

**Innovative, Intuitive, Flexible.**

Luminex Flow Cytometry Solutions  
with Guava® and Amnis® Systems

Learn More >



**Luminex**  
complexity simplified.



## Direct Inhibition of Human RANK<sup>+</sup> Osteoclast Precursors Identifies a Homeostatic Function of IL-1 $\beta$

This information is current as  
of November 7, 2022.

Bitnara Lee, Tae-Hwan Kim, Jae-Bum Jun, Dae-Hyun Yoo,  
Jin-Hyun Woo, Sung Jae Choi, Young Ho Lee, Gwan Gyu  
Song, Jeongwon Sohn, Kyung-Hyun Park-Min, Lionel B.  
Ivashkiv and Jong Dae Ji

*J Immunol* 2010; 185:5926-5934; Prepublished online 8  
October 2010;  
doi: 10.4049/jimmunol.1001591  
<http://www.jimmunol.org/content/185/10/5926>

**Supplementary Material** <http://www.jimmunol.org/content/suppl/2010/10/08/jimmunol.1001591.DC1>

**References** This article **cites 47 articles**, 16 of which you can access for free at:  
<http://www.jimmunol.org/content/185/10/5926.full#ref-list-1>

**Why *The JI*? Submit online.**

- **Rapid Reviews! 30 days\*** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Fast Publication!** 4 weeks from acceptance to publication

*\*average*

**Subscription** Information about subscribing to *The Journal of Immunology* is online at:  
<http://jimmunol.org/subscription>

**Permissions** Submit copyright permission requests at:  
<http://www.aai.org/About/Publications/JI/copyright.html>

**Email Alerts** Receive free email-alerts when new articles cite this article. Sign up at:  
<http://jimmunol.org/alerts>

*The Journal of Immunology* is published twice each month by  
The American Association of Immunologists, Inc.,  
1451 Rockville Pike, Suite 650, Rockville, MD 20852  
Copyright © 2010 by The American Association of  
Immunologists, Inc. All rights reserved.  
Print ISSN: 0022-1767 Online ISSN: 1550-6606.



# Direct Inhibition of Human RANK<sup>+</sup> Osteoclast Precursors Identifies a Homeostatic Function of IL-1 $\beta$

Bitnara Lee,<sup>\*,1</sup> Tae-Hwan Kim,<sup>\*,1</sup> Jae-Bum Jun,<sup>\*</sup> Dae-Hyun Yoo,<sup>\*</sup> Jin-Hyun Woo,<sup>†</sup> Sung Jae Choi,<sup>†</sup> Young Ho Lee,<sup>†</sup> Gwan Gyu Song,<sup>†</sup> Jeongwon Sohn,<sup>‡</sup> Kyung-Hyun Park-Min,<sup>§</sup> Lionel B. Ivashkiv,<sup>§</sup> and Jong Dae Ji<sup>†</sup>

IL-1 $\beta$  is a key mediator of bone resorption in inflammatory settings, such as rheumatoid arthritis (RA). IL-1 $\beta$  promotes osteoclastogenesis by inducing RANKL expression on stromal cells and synergizing with RANKL to promote later stages of osteoclast differentiation. Because IL-1Rs share a cytosolic Toll-IL-1R domain and common intracellular signaling molecules with TLRs that can directly inhibit early steps of human osteoclast differentiation, we tested whether IL-1 $\beta$  also has suppressive properties on osteoclastogenesis in primary human peripheral blood monocytes and RA synovial macrophages. Early addition of IL-1 $\beta$ , prior to or together with RANKL, strongly inhibited human osteoclastogenesis as assessed by generation of TRAP<sup>+</sup> multinucleated cells. IL-1 $\beta$  acted directly on human osteoclast precursors (OCPs) to strongly suppress expression of RANK, of the costimulatory triggering receptor expressed on myeloid cells 2 receptor, and of the B cell linker adaptor important for transmitting RANK-induced signals. Thus, IL-1 $\beta$  rendered early-stage human OCPs refractory to RANK stimulation. Similar inhibitory effects of IL-1 $\beta$  were observed using RA synovial macrophages. One mechanism of RANK inhibition was IL-1 $\beta$ -induced proteolytic shedding of the M-CSF receptor c-Fms that is required for RANK expression. These results identify a homeostatic function of IL-1 $\beta$  in suppressing early OCPs that contrasts with its well-established role in promoting later stages of osteoclast differentiation. Thus, the rate of IL-1-driven bone destruction in inflammatory diseases, such as RA, can be restrained by its direct inhibitory effects on early OCPs to limit the extent of inflammatory osteolysis. *The Journal of Immunology*, 2010, 185: 5926–5934.

