TNF-α, whereas overall cell numbers were unaffected (Fig. 3 and legend). This suggests that even those TRAP-positive cells formed by TNF-α alone might be dependent on endogenous TGF-β in these cultures. Without TNF-α, TGF β_1 did not induce TRAP-positive cells (data not shown).

We noted that the proportion of TRAP-positive cells in cultures prepared as above, in which macrophages were first allowed to

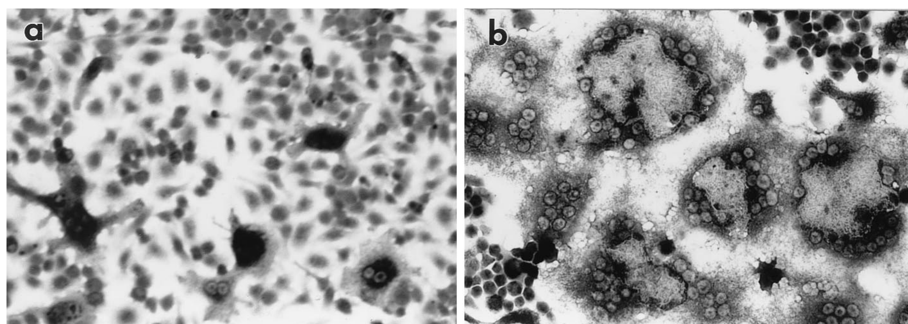

FIGURE 5. Photomicrograph of cultures of nonadherent bone marrow cells incubated in M-CSF plus TNF-α (10 ng/ml; *a*) or TNF-α + TGF-β_1 (0.1 ng/ml; *b*). Note many and better-spread multinuclear cells in culture incubated with TNF-α with TGFβ_1 vs TNF-α alone.

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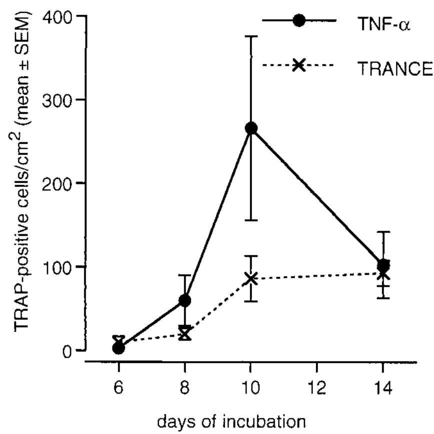

FIGURE 6. Effect of TNF-α (●) and TRANCE (✕) (both 30 ng/ml) on TRAP-positive cell production in cultures of resident peritoneal macrophages. $n = 18$ cultures per variable.

develop for several days in M-CSF without TNF-α, was low (see Fig. 1). We have previously found that prolonged incubation in M-CSF before addition of TRANCE similarly reduced osteoclastic differentiation, compared with cultures to which TRANCE was added at the beginning of the culture (26). It might be that differentiation into macrophages is associated with resistance to osteoclast-induction. Therefore, we tested the ability of TNF-α and TGF-β_1 to produce osteoclasts when added to bone marrow cells immediately after these had been incubated for 24 h in M-CSF to remove stromal cells. Under these circumstances, TNF-α induced many more TRAP-positive cells comparable to cultures similarly incubated early in TRANCE (see Ref. 26); TGF-β_1 increased TRAP-positive cell formation. Not only did TGF-β_1 increase the number of TRAP-positive multinuclear cells formed and increase the proportion of nuclei that were within strongly TRAP-positive cells (Figs. 4 and 5), but TRAP-positive cells were induced at a lower concentration of TNF-α (1 ng/ml) than we had previously observed (Fig. 1). This suggests that TGF-β_1 sensitized bone marrow precursors to osteoclastic differentiation by TNF-α. At least 90% of the TRAP-positive multinuclear cells in these cultures, and some TRAP-positive mononuclear cells, also expressed calcitonin receptors, another marker for osteoclasts, by autoradiography (data not shown; see Refs. 17 and 26). Thus, a major phenotype of immature mononuclear phagocytes incubated in TNF-α with TGF-β_1 is osteoclastic.

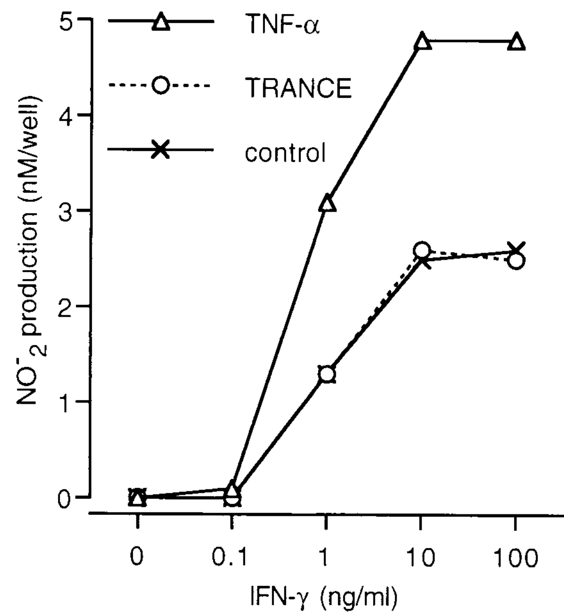

FIGURE 8. Dose responsiveness of effect of IFN-γ on NO_2^- release by resident peritoneal macrophages incubated for 3 days in M-CSF with TNF-α (10 ng/ml; △), TRANCE (100 ng/ml; ○), or M-CSF alone (✕). This experiment was repeated twice with very similar results.

To determine whether this propensity for osteoclastic differentiation was unique to bone marrow mononuclear phagocytes, we tested the ability of TNF-α to induce osteoclasts from resident peritoneal macrophages. Both TRANCE and TNF-α induced production of strongly TRAP-positive cells (Fig. 6), of which ~3–5% were multinuclear. OPG suppressed TRANCE-induced TRAP-positive cell formation completely, but did not suppress TNF-α-induced TRAP-positive cell production (there were 11 ± 4, 304 ±

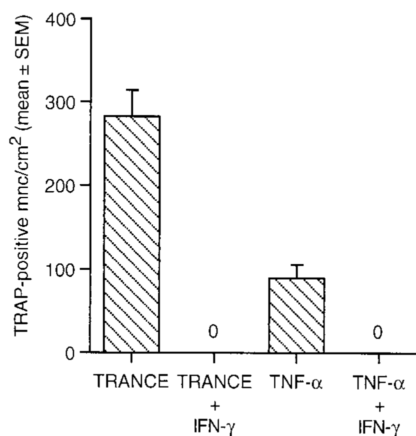

FIGURE 7. Effect of IFN-γ on TRAP-positive multinuclear cell (mnc) formation from nonadherent bone marrow cells incubated for 24 h in M-CSF before continued incubation for 6 days in M-CSF along with/without IFN-γ (0.1 ng/ml), TNF-α (10 ng/ml), or TRANCE (10 ng/ml). $n = 6$ cultures per variable. No multinucleated TRAP-positive cells were observed in any cultures with IFN-γ. No TRAP-positive cells were observed in IFN-γ alone (data not shown).

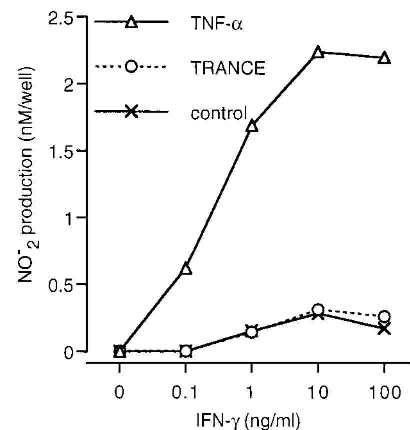

FIGURE 9. Dose responsiveness of effect of IFN-γ on NO_2^- release by bone marrow macrophages. Nonadherent bone marrow cells were incubated in M-CSF for 4 days, followed by 3 days in serial dilutions of IFN-γ along with TNF-α (10 ng/ml) and M-CSF (△), TRANCE (100 ng/ml) and M-CSF (○) or M-CSF alone (✕). This experiment was performed three times after each of 3-, 5-, and 10-day preincubations with M-CSF, with very similar results.

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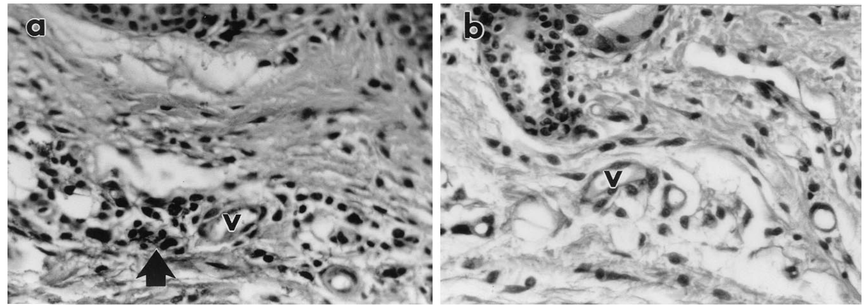

FIGURE 10. Photomicrograph of mouse footpad 24 h after injection of TNF-α (100 ng; *a*) or TRANCE (100 ng; *b*). Perivascular leukocytes are observed (arrow) around small vessels (v) after TNF-α but not after TRANCE.

172, and 210 ± 180 TRAP-positive cells/cm^2 in cultures incubated with TNF-α and OPG (100 ng/ml) for 8, 10, and 14 days, respectively).

