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CXC Chemokine Ligand 2 Induced by Receptor Activator of NF-κB Ligand Enhances Osteoclastogenesis

Jeongim Ha,* Hyo-Sun Choi,* Youngkyun Lee,* Hyung-Joo Kwon,†‡ Yeong Wook Song,§ and Hong-Hee Kim*†

CXCL2 has been known to regulate immune functions mainly by chemo-attracting neutrophils. In this study, we show that CXCL2 can be induced by receptor activator of NF-κB ligand, the osteoclast (OC) differentiation factor, through JNK and NF-κB signaling pathways in OC precursor cells. CXCL2 in turn enhanced the proliferation of OC precursor cells of bone marrow-derived macrophages (BMMs) through the activation of ERK. Knockdown of CXCL2 inhibited both the proliferation of and the ERK activation in BMMs. During osteoclastogenesis CXCL2 stimulated the adhesion and the migration of BMMs. Moreover, the formation of OCs from BMMs was significantly increased on treatment with CXCL2. Conversely, the CXCL2 antagonist reper- 

activation in BMMs. During osteoclastogenesis CXCL2 stimulated the adhesion and the migration of BMMs. Moreover, the pathways in OC precursor cells. CXCL2 in turn enhanced the proliferation of OC precursor cells of bone marrow-derived 

Bone is a dynamic tissue that is continuously formed and replaced via a process called bone remodeling. Osteoblasts, derived from mesenchymal stem cells, produce the bone matrix, and osteoclasts (OCs), originated from hematopoietic progenitor cells, dissolve (resorb) bone (1). The generation of OCs from progenitor cells is governed by the differentiation factor receptor activator of NF-κB ligand (RANKL) with the assistance of macrophage CSF (M-CSF) that supports cell survival during differentiation (2). A series of steps, including OC precursor development, migration and adhesion of precursors to bone surfaces, differentiation into mature OCs, and the secretion of protons and lysosomal enzymes into the resorption site regulate osteoclastic bone removal (3).

Some chemokines have been shown to be associated with the generation and function of OCs. RANKL induces the expression of C-C chemokines such as CCL2, CCL3, CCL5, and CCL9, and C-X-C chemokines, such as CXCL10 (4–7). CCL2 also rescues bone resorption suppressed by granulocyte M-CSF (4). RANKL upregulates the expression of CCL3 and its receptor CCR1 in an NFATc1-dependent manner, which enhances migration of differentiating OCs (5). CCL9/MIP-1γ stimulates cytoplasmic motility and cell spreading of OCs, and CXCL12/SDF-1 increases osteoclastogenesis and resorption activity (6, 8). CXCL10/IP-10, induced by RANKL, plays critical roles in bone-erorative experimental arthritis via a CD4+ T cell-mediated mechanism (7).

CXCL2 was first identified as a major chemokine produced by endotoxin-treated macrophages and acts as a mediator of inflammation (9). The principal roles of CXCL2 are chemotaxis of neutrophils (10), regulation of endotoxin-induced transmigration and extravascular tissue accumulation of leukocytes (10), proliferation and apoptosis protection of hepatocytes (10, 11), and regulation of ischemia/reperfusion-induced leukocyte adhesion (12).

In the current study, we show that the osteoclastogenic factor RANKL could promote CXCL2 expression in OC precursors. We further provide evidence that CXCL2 has critical roles in osteoclastogenesis in vitro and in bone erosion in vivo. Therefore, targeting CXCL2 might be a new therapeutic strategy for antiresorptive drug development.

Materials and Methods

Materials

Recombinant RANKL, M-CSF, and CXCL2 were purchased from Peprotech (London, U.K.). Polyclonal CXCL2 Ab was purchased from Abcam (Cambridge, U.K.). Monoclonal anti-CXCR2 Ab was purchased from R&D Systems (Minneapolis, MN). PE-conjugated anti-RANK Ab was from Biologend (San Diego, CA). Anti-c-Jun and anti-p65 Abs for chromatin immunoprecipitation (ChIP) assays were from Upstate (Billerica, MA) and Santa Cruz Biotechnology (Santa Cruz, CA), respectively. Polyclonal CXCL2 Ab was purchased from Abcam (Cambridge, U.K.). Monoclonal anti-CXCR2 Ab was purchased from Cell Signaling Technology (Cambridge, MA). Anti-mouse and anti-rabbit IgG-conjugated HRP and anti-mouse actin Abs were obtained from Sigma-Aldrich (St Louis, MO). FITC-labeled mouse secondary Ab was purchased from Jackson ImmunoResearch Laboratories (West Grove, PA). Mouse and human CXCL2 ELISA kits were from R&D Systems and Peprotech, respectively. SB203580, SP600125, PD98059, LY294002, repertaxin, and BrdU assay kits were purchased from Calbiochem (San Diego, CA). Parthenolide (PAR) and Bay11-7082 were purchased from Alexis Biochemicals (Grünenberg, Germany). The luciferase assay system kit was purchased from Promega (Madison, WI). Lipofectamine 2000

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BMM, bone marrow-derived macrophages; ChIP, chromatin immunoprecipitation; M-CSF, macrophage CSF; μCT, microcomputer tomography; OA, osteoarthritis; OC, osteoclast; PAR, parthenolide; RA, rheumatoid arthritis; pOC, prefusion OC; RANKL, receptor activator of NF-κB ligand; siRNA, small interfering RNA; TRAP, tartrate-resistant acid phosphatase.

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Abbreviations used in this paper: BMM, bone marrow-derived macrophages; ChIP, chromatin immunoprecipitation; M-CSF, macrophage CSF; μCT, microcomputer tomography; OA, osteoarthritis; OC, osteoclast; PAR, parthenolide; RA, rheumatoid arthritis; pOC, prefusion OC; RANKL, receptor activator of NF-κB ligand; siRNA, small interfering RNA; TRAP, tartrate-resistant acid phosphatase.

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and Stealth RNAi were from Invitrogen (Carlsbad, CA). Transwell was purchased from Corning Costar (Cambridge, MA). Cy5-labeled phalloidin and the TRAP staining kit were obtained from Sigma-Aldrich. Materials for real-time PCR were purchased from Applied Biosystems (Foster City, CA).

Patient samples

Synovial fluid and serum were drawn from 25 patients with rheumatoid arthritis (RA) and from 16 patients with osteoarthritis (OA) after obtaining informed consent. All samples were stored at −70°C before use. The study was approved by the ethics committee of Seoul National University Hospital.

Cell culture

Mouse bone marrow cells were isolated by flushing the bone marrow space of femora and tibiae of 6- to 8-wk-old ICR mice ( Orient Bio, Seongnam, Korea) (13). Cells were incubated with α-MEM (JBI, Daegu, Korea) containing 10% FBS, penicillin (100 U/ml) and streptomycin (100 µg/ml) for 24 h in 5% CO2 at 37°C. Nonadherent cells were plated on noncoated petri dishes with M-CSF (20 ng/ml) for 3 d. Adherent cells were considered to be bone marrow-derived macrophages (BMMs) and were used as OC precursor cells. OC differentiation was induced by treatment of BMMs with M-CSF (20 ng/ml) and RANKL (150 ng/ml). After culturing for 2 d, most cells became TRAP-positive mononucleated prefusion OCs (pOCs). TRAP-positive multinucleated OCs were formed after culturing for 5 d.

RT-PCR and real-time PCR

RNA (3 µg) extracted using an RNeasy mini kit (Qiagen, Valencia, CA) was reverse transcribed into cDNA using Superscript II (Invitrogen) in a total reaction volume of 20 µl. PCR was performed with 1 µl cDNA using genespecific oligonucleotide primers: mCCL2, 5'-TCCCAATAGTAGGCTCTCCAG-3' and 5'-TCTTGAGACCCATTTCCCTGTG-3'; mCCL3, 5'-AGGTCAGCTTCCATTTCCCTGTG-3'; mCCL4, 5'-TTGTTCCTCTGGAAGGCTG-3'; mCCL5, 5'-GCTGGAGGCCTCTCCCTGTG-3'; mCCL10, 5'-CCCCAGTGTTGTAGCTCTCCACACCTG-3'; mCCL11, 5'-GAAGCTCTCCCTGTG-3'; mCXCL2, 5'-ACAGAAGTCAATCGCAAGCCCTTGTGACCC-3' and 5'-GAGGAGGCCTCTCCCTGTG-3'; mCCL3, 5'-CTTTGAGGAGGCCTCTCCCTGTG-3'; mCCL5, 5'-GCTGGAGGCCTCTCCCTGTG-3'; mCCL10, 5'-CTTTGAGGAGGCCTCTCCCTGTG-3'; mCCL11, 5'-CTTTGAGGAGGCCTCTCCCTGTG-3'. For the input control, 1% of the sonicated DNA was directly purified before immunoprecipitation and subjected to PCR with the same primers.

Western blotting

BMMs (5 × 10⁵ cells per well in 6-well plates) were treated with M-CSF (20 ng/ml), RANKL (150 ng/ml), or M-CSF plus RANKL for 6 h. To detect CXCL2, 50 µl of the 1 ml culture supernatant was used for Western blotting (14). After treating BMMs with CXCL2 (50 ng/ml) in the absence or presence of M-CSF (10 ng/ml) for the indicated times, cell lysates were subjected to Western blotting to detect ERK and Akt phosphorylation. BMMs transfected with control- or CXCL2-small interfering RNA (siRNA) were also analyzed.

ELISA

CXCL2 levels in synovial fluid and serum from patients with RA and OA, as well as levels in cell culture supernatants, were measured using human and mouse CXCL2 ELISA kits, following the manufacturer’s instructions.

Luciferase assay

RAW264.7 cells were transfected with the CXCL2-luciferase construct (15) for 5 h in serum-free DMEM. After 24 h of incubation with DMEM containing 10% FBS, cells were stimulated with increasing doses of RANKL for 5 h. Cells were lysed with Glo lysis buffer and were subjected to a luciferase assay using a FLUOstar OPTIMA luminometer (BMG Labtech, Offenburg, Germany).

ChIP assay

BMMs were treated with M-CSF (20 ng/ml) plus RANKL (150 ng/ml) for 1 h. The procedure for ChIP assays was based on the description in the EZ ChIP kit (17-371, Upstate). The 5 µg anti-c-Jun or anti-p65 was used for immunoprecipitation. After eluting DNA from the precipitated immune complex, PCR reaction was performed using specific primers: c-Jun, 5'-CTCGTGTCTAGTACACCGCA-3' and 5'-GACGCTGTCATTTCCCTGTG-3'; p65, 5'-AACCCACGCTACCATAGGGGGGGTCA-3' and 5'-TTGGTGACTGAAGACGTACGGGG-3'. For the input control, 1% of the sonicated DNA was directly purified before immunoprecipitation and subjected to PCR with the same primers.

Flow cytometry

To detect the surface expression of CXCR2, cells were incubated with monoclonal anti-mouse CXCR2 Ab for 20 min on ice after blocking non-specific binding by treating with goat serum. Cells were washed with PBS three times before incubation with FITC-labeled secondary Ab for 20 min. To detect the surface expression of RANK, BMMs were incubated with PE-conjugated anti-RANK for 30 min. Flow cytometric analysis was performed using a FACS Calibur flow cytometer (Becton Dickinson, San Jose, CA). Acquisition and analysis were performed using CellQuest software.

CXCL2-siRNA transfection

CXCL2 gene-specific double-stranded stealth RNAi was designed using software from Invitrogen (si-CXCL2 no. 1, UUGAAGUCAACCUUGUGAGGGCGUCU; si-CXCL2 no. 2, AGACAGAAATGATAGCCACCTTCTTCACA; and si-CXCL2 no. 3, GAACATGGCTGATAATCGGGAGAAA). BMMs were transfected with CXCL2-siRNA (20 nM) using lipofectamin 2000
BMMs were cultured with M-CSF (20 ng/ml) plus RANKL (150 ng/ml) and dexamethasone (1 μM) for 5 d, whereas those of CCL5 and CXCL2, 10, and 11 were greatly induced by 150 ng/ml RANKL treatment for 3 h (Fig. 1C). In contrast to a previous report (4), CCL2 mRNA expression did not change in the presence of RANKL (Fig. 1A).

Results

**RANKL induced CXCL2 through JNK and NF-κB signaling pathways**

We screened chemokines that could be induced by the osteoclastogenic factor RANKL in mouse primary OC precursor cells, BMMs. The expressions of CCL3 and CCL4 were slightly increased, whereas those of CCL5 and CXCL2, 10, and 11 were greatly induced by 150 ng/ml RANKL treatment for 3 h (Fig. 1A). In contrast to a previous report (4), CCL2 mRNA expression did not change in the presence of RANKL (Fig. 1A). Because the role of CXCL2 in osteoclastogenesis has not been addressed, we...
further elevated the luciferase activity (Fig. 2A, 2B). The possibility of regulation of CXCL2 mRNA stability was also explored. As shown in Fig. 2C, mRNA stability of CXCL2 was increased by M-CSF plus RANKL (Fig. 2C). When we tested the single treatment of RANKL or M-CSF, CXCL2 mRNA stabilization was greater by RANKL than by M-CSF (data not shown). We next examined whether RANKL-induced CXCL2 expression would require new synthesis of other proteins. Interestingly, CXCL2 mRNA expression was not suppressed, rather increased, by the protein synthesis inhibitor cycloheximide (Fig. 2D), suggesting that RANKL directly stimulates CXCL2 expression and that RANKL might also promote synthesis of other proteins inhibitory to CXCL2 induction. To gain insight into the signaling pathways involved in RANKL-dependent CXCL2 induction, BMMs were stimulated with M-CSF plus RANKL in the presence of chemical inhibitors of MAPK, PI3K/Akt, and NF-κB pathways as those signaling molecules were activated by M-CSF and RANKL was almost completely abolished by the JNK inhibitor, SP600125, and NF-κB inhibitors, Bay 11-7082 and PAR, whereas the p38 inhibitor, SB203580, and the PI3K inhibitor, LY294002, slightly reduced CXCL2 mRNA expression (Fig. 2E). Similarly, JNK and NF-κB inhibitors abolished the upregulation of CXCL2 protein by M-CSF and RANKL in BMMs (Fig. 2F). The recruitment of JNK-dependent transcription factor c-Jun and the NF-κB subunit p65 to the CXCL2 promoter was also found to be stimulated by M-CSF plus RANKL (Fig. 2G, 2H). Taken together, these results demonstrate that JNK and NF-κB signaling pathways play a major role in RANKL-induced CXCL2 expression.

FIGURE 4. Migration and adhesion of OC precursors by CXCL2. A. A migration assay was performed using the transwell system. Serum-deprived BMMs were added to the upper chamber and serum-free α-MEM containing CXCL2 was added to the lower chamber. After a 3-h incubation, migrated cells were fixed, stained with H&E, and counted under a microscope. B. CXCL2 was added to serum-starved BMMs on the cover slip for 10 min. Nonadherent cells were washed vigorously with PBS. Adherent cells were fixed, stained with phalloidin-Cy5, and observed under a confocal microscope (top panel). Mean fluorescence intensity was measured using an LSM program (bottom panel). Data are expressed as means ± SD. *p < 0.05 versus untreated control.
CXCL2 stimulated the proliferation of OC precursors

CXCR2 is known to be the major receptor for CXCL2. FACS analysis using anti-CXCR2 Ab revealed that BMMs expressed CXCR2 at a high level (Fig. 3A). Thus, we hypothesized that CXCL2 released on RANKL stimulation of BMMs could function in an autocrine manner. To explore this possibility, we first investigated the effect of CXCL2 on the proliferation of BMMs. During the early stage of osteoclastogenesis, enhanced proliferation was observed when BMMs were cultured with M-CSF plus RANKL compared with that of M-CSF alone (Fig. 3C). To test whether RANKL-induced CXCL2 production is involved in the proliferation, BMMs were transfected with CXCL2-specific siRNA oligonucleotides. Two functional (no. 1 and no. 3) and one non-functional (no. 2) siRNAs were used (Fig. 3B, data not shown). CXCL2 knockdown significantly reduced BMM proliferation on M-CSF stimulation or M-CSF plus RANKL treatment (Fig. 3C).
data not shown). As the ERK and Akt signaling pathways have been implicated in cell proliferation, we next examined the effects of CXCL2 siRNA on ERK and Akt phosphorylation. Although RANKL stimulation of BMMs induced sustained activation of Akt and ERK, knockdown of CXCL2 inhibited ERK phosphorylation (Fig. 3D, data not shown). The activation of Akt was indistinguishable between si-control and si-CXCL2 transfected cells. Next, we tested whether CXCL2 could augment M-CSF–induced BMM proliferation in the absence of RANKL. As shown in Fig. 3E, the addition of CXCL2 significantly elevated BMM proliferation in the presence of M-CSF. Again, CXCL2 enhanced sustained ERK phosphorylation, with little effect on Akt activation in BMMs treated with M-CSF (Fig. 3F).

**CXCL2 promoted the migration and adhesion of OC precursors**

CXCL2 has been suggested as a chemoattractant for various types of cells (10). Thus, the effect of CXCL2 on the migration of OC precursors was examined using the transwell system. CXCL2 significantly increased the number of migrating BMMs (Fig. 4A). In a bone microenvironment, adhesion of OC precursors is another critical step during the early stages of osteoclastogenesis. Notably, increasing concentrations of CXCL2 dramatically stimulated the adhesion of BMMs on the glass cover slips (Fig. 4B), as well as on tissue culture plastics (data not shown). In addition, CXCL2 treatment at higher concentrations enhanced the cell spreading of the BMMs (data not shown).

**CXCL2 increased the formation and survival of OCs in vitro**

We next investigated the effect of CXCL2 on OC differentiation from mouse BMMs. When CXCL2 was added to BMMs in osteoclastogenic medium containing RANKL and M-CSF, the generation of TRAP-positive multinucleated OCs was enhanced (Fig. 5A, 5B). Moreover, the size of the OCs formed was also increased by CXCL2 (Fig. 5A), and the number of nuclei per cell was also higher in CXCL2-treated OCs (Fig. 5C). To further confirm the role of CXCL2 during RANKL-induced OC differentiation, CXCL2-neutralizing Ab was included during in vitro osteoclastogenesis. As shown in Fig. 5D, addition of a CXCL2-neutralizing Ab almost completely inhibited OC differentiation. In addition, the CXCR2-specific antagonist receptorinax also blocked osteoclastogenesis (Fig. 5E). These data indicate that the CXCL2 induced by RANKL in OC precursors acts on its receptor in an autocrine fashion to augment osteoclastogenesis. To examine whether the OCs generated in the presence of CXCL2 are functionally competent, we performed bone resorption assays. BMMs were cultured with M-CSF plus RANKL in the presence of increasing doses of CXCL2 on dentin slices. The resorption pit areas were increased in CXCL2-treated dentine slices (Fig. 6A, 6B). We next investigated whether CXCL2 could also regulate the survival of differentiated OCs. It has been reported that RANKL sustains the survival of mature OCs (16). When a neutralizing CXCL2 Ab was added in the presence of RANKL to differentiated mature OCs, the number of live cells was significantly decreased (Fig. 6C).

**CXCL2 elicited bone erosion in vivo**

We investigated the effect of CXCL2 on bone resorption in vivo. PBS- or RANKL-soaked collagen sponges were implanted onto mice calvariae, and CXCL2 or a control vehicle was injected three times onto the calvariae to assess bone erosion. The µ-CT analyses revealed that RANKL significantly increased bone loss in calvariae (they appeared black in the µ-CT image) compared with PBS (Fig. 7A). Notably, the addition of CXCL2 elicited dramatic bone loss both in the absence and the presence of RANKL (Fig. 7B). To confirm the involvement of CXCL2 in the RANKL-induced bone resorption, a CXCL2 neutralizing Ab was administered into calvariae. The CXCL2 Ab significantly reduced the bone loss induced by RANKL (Fig. 7C, 7D). To gain further insights into the role of CXCL2 in bone-destructive diseases, we measured CXCL2 levels in synovial fluids and sera from RA patients. RA synovial fluids contained significantly higher levels of CXCL2 compared with those of OA (Fig. 7E). A similar increase in the CXCL2 protein levels was also evident in serum samples from RA patients (Fig. 7F). These data suggest that increased CXCL2 in synovial fluids might have a role in bone destruction during RA pathogenesis.

**Discussion**

The pathogenesis of bone destruction in RA is initiated by the upregulation of RANKL as well as TNF-α and IL-1 in the synovial tissue. These cytokines induce other cytokines that promote the differentiation and activation of OCs and recruit immune cells, leading to massive destruction of cartilages and bones (17). To uncover the role of chemokines during this process, we investigated the regulation of chemokines by the osteoclastogenic factor RANKL in primary OC precursor cells. RANKL rapidly induced the C-C chemokines CCL3, CCL4, and CCL5 as well as the C-X-C chemokine CXCL10 (Fig. 1). A number of studies have shown that some of these chemokines and their receptors play roles in OC differentiation and activation (4–8). Interestingly, RANKL also significantly increased the expression of CXCL2 that has never been associated with OCs. In this study, we...
clearly showed that the RANKL-induced CXCL2 in OC precursors play key roles in the attachment, migration, differentiation, and function of OCs. Because mouse bone marrow OC precursors (BMMs) highly expressed the CXCL2 receptor CXCR2 (Fig. 3), it was likely that the CXCL2-dependent augmentation of osteoclastogenesis was mediated via an autocrine/paracrine mechanism.

RANKL seemed to increase the mRNA level of CXCL2 not only by direct stimulation of CXCL2 promoter activity, but also by stabilizing the mRNA. Although the CXCL2 promoter-dependent luciferase activity was only 1.5-fold enhanced by RANKL, there was >4-fold induction of CXCL2 mRNA (Figs. 1, 2). Indeed, the presence of RANKL significantly delayed the degradation of CXCL2 mRNA (Fig. 2). Interestingly, inhibition of protein synthesis by cycloheximide seemed to increase CXCL2 mRNA level, suggesting that there might exist RANKL-induced protein(s) that negatively regulate CXCL2 mRNA. Further studies are required to reveal whether these proteins are repressors that regulate transcription, or involved in the mRNA degradation.

CXCL2 induction by RANKL in OC precursors was significantly impaired in the presence of JNK and NF-κB inhibitors (Fig. 2). In fact, c-Jun and p65 transcription factors were recruited to CXCL2 promoter by RANKL stimulation. These data concur with a previous report that showed the existence of two AP-1 binding sites in the CXCL2 promoter (15). Because c-Jun and NF-κB binding sites were also regulated LPS-dependent CXCL2 induction (15), it is likely that the same signaling mechanisms are shared by TLR and RANK receptors in terms of CXCL2 production. Interestingly, we found that p38 and P38K inhibitors slightly inhibited CXCL2 mRNA expression, but they did not affect CXCL2 protein production. The extent of mRNA reduction by the p38 and P38K inhibitors might have not been sufficient to mitigate CXCL2 protein levels. Alternatively, these inhibitors may have additional suppressive effects on a mechanism that destabilizes the CXCL2 protein.