**B**one resorption and osteolysis are a prominent feature and a cause of substantial morbidity in several inflammatory diseases, including rheumatoid arthritis (RA), periodontitis, and peri-prosthetic loosening (1–3). Osteoclasts are the primary bone-resorbing cells and are essential for bone destruction in these inflammatory diseases. Osteoclasts are multinucleated giant cells that are differentiated from hematopoietic cells of myeloid lineage. RANKL and M-CSF are essential molecules for differentiation of osteoclasts from their precursors, and these osteoclastogenic molecules are abundantly expressed in inflammatory conditions, such as RA and periodontitis (4, 5). M-CSF binds to the surface receptor c-Fms (also termed colony-stimulating factor 1 receptor), which is responsible for early differentiation

of osteoclasts and acts as a potent stimulator of RANK expression (6). RANKL binds to RANK on the cell surface of osteoclast precursors (OCPs) and induces the full differentiation of osteoclasts and their bone resorbing activity. Osteoprotegerin is another receptor for RANKL and a potent inhibitor of osteoclastogenesis that acts as a decoy receptor for RANKL. Other inflammatory molecules also positively or negatively contribute to bone destruction by regulating the differentiation of osteoclasts. Therefore, the extent of bone destruction is determined by the balance between stimulatory and inhibitory factors of osteoclastogenesis in inflammatory conditions.

In RA, several inflammatory molecules, such as TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-17, and PGs play a vital role in osteoclastogenesis and bone resorption. These molecules promote osteoclastogenesis indirectly by increasing expression of RANKL and M-CSF by stromal cells and T cells, and also by acting directly on OCPs to synergize with RANKL in driving osteoclastogenesis (1, 2). Among these molecules, TNF- $\alpha$  is the most important osteoclastogenic molecule in pathologic conditions, such as RA. TNF- $\alpha$  increases osteoclastogenesis through several different mechanisms (7). TNF- $\alpha$  increases the pool size of marrow OCPs, enhances the RANKL-induced osteoclastogenic actions, and increases expression of RANKL in synovial cells, T cells, and osteoblast/stromal cells.

IL-1 is a multifunctional cytokine that has predominately proinflammatory properties but can also engage feedback inhibitory mechanisms (e.g., induction of glucocorticoid production) that restrain and balance its proinflammatory function (8). This cytokine was initially described as an osteoclast-activating factor due to its potent bone-resorbing activity (9). Like TNF- $\alpha$ , IL-1 $\beta$  also plays an essential role in the pathogenesis of bone destruction in RA. Although IL-1 alone does not induce osteoclastogenesis, it

\*Hospital for Rheumatic Diseases, Hanyang University; <sup>†</sup>Division of Rheumatology and <sup>‡</sup>Department of Biochemistry, College of Medicine, Korea University, Seoul, South Korea; and <sup>§</sup>Arthritis and Tissue Degeneration Program, Hospital for Special Surgery, New York, NY 10021

<sup>1</sup>B.L. and T.-H.K. contributed equally to this work.

Received for publication May 14, 2010. Accepted for publication September 8, 2010.

This work was supported by Grant A084224 from the Korea Healthcare Technology R&D Project, Ministry for Health, Welfare and Family Affairs, Republic of Korea (to J.D.J.) and by grants from the National Institutes of Health (to L.B.I.) and the Arthritis Foundation (to K.-H.P.-M.).

Address correspondence and reprint requests to Dr. Jong Dae Ji, Division of Rheumatology, College of Medicine, Korea University, 126-1, Anam-Dong 5-Ga, Sungbuk-Ku, Seoul 136-705, South Korea. E-mail address: jjdimesy@korea.ac.kr

The online version of this article contains supplemental material.

Abbreviations used in this paper: BLNK, B cell linker; COX-2, cyclooxygenase-2; IL-1Ra, IL-1R antagonist; MMP, matrix metalloproteinase; OCP, osteoclast precursor; Pam3Cys, Pam<sub>3</sub>CysSer(Lys)<sub>4</sub>; PKC, protein kinase C; RA, rheumatoid arthritis; TRAP, tartrate-resistant acid phosphatase; TREM-2, triggering receptor expressed on myeloid cells 2.

Copyright © 2010 by The American Association of Immunologists, Inc. 0022-1767/10/\$16.00

augments RANKL-induced osteoclast differentiation and promotes osteoclast activation and survival (10). IL-1 also mediates TNF-induced bone resorption (11). The IL-1 gene family has several members, such as IL-1 $\alpha$ , IL-1 $\beta$ , and the IL-1R antagonist (IL-1Ra) (8). IL-1 $\alpha$  and IL-1 $\beta$  are agonists, and IL-1Ra is a specific receptor antagonist. There are two members of the IL-1R gene family. The type I receptor IL-1RI transduces signals, whereas IL-1RII does not transduce signals and instead works as a decoy receptor. IL-1 exerts its biological effects by forming a complex with the IL-1RI and IL-1R accessory protein. IL-1 uses the adaptor molecule MyD88 to activate signaling pathways leading to the activation of NF- $\kappa$ B and MAPKs and downstream transcription factors that drive inflammatory gene expression (12).

While inflammatory molecules, such as TLR ligands, drive bone destruction, these molecules also engage potent homeostatic mechanisms to limit damage associated with inflammation, and these mechanisms may limit the extent of bone resorption. Direct stimulation of various TLRs on OCPs inhibits RANKL-induced osteoclastogenesis in mouse cells (13–16), and we also found that TLR ligands inhibit human osteoclast differentiation by acting directly on OCPs (17). Generally, TLR ligands and other inflammatory molecