Natural killer cells trigger osteoclastogenesis and bone destruction in arthritis

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Osteoclasts are bone-eroding cells that develop from monocytic precursor cells in the presence of receptor activator of NF-κB ligand (RANKL) and macrophage colony-stimulating factor (M-CSF). Osteoclasts are essential for physiological bone remodeling, but localized excessive osteoclast activity is responsible for the periarticular bone destruction that characteristically occurs in patients with rheumatoid arthritis (RA). The origin of osteoclasts at sites of bone erosion in RA is unknown. Natural killer (NK) cells, as well as monocytes, are abundant in the inflamed joints of patients with RA. We show here that such NK cells express both RANKL and M-CSF and are frequently associated with CD14$^+$ monocytes in the RA synovium. Moreover, when synovial NK cells are cocultured with monocytes in vitro, they trigger their differentiation into osteoclasts, a process dependent on RANKL and M-CSF. As in RA, NK cells in the joints of mice with collagen-induced arthritis (CIA) express RANKL. Depletion of NK cells from mice before the induction of CIA reduces the severity of subsequent arthritis and almost completely prevents bone erosion. These results suggest that NK cells may play an important role in the destruction of bone associated with inflammatory arthritis.

macrophage colony-stimulating factor | receptor activator of NF-κB ligand | rheumatoid arthritis

An early advance in the understanding of osteoclastogenesis was the finding that coculture of osteoclast precursors and stromal cells yielded osteoclasts (1). This ultimately led to the discovery of two factors produced by stromal cells that are necessary and sufficient for osteoclast formation, namely macrophage colony-stimulating factor (M-CSF) and receptor activator of NF-κB ligand (RANKL), which act on osteoclast precursor cells to induce their differentiation (2). The importance of these factors is further supported by studies in animal models showing enhanced bone erosion on administration of M-CSF (3) or RANKL (4), and in animals rendered deficient for osteoprotegerin (OPG) (5), a decoy receptor that binds RANKL, preventing its binding to (RANK). Conversely, mutation or gene ablation of M-CSF (6), RANKL (7), or RANK (8) or overexpression of OPG (9) leads to increased bone thickness and decreased capacity to generate osteoclasts. In addition, an antibody to RANKL is effective in the treatment of osteoporosis in humans (10).

Pathological bone resorption occurs in patients with immune-mediated arthritis and is particularly prominent at the interface of inflamed synovial tissue and bone (11). There is evidence that osteoclasts are responsible for such periarticular erosions, but the origin of osteoclasts at this site is incompletely understood (11). It is known, however, that ostecoclasts can be derived from circulating CD14$^+$ monocytes, which are recruited to inflamed joints, and that osteoclastogenesis is likely influenced by the relatively high levels of RANKL and M-CSF and low levels of OPG in the inflammatory mileu (11, 12). Indeed, the recent demonstration that a monoclonal antibody against RANKL inhibits the development and progression of bone erosion in patients with rheumatoid arthritis (RA) confirms an important role for RANKL in this disorder (13).

Natural killer (NK) cells are known to participate in the clearance of virus-infected, aberrant, or transformed cells (14). Moreover, NK cells are poised for a rapid release of cytokines and growth factors that might influence the initiation and development of immune responses mediated by T and B cells (15–17). NK cells can be detected in the inflamed synovial tissue at an early stage of the disease, and they constitute up to 20% of all lymphocytes in the synovial fluid (SF) of patients with established RA (18, 19). Such SF NK cells express CD56 and CD94/NKG2A at bright levels, but essentially lack expression of other common NK cell–associated markers, such as CD16 and killer cell Ig-like receptors (18). Thus, the SF NK cells are phenotypically similar to a minor population of NK cells found in the blood of patients with RA and normal controls. Recent evidence shows that this CD56^{bright} NK cell subset produces high levels of cytokines and might play a role in immunoregulation (15). Moreover, this NK cell subset has an up-regulated expression of several chemokine receptors and adhesion molecules that may participate in its preferential recruitment into the inflamed synovium (20), and enable the cells to engage and subsequently activate monocytes through a variety of receptor–ligand interactions (18, 21, 22). Here we asked whether the interaction between NK cells and monocytes results in the differentiation of monocytes into osteoclasts.

Results

Synovial NK Cells Induce Differentiation of Monocytes Into Osteoclasts In Vitro. We analyzed RA synovial tissue sections for the presence of NK cells and monocytes. Compared with monocytes, NK cell numbers were relatively low, and a fraction of NK cells were juxtaposed with monocytes (Fig. 1*A* and Fig. S1), enabling interactions that might result in reciprocal cellular activation. To assess this possibility, we isolated SF NK cells and CD14$^+$ SF monocytes and cocultured them in vitro at a NK cell:monocyte ratio of 1:10. Cocultures were performed in medium alone or in medium supplemented with IL-15, a cytokine produced in the inflamed synovium (23) that is known to activate NK cells (24). In the presence of IL-15, such cocultures yielded many large, round, adherent cells that appeared only when NK cells and monocytes were placed in direct contact with each other (Fig. 1*B*). As shown in Fig. 1*C*, these large cells are multinucleated and express tartrate-resistant acid phosphatase (TRAP), a marker associated with osteoclasts. Moreover, as shown in Fig. 1*D*, the multinucleated cells express receptors for calcitonin (CTR) and F-actin, markers associated with functional resorbing osteoclasts.

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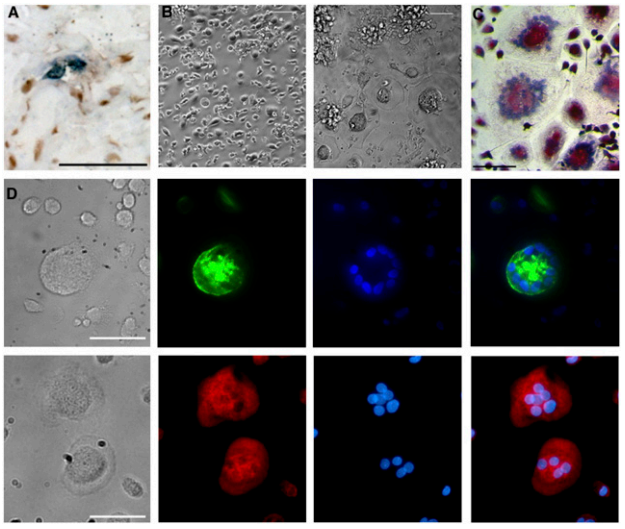

Fig. 1. NK cells are juxtaposed with monocytes in RA synovial tissue and induce their differentiation into osteoclasts in vitro. (*A*) Representative immunohistochemical staining of a patient with RA (n = 3) shows NK cells (blue) and $CD14^+$ monocytes (brown) in inflamed synovial tissue. (Scale bar: 50 μm.) (*B*) Phase-contrast microscopy (original magnification 100×, 10× objective lens) showing $CD14^+$ monocytes cultured for 6 d in direct contact with autologous SF NK cells (*Right*), or cultured in the same well as NK cells but on opposite sides of a transwell membrane (*Left*). Results are from one of three separate experiments. (Scale bar: 50 μm.) (*C*) SF monocytes were cocultured with autologous SF NK cells in the presence of 10 ng/mL of IL-15 for 6 d. Several multinucleated (i.e., hematoxylin; blue nuclei) adherent cells that express TRAP (purple) can be seen. (Original magnification 400×, 40× objective lens.) (Scale bar: 50 μm.) (*D*) Osteoclasts derived from monocytes in coculture with NK cells are multinucleated (i.e., DAPI; blue nuclei), stain brightly for F-actin (green; *Upper*) and express calcitonin receptors (red; *Lower*). Corresponding light microscopic image is shown on the left (original magnification 400×, 40× objective lens), and overlays of respective fluorescent images are shown on the right. (Scale bar: 50 μm.)

We tested whether the osteoclasts formed from monocytes in coculture with NK cells are capable of degrading bone minerals by culturing the cells on calcium phosphate–coated discs. When synovial monocytes were cocultured with SF NK cells in the presence of IL-15, many large eroded areas could be distinguished on these discs; a representative result is shown in Fig. 2*A*. In addition, bone erosion was observed on dentine slices where resorption zones formed in the presence, but not in the absence, of IL-15–activated NK cells (Fig. 2*B*). Taken together, these results provide evidence that human monocytes can differentiate into functional osteoclasts in the presence of SF NK cells.

We next induced the formation of osteoclasts from $CD14^+$ peripheral blood (PB) monocytes with soluble M-CSF and RANKL, a standard method for generating osteoclasts in vitro, and compared the osteoclast-forming capacity with that of cocultures with SF NK cells. Interestingly, SF NK cells appeared more efficient in triggering osteoclast differentiation compared with exogenous M-CSF and RANKL, as indicated by both the greater number and larger size of $TRAP^+$ multinucleated osteoclasts (Fig. S2 *A* and *B*).

RANKL and M-CSF Mediate Osteoclastogenesis Induced by NK Cells. Because M-CSF and RANKL are required and sufficient for osteoclastogenesis (2), we tested whether SF NK cells express these molecules. As shown in Fig. 3*A*, a large fraction of $CD3^-CD56^+$ SF NK cells (mean ± SEM, 54.9% ± 9.6%; n = 3), but not of $CD3^+CD56^-$ SF T cells (2.5% ± 1.8%), express cell-surface RANKL. Notably, a small proportion of the SF NK cells appears to express low levels of M-CSF on the cell surface (Fig. 3*A*), and on prolonged culture of SF NK cells in IL-15, cell surface expression of both M-CSF and RANKL are further up-regulated (Fig. 3*B*).

We next analyzed RANKL expression on NK cells derived from healthy PB. In line with a previous report (25), we found that RANKL is associated mainly with the minor $CD56^{bright}$ subset (Fig. 3*C* and Fig. S3). Moreover, freshly isolated $CD56^{bright}$ NK cells from healthy PB appear to secrete similar amounts of M-CSF as SF NK cells when stimulated with IL-15 (Fig. 3*D*). Low levels of soluble RANKL (100 pg/mL) could be detected only when SF NK cells and $CD56^{bright}$ PB NK cells were stimulated with the highest dose of IL-15 (i.e., 100 ng/mL), suggesting that RANKL is not efficiently shed from the NK cell surface. In line with these findings, we also observed that isolated $CD56^{bright}$ PB NK cells from healthy donors are more potent than the $CD56^{dim}$ PB NK subset in promoting the formation of $TRAP^+$ multinucleated cells from autologous $CD14^+$ PB monocytes (Fig. 3*E*).

To determine whether M-CSF and RANKL mediate NK cell–induced osteoclastogenesis, we evaluated the effect of inhibiting these molecules with soluble M-CSF receptor (sM-CSFR) and OPG, respectively. SF NK cells were cultured in the presence of one or the other of these inhibitors together with autologous $CD14^+$ SF monocytes. As shown in Fig. 4 *A* and *B*, reduced osteoclastogenesis was observed on blocking of M-CSF or RANKL. IFN-γ is known to inhibit osteoclastogenesis (26), but blocking of IFN-γ in the cocultures did not further enhance the capacity of SF NK cells to trigger osteoclastogenesis, a finding that may be explained by the relatively poor capacity of NK cells to produce IFN-γ in response to exogenous IL-15 (Fig. S4) (27).

The finding that a small number of erosion zones form when RA synovial monocytes are cultured in the absence of NK cells (Figs. 2*A* and 4*B*) suggests that osteoclasts can appear spontaneously, an observation in line with previous studies (28, 29).

NK Cells in the Joints of Mice with Collagen-Induced Arthritis Express RANKL, and NK Cell Depletion Reduces Signs of Arthritis and Bone Erosion. To further evaluate the role of NK cells in osteoclastogenesis in arthritis, we studied their effect on bone erosion in collagen-induced arthritis (CIA) in DBA/1 mice, a model of human RA. Consistent with an important role for NK cells as mediators of osteoclast formation, many $RANKL^+$ cells within the inflamed tissue of CIA mice appear to be NK cells, and such NK cells can be seen adjacent to eroded bone surfaces (Fig. 5*A*). To directly study the effect of NK cells in CIA, we used anti-asialo GM1 antibodies to deplete these cells from mice immunized with type II collagen, before the onset of arthritis. Two days after treatment with anti-asialo GM1 antibodies, the frequency of $DX5^+CD3^-$ NK cells in spleen was 0.4 ± 0.2% (mean ± SD) compared with 2.4 ± 0.6% in IgG-treated mice, whereas the frequencies of $DX5^+CD3^+$ and $DX5^-CD3^-$ T cells remained unchanged, indicating that NK cells, but not the T or NK T cell populations, were specifically depleted, confirming previous reports (16, 30). Although the incidence of disease was 100% in all groups, the resultant NK cell–depleted mice showed significantly reduced signs of arthritis (Fig. 5*B*) and greatly reduced bone erosion, synovitis, and pannus formation (Fig. 5 *C* and *D*) compared with animals treated with rabbit Ig or PBS. These results indicate that NK cells not only play a pathogenic role in CIA-associated bone destruction, but also contribute to other manifestations of the disease.

Discussion

The major underlying immunologic events responsible for enhanced bone erosion in RA are still largely unknown. In this study, we have shown that NK cells in the SF of RA patients efficiently trigger formation of osteoclasts from monocytes. Such NK cells express both M-CSF and RANKL, which are responsible for osteoclastogenesis, and both molecules are further up-regulated

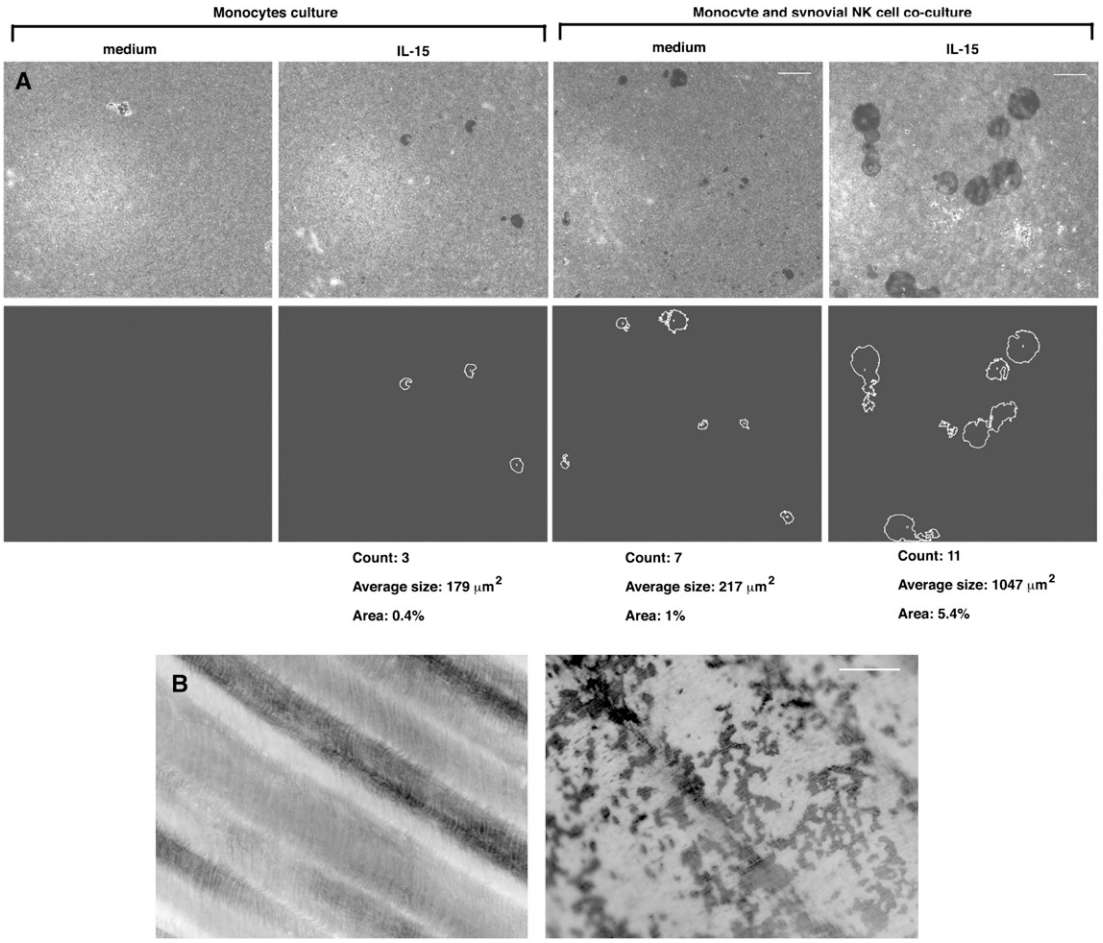

Fig. 2. Osteoclasts that form on coculture between SF NK cells and monocytes erode bone substrates. (*A*) (*Upper*) Phase-contrast micrographs of osteologic discs that have been exposed to (left to right): freshly isolated SF monocytes from an RA patient cultured in medium alone, monocytes cultured in the presence of IL-15, monocytes cocultured with NK cells in medium alone, and monocytes cocultured with NK cells in the presence of IL-15, as indicated. The dark zones represent erosions. (*Lower*) ImageJ micrographs of the upper panel used for analysis. The data in each micrograph denote the number of pits and total erosion area. (*B*) Light micrographs of dentine slices, on which monocytes were cocultured with IL-15 preactivated NK cells (*Right*) or in the absence of NK cells (*Left*). Several erosion zones can be seen at day 7. (Original magnification 100×, 10× objective lens.) (Scale bar: 50 μm.)

on NK cells by IL-15, a cytokine abundantly expressed in the RA joint (23).

Emerging data indicate that IL-15 may play an important pathological role in the initiation of inflammatory arthritis. For example, a recent report has linked elevated levels of IL-15 in the SF of patients with early synovitis and the subsequent development of RA (31), and neutralization of IL-15 in mice with CIA was found to profoundly suppress the development of disease and prevented bone erosion (32). Although IL-15 can be produced by several cell types, its expression in RA synovial tissue tends to be associated with activated endothelial cells in the vicinity of inflammatory infiltrates (33). Interestingly, the $CD56^{bright}$ NK cells bear high-affinity receptors for IL-15 (24) and express a chemokine receptor pattern similar to that of monocytes (20). This subset predominates in the RA joint, as well as in the joints of patients with early synovitis (18), and its accumulation may depend on IL-15 associated with inflamed vascular endothelial cells, because this cytokine helps mediate increased NK cell adhesion, activation, and subsequent migration across endothelium (34). It is noteworthy that NK cells within the RA tissue can be found in close proximity to $CD14^{+}$ monocytes (22) (Fig. 1*A*), supporting the view that NK cell interaction with monocytes in the RA synovium may result in the triggering of osteoclast differentiation. We hypothesize that the enhancing effect of IL-15 on inflammation and bone erosion might be due to its stimulation of M-CSF and RANKL expression by NK cells. IL-15 has been reported to stimulate the differentiation of rat osteoclast progenitors into preosteoclasts in vitro (35). In this study, however, IL-15 alone failed to increase osteoclast formation from isolated monocytes, whereas this formation was dramatically enhanced in the presence of NK cells and IL-15, indicating that the effect of IL-15 is mediated predominantly through NK cells in our system.

Immature dendritic cells (DCs) are capable of transdifferentiation into osteoclasts in vitro on subsequent culture with M-CSF and RANKL, a process that is further enhanced by TNF-α (36). Because SF NK cells can trigger differentiation of monocytes into immature DCs (22), we cannot exclude the possibility that osteoclasts generated from monocytes in our coculture system might be derived from immature DC-like cells undergoing transdifferentiation, particularly because SF NK cells are potent inducers of monocyte TNF-α synthesis (21). Further experiments using specific TNF-α inhibitors are needed to evaluate TNF-α's contribution to NK cell–mediated osteoclastogenesis.

IFN-γ inhibits osteoclastogenesis through a counterbalancing effect on RANKL (26). However, blockade of IFN-γ had only a minor effect on osteoclast formation in our assays, which is consistent with the poor capacity of IL-15 to stimulate IFN-γ

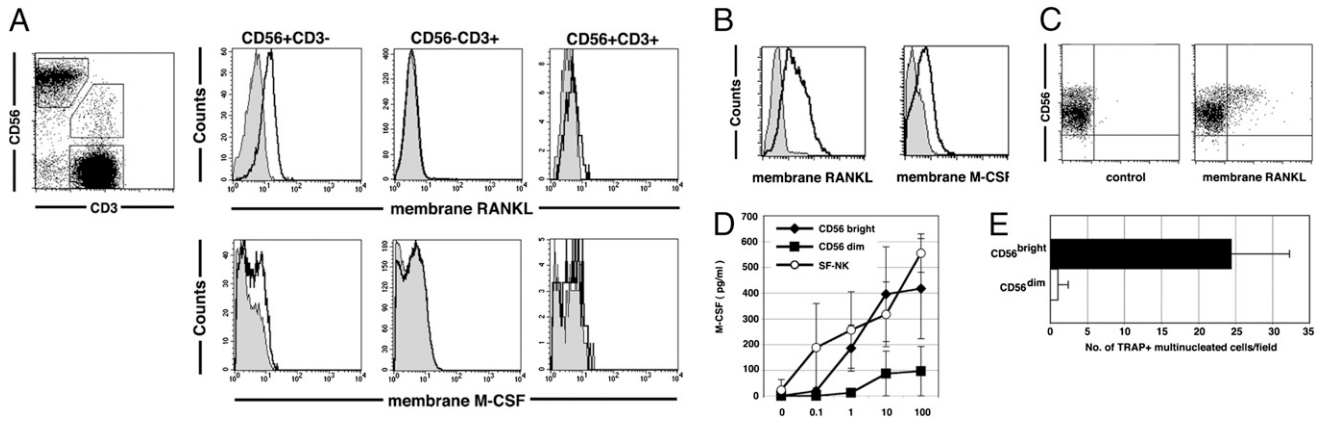

Fig. 3. RANKL and M-CSF expression by NK cells. (*A*) Histograms showing membrane expression of RANKL (*Top*) and M-CSF (*Bottom*) on freshly isolated SF NK cells (gated on $CD56^{+}CD3^{-}$ cells), $CD56^{-}CD3^{+}$ SF T cells (*Middle*), or $CD56^{+}CD3^{+}$ SF T cells (*Right*). The cells were stained with anti-CD56 and anti-CD3 along with either isotype control mAbs or mAb against RANKL or M-CSF. The expression of RANKL and M-CSF (bold lines) are compared with the isotype controls (gray histogram) in each gated subpopulation. Shown are data from a representative RA patient. (*B*) RANKL and M-CSF expression on a SF NK cell line cultured in vitro in the presence of 10 ng/mL of IL-15. (*C*) Membrane RANKL expression on freshly isolated PB NK cells from a healthy individual (*Right*) versus a control (*Left*). A representative staining out of three is shown. (*D*) NK cells were purified from RA SF or healthy PB and cultured in the presence of the indicated concentrations of IL-15. Secreted M-CSF was measured by ELISA after 72 h. (*E*) Sorted $CD56^{bright}$ or $CD56^{dim}$ PB NK cells were cocultured with autologous monocytes in the presence of 10 ng/mL of IL-15. Tthe number of $TRAP^{+}$ multinucleated cells was determined on day 6. The data represent mean ± SEM.

release by NK cells (27). Notably, low levels of IFN-γ are present in the RA synovium (37), and clinical studies have failed to demonstrate that IFN-γ has an effect on bone resorption (38), suggesting that this cytokine plays a minor role in counterbalancing the effect of RANKL in vivo.

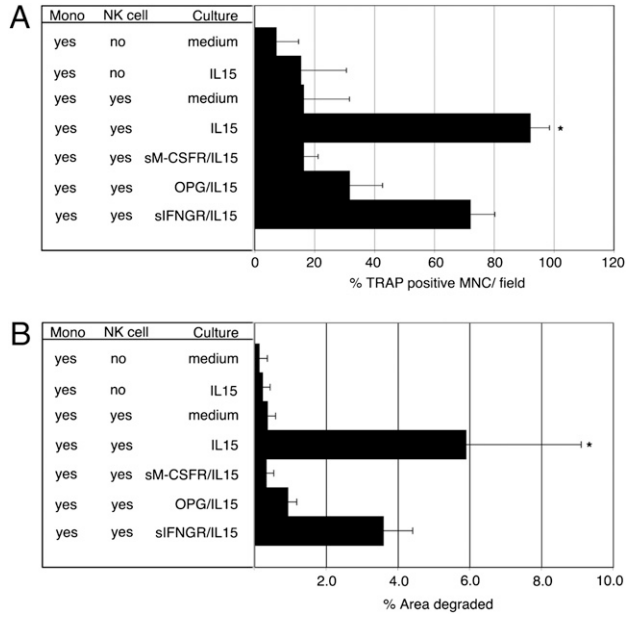

Fig. 4. Neutralization of either M-CSF or RANKL suppresses osteoclastogenesis induced by SF NK cells cocultured with autologous SF monocytes. (*A*) Autologous SF monocytes from patients with RA ($n = 3$) were cultured in the presence or absence of autologous SF NK cells in 24-well culture plates in medium alone or medium supplemented with 10 ng/mL of IL-15 as indicated. M-CSF, RANKL, and IFN-γ were blocked using sM-CSFR, OPG, and soluble IFN-γR, respectively, as indicated. The number of $TRAP^{+}$ multinucleated cells among adherent cells per high-power field was determined on day 6. (*B*) Autologous $CD14^{+}$ SF monocytes from patients with RA ($n = 2$) were cultured in the presence or absence of autologous SF NK cells on osteologic discs in medium alone or medium supplemented with 10 ng/mL of IL-15 as indicated. M-CSF, RANKL, and IFN-γ were blocked using sM-CSFR, OPG, and soluble IFN-γR, respectively, as indicated. After 6 d, the percentage of degraded area was quantified. The data represent mean ± SEM. Student's *t* test was used to calculate the *P* values (*<0.05) compared with sM-CSFR– and OPG-treated cultures.

Evidence of a direct role for T cells in mediating osteoclastogenesis is mixed (39–41). $RANKL^{+}$ $CD4^{+}$ PB T cells isolated from patients with early RA, if cultured with monocytes for 2 wk at a high T cell:monocyte ratio (4:1), can induce osteoclast differentiation (41). Other studies have shown that activated PB T cells induce RANKL-dependent osteoclastogenesis from adherent PBMCs if these cultures are pretreated with M-CSF (40, 42). Although a direct role for T cells in inducing osteoclastogenesis remains controversial, recent studies have established an indirect role for the Th17 cell subset that up-regulates RANKL expression by osteoblasts (43).

We detected little RANKL on human SF T cells. Similarly, we detected little RANKL on human PB T cells or on $CD56^{dim}$ PB NK cells compared with $CD56^{bright}$ PB NK cells. In line with the crucial role for RANKL in osteoclast formation, the $CD56^{bright}$ subset is more potent in this regard than both $CD56^{dim}$ NK cells (Fig. 4) and PB T cells derived from healthy donors (Fig. S5). However, a critical evaluation of whether SF NK cells are main contributors to processes leading to osteoclast formation requires a side-by-side comparison of SF NK cells and other SF cell subsets, including activated T cell subsets, such as SF-derived Th17 cells.

As another approach to evaluate the relative importance of NK cells in arthritis and bone erosion, we established CIA in DBA/1 mice, a well-characterized model for human RA. The destruction of bone in CIA, as in RA, appears to be largely regulated by RANKL (13, 44). Our results show that a large fraction of synovial NK cells in CIA, as well as in RA, express RANKL, suggesting that NK cells are important in the bone erosive processes associated with CIA. Indeed, we show that depletion of NK cells before the induction of disease almost completely prevented bone erosion in CIA. Interestingly, such mice also displayed significantly reduced inflammation, pannus formation, and synovitis. It should be noted that previous studies have shown that neutralization of RANKL in CIA, as well as in RA, reduces bone loss but affects inflammation only minimally (13, 44, 45). Given our finding that NK cell depletion reduces both inflammation and bone erosion in CIA, it is possible that inflammation and bone erosion might be affected by distinct NK cell subsets, where $RANKL^{+}$ NK cells may be primarily involved in the latter path-