Osteoclastogenesis requires proper regulation of sequential processes, including the proliferation, adhesion, migration, and fusion of precursors and differentiating cells. Our data showed that CXCL2 promoted the proliferation of BMMs (Fig. 3). The knockdown of CXCL2 significantly reduced BMM proliferation and ERK activation on M-CSF or RANKL stimulation, whereas exogenous CXCL2 augmented both proliferation and ERK activity. A similar ERK-dependent stimulation of cell proliferation by CXCL2 was observed in esophageal cancers that highly expressed both CXCL2 and CXCR2 compared with adjacent normal esophageal tissues (18). CXCL2 has been shown to potently chemo-attract neutrophils (19). CXCL2 not only stimulated the adhesion but also induced the migration of OC precursors in our study (Fig. 4). Moreover, cell spreading was enhanced by CXCL2 treatment (data not shown). Thus, CXCL2 likely plays a crucial role by regulating various cellular responses during the early stages of osteoclastogenesis. Indeed, CXCL2-neutralizing Ab and the CXCR2 antagonist repertaxin almost completely inhibited osteoclastogenesis when they were included during the early stages of osteoclastogenic culture (Fig. 5). In addition, a significant increase in the number of nuclei per OC was observed by CXCL2 treatment (Fig. 5), suggesting that CXCL2 might also be involved in the fusion of OC precursors. Furthermore, CXCL2 increased the number of active OCs (Fig. 6). Finally, CXCL2 blockade by neutralizing Ab significantly decreased OC survival. All these data suggest that the RANKL-induced production of CXCL2 has influence on various cellular responses involved in OC differentiation and function in vitro.

The osteoclastogenesis-enhancing effects of CXCL2 were further corroborated by our investigation with an in vivo bone resorption model. Notably, CXCL2 alone induced significant bone loss in mice calvariae similar to that by RANKL (Fig. 7). Because CXCL2 alone in the absence of RANKL did not induce OC differentiation in vitro, this might be explained by the CXCL2 stimulation of osteoclastogenesis from pre-existing RANKL-primed precursors. However, we cannot rule out the possibility that CXCL2 might have caused the recruitment of inflammatory immune cells that contributed to osteoclastogenesis. The RANKL-induced bone destruction was significantly blocked by administration of CXCL2 neutralizing Ab (Fig. 7). Therefore, it was likely that CXCL2 might be one of the important mediators in response to RANKL stimulation that promote bone destruction. In support of this notion, CXCL2 was significantly upregulated in synovial fluids and sera from RA patients (Fig. 7). Combined with the data demonstrating the ability of CXCL2 to evoke bone resorption in mice calvariae, our study suggests that CXCL2 might be involved in bone destruction during the pathogenesis of RA. Therapies targeting CXCL2/CXCR2 have been tested in animal models of arthritis with concomitant reduction in neutrophil recruitment and TNF-α production (20), and immunization against CXCL2 was efficient in delaying the onset of arthritis and reducing the disease severity in a murine collagen-induced arthritis model (21). In addition, an antagonist of CXCR2 inhibited arthritis in rabbits (22). Our results imply that targeting CXCL2 as a strategy to treat RA would be beneficial in protection from bone destruction by directly inhibiting bone resorption, in addition to the already suggested anti-inflammatory effects.

In conclusion, RANKL induced CXCL2 in OC precursors in both JNK- and NF-κB–dependent mechanisms. CXCL2 promoted the proliferation, adhesion, and migration of OC precursors and augmented the formation of OCs in vitro. Furthermore, CXCL2 induced significant bone destruction on in vivo administration, and its level was found to be high in RA patient fluids. These results uncover a previously unappreciated role of CXCL2 during osteoclastogenesis and suggest CXCL2 blockade as a novel strategy for the treatment of inflammatory bone destructive diseases.

Disclosures
The authors have no financial conflicts of interest.

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