IFN-γ primes macrophages for activation of cytocidal capacity by TNF-α. IFN-γ is also known to suppress osteoclast formation in bone marrow cultures (31). This latter action might occur through effects of IFN-γ on osteoclast precursors, or stromal cells, or both. To test whether IFN-γ has a direct inhibitory effect on osteoclast-induction in uncommitted macrophage-osteoclast precursors, we tested the effect of IFN-γ on osteoclast formation by TRANCE and TNF-α in cultures of bone marrow cells depleted of stromal cells by incubation for 24 h in M-CSF. IFN-γ strongly suppressed osteoclast induction by either agent (Fig. 7).

Because TNF-α, a proinflammatory cytokine, like TRANCE induced osteoclasts, and because TGF-β_1, a macrophage deactivator, augmented osteoclast formation by both agents, it seemed possible that TRANCE might also be proinflammatory. Therefore, we tested the ability of TRANCE to induce NO production in bone marrow and peritoneal macrophages in the presence/absence of IFN-γ. TNF-α strongly activated NO production in both cell populations, but TRANCE showed no effect whatsoever on either population (Figs. 8 and 9). TRANCE was similarly unable to significantly increase the ability of bone marrow cells to generate a respiratory burst (nmol O_2^- per 10^6 cells; mean ± SD: M-CSF alone: 26.8 ± 3.8; with TNF-α (100 ng/ml): 188 ± 18.5; with TRANCE (100 ng/ml): 36.6 ± 10.6). Moreover, while inflammation in murine footpads was induced by injection of 50 ng TNF-α, TRANCE, like heat-treated TNF-α, did not increase perivascular leukocytes, even at the highest dose used (500 ng) compared with controls (Fig. 10).

Discussion

TNF-α is a proinflammatory cytokine, capable of activating many of the components of host defense, including the induction in macrophages of cytocidal activity (18 and 19). Surprisingly, it was recently found to induce osteoclast formation from bone marrow macrophages (17): bone-resorbing osteoclasts and bacteriocidal macrophages seem phenotypically and functionally to be very different cell types. If they are, then lineage regulation requires additional signals. We found that TGF-β_1 strongly promotes TRAP-positive cell formation in response to TNF-α, whereas IFN-γ activates NO production while strongly inhibiting TRAP-positive cell formation.

IFN-γ and TGF-β have opposing effects on diverse cellular functions (25, 32–35). Cytokines that signal, like IFN-γ, through Janus kinase-STAT pathways (36–38) are generally antagonistic to TGF-β, which signals through SMADS-2 and -3 (39, 40), in the regulation of hemopoietic and immune-cell function (25, 41). Transmodulation of TGF-β/SMAD signaling by the IFN-γ/STAT pathway might occur through the IFN-γ-induced SMAD-7, an antagonist SMAD (42). The signaling systems might also cooperate for the expression of some genes through interactions with p300 (43).

Whatever the mechanism by which IFN-γ and TGF-β act and interact, these cytokines clearly have a potent ability to modulate osteoclast vs cytocidal macrophage differentiation. This suggests that the osteoclast is an alternative destiny for mononuclear phagocyte precursors activated by TNF-α: while IFN-γ primes for cytocidal activity (28, 44–46), TGF-β_1 promotes osteoclast formation (Fig. 11).

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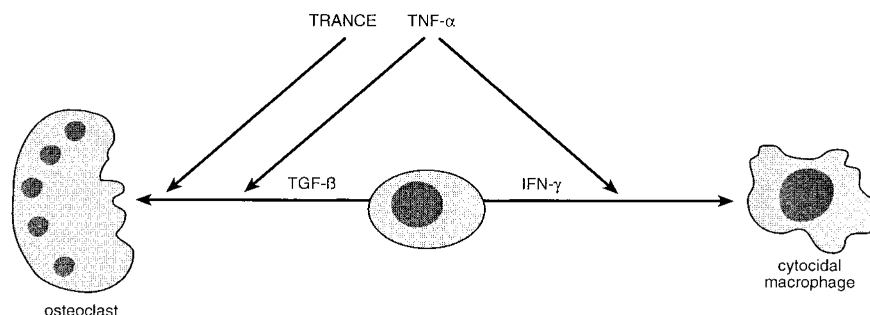

FIGURE 11. Prototype pathways for mononuclear phagocyte lineages. TGF-β and IFN-γ direct responsive, M-CSF-dependent bone marrow precursors toward remodeling or cytocidal activities, respectively. TRANCE might be needed to induce remodeling macrophages to resorb bone because removal of bone requires a special activation level, but it might also assist remodeling macrophages elsewhere (e.g., scavengers of apoptotic cells and redundant matrix). Just as TGF-β_1 deactivates cytocidal macrophages, inflammatory cytokines, such as IFN-γ, GM-CSF, and IL-4, deactivate the osteoclast pathway. In vitro, TRANCE and TNF-α have a similar osteoclast-inductive capacity. This is unlikely to be the case in vivo, where the very presence of TNF-α implies that inflammation is present. In this scheme, the special characteristic of TRANCE is that it activates macrophages but, unlike TNF-α, is not proinflammatory.