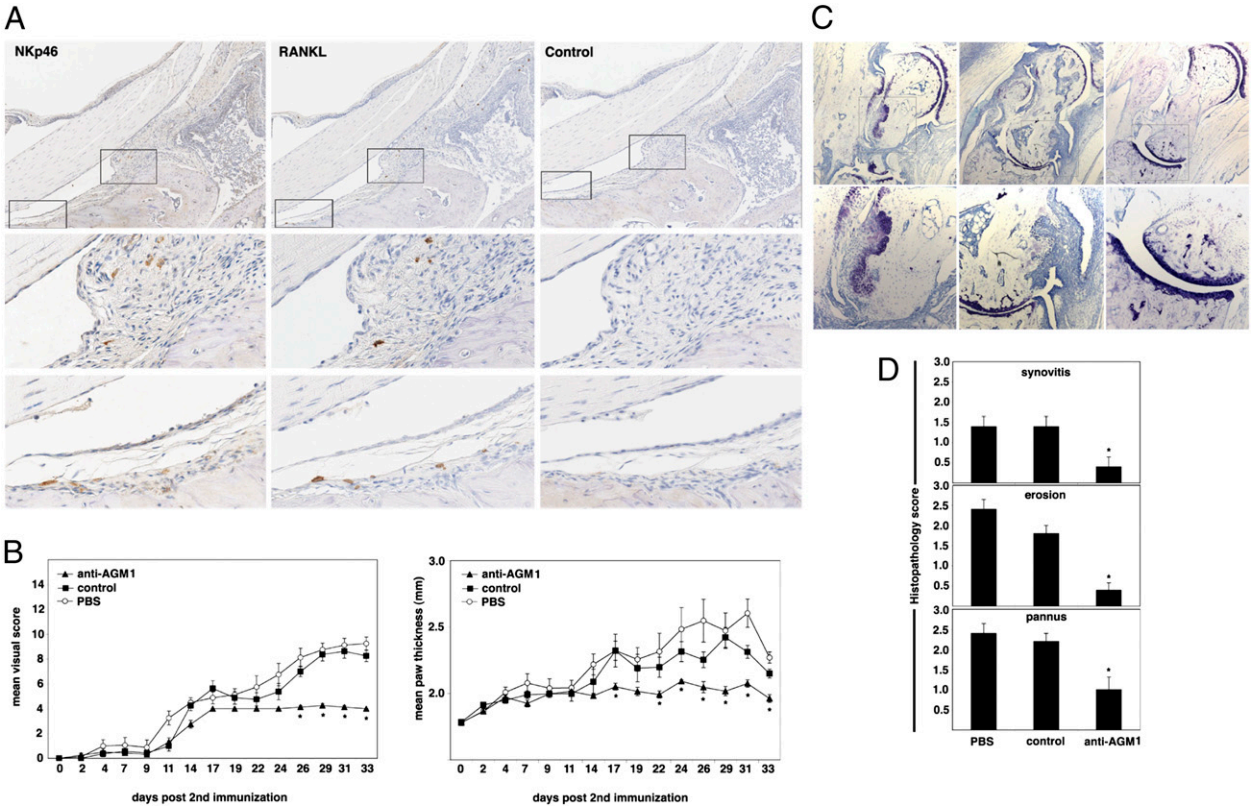

Fig. 5. Synovial tissue NK cells of mice with CIA express RANKL and NK cell–depletion ameliorates disease. (*A*) Adjacent sections of an ankle joint stained with antibody against NK cells (NKp46; *Left*), RANKL (*Center*), or control (*Right*) show RANKL$^+$ NK cells in synovial tissue and close to the bone. The boxes in the upper pictures indicate the areas shown below in 200× original magnification. (*B*) Severity of arthritis in NK cell–depleted mice ($n = 8$) versus rabbit Ig–treated mice (control, $n = 8$) and PBS-treated mice ($n = 8$) as assessed over time by visual scoring (*Left*) and measurements of paw thickness (*Right*). The data represent mean ± SEM $^*P < 0.01$ compared with control mice. (*C*) Representative H&E-stained joint sections from mice that had received PBS (*Left*), rabbit Ig (*Center*), or anti-asialo GM1 (*Right*). The boxes in the top sections (original magnification 40×) indicate the areas shown below in 400× original magnification. (*D*) Histopathological scores of synovitis (*Top*), bone and cartilage erosion (*Middle*), and pannus formation (*Bottom*) in CIA mice that had received PBS, rabbit Ig (control), or anti-asialo GM1. The data represent mean ± SEM $^*P < 0.01$ compared with control treated mice.

way. Another interpretation is that the NK cell-dependent inflammatory process is independent of the expression of RANKL, and that the reduction in bone erosion is an indirect consequence of the reduced inflammation necessary to set the stage for erosive processes.

It should be noted that although subsets of T cells express asialo GM1, in vivo administration of anti-asialo GM1 antibody specifically reduced the number of NK cells without depleting conventional T cells or NKT cells, confirming previous reports (16, 30). This effect is likely due to the relatively high levels of asialo GM1 on NK cells compared with T cells (46). We have shown that reducing the number of NK cells in DBA/1 mice using this antibody prevented bone erosion and ameliorated CIA, which is in sharp contrast to a recent report by Lo et al. (30) that anti-asialo GM1 treatment exacerbated CIA. Based on these divergent results, future studies aimed at further defining the role of NK cells in arthritis probably should use animal models in which NK cells can be depleted with a different method. Transgenic mice expressing the diphtheria toxin (DT) receptor in NK cells as described by Walzer et al. (47), in which DT injection leads to complete and selective NK cell ablation, might prove useful for this purpose. Moreover, generation of mice that specifically lack RANKL expression on NK cells may shed further light on the specific role for RANKL$^-$ versus RANKL$^+$ NK cells in inflammation and bone erosion.

Methods

Cell Isolation and Culture. Mononuclear cells from RA SF and healthy PB were obtained by Ficoll density gradient centrifugation, and CD14$^+$ monocytes and CD56$^+$CD3$^-$ NK cells were isolated and cultured as described previously (22). See *SI Methods* for details.

Analysis of in Vitro Osteoclast Differentiation and Function. In vitro osteoclast differentiation and osteoclast function were analyzed according to standard methods with minor modifications. See *SI Methods* for details.

Immunofluorescence. Cells were preincubated with FcR-blocking reagent (Miltenyi) according to the manufacturer's instructions, and staining was performed using FITC-, phycoerythrin (PE)-, or allophycocyanin (APC)-conjugated mAbs against CD3, CD14, and CD56 (all from BD Biosciences); PE-conjugated anti-RANKL (mouse IgG2b, clone MIH24) and mouse IgG2b control (eBioscience); APC-conjugated anti–M-CSF (R&D Systems), and isotype-matched controls (BD Biosciences) and analyzed on a FACSCalibur flow cytometer (BD Biosciences).

ELISA. Secretion of RANKL (Peprotech) and M-CSF (R&D Systems) was measured by ELISA according to the manufacturer's instructions. In brief, NK cells were cultured in 96-well plates (Corning) at 10^5 cells in 200 μL, and cell-free supernatant was harvested and assayed after 72 h.

Immunohistochemistry. Frozen synovial tissue sections were derived from two female RA patients (age 58 and 72 y), and one male (age 68 y) treated with conventional DMARDs at the time of knee joint replacement surgery. The patients met the American College of Rheumatology criteria and the tissues were collected after informed consent under local Institutional Review Board-

approved protocols. The tissue sections were incubated with an NK cell-specific mAb and mouse anti-human CD14 mAb (Dako Cytomation) as described previously (22).

CIA Studies. Collagen induced arthritis (CIA) was induced in DBA/1 mice. See *SI Methods*.

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