It is clear that the osteoclast derives from the mononuclear phagocyte lineage, but it remains uncertain at what level of maturation it diverges. TNF-α induces TRAP-positive cells even from M-CSF-dependent bone marrow cells that have been allowed to differentiate into macrophages for several days in culture (17), but we noted that the proportion of such cells that become TRAP-positive is substantially smaller than that observed if the precursors are incubated in TNF-α throughout. This is similar to our previous experience of osteoclast-induction by TRANCE (26), and consistent with the notion that precursors become increasingly resistant to osteoclast-induction as macrophage differentiation proceeds (26).

The ability of TNF-α to induce osteoclast-like cell formation through a direct effect on precursors might be the mechanism by which inflammation leads to osteolysis in diseases such as rheumatoid arthritis. Against this, OPG, the decoy receptor for TRANCE, was recently reported to abolish bone erosion in adjuvant arthritis (47). This supports the model in which TNF-α stimulates resorption through induction of TRANCE in osteoblastic cells (48). The experiment (47) does not, however, exclude a direct effect of TNF-α on osteoclast precursors, because TRANCE might be crucial for the induction of TNF-α itself in adjuvant arthritis.

Although the ability of TNF-α to induce osteoclast-like cells directly is a plausible explanation for inflammatory osteolysis, TNF-α is a major mediator of inflammation not only in bone, but in all tissues. Moreover, the effect of TNF-α is not limited to bone marrow macrophage precursors; we found that peritoneal macrophages can also be induced to form TRAP-positive cells. If mononuclear phagocytes outside bone are susceptible to osteoclast-induction by TNF-α, why are not osteoclasts a common feature of inflammation? Presumably, TNF-α coexists in inflammatory exudates with other inflammatory cytokines, such as GM-CSF, IL-4, and IFN-γ, which inhibit osteoclast formation (49–52). Because TNF-α is proinflammatory, whenever TNF-α is present, so will be such inflammatory cytokines; and when inflammation is succeeded by debridement and repair, levels of TNF-α will fall. However, whether or not TNF-α induces osteoclast formation in vivo, its ability to generate osteoclast-like cells in vitro has implications for macrophage and osteoclast biology.

First, if induction of osteoclastic cells by TNF-α in vitro reflects the known ability of TNF-α to activate macrophages, the corollary is that TRANCE too might have a wider role as a macrophage activator. The number and diversity of tissues that express TRANCE imply actions beyond those described in lymphoid and bone biology. Moreover, monocytes express RANK (53). What is the effect of extraosseous TRANCE on these cells? One possibility is that, just as TNF-α is capable of activation of mononuclear phagocytes both to cytocidal and osteoclastic phenotypes, TRANCE might share this capacity. However, we found that TRANCE is unable to induce NO production or heighten the potential for respiratory boost activity in macrophages, and is not proinflammatory in vivo. TRANCE might therefore have the special ability to activate macrophages without causing inflammation. Its expression by stromal cells in bone and soft tissues could then provide a mechanism by which macrophages are activated to debride apoptotic cells and remodel tissues, including bone.

Second, the capacity of TGF-β_1 to inhibit cytocidal activity in macrophages has led to its being considered to be primarily a macrophage-deactivator (25). In this model, its ability to facilitate formation of osteoclastic cells by TNF-α would be attributed to suppression of the alternative, cytocidal lineage in precursors. However, TGF-β_1 also promotes osteoclast formation by TRANCE (54, 55). Because TRANCE, unlike TNF-α, does not activate cytocidal functions in mononuclear phagocytes, this suggests that TGF-β_1 promotes formation of osteoclastic cells not only by inhibiting cytocidal behavior, but also by actively directing TNF-α-activation of precursors toward osteoclasts. The corollary is that TGF-β_1 deactivation of cytocidal to inflammatory or debriding macrophages (21–23; see refs. 24 and 25 for reviews) might reflect active induction by TGF-β_1 of the debriding phenotype in macrophages, equivalent to priming by IFN-γ of macrophages for cytocidal activity. From this perspective, the osteoclast could be envisaged as a special or activated version of debriding macrophage, and TGF-β_1 as the cytokine that determines the debriding lineage. Our data suggest that the osteoclast is a mononuclear phagocyte, directed toward a debriding function by TGF-β_1 and activated for this function by TNF-α/TRANCE.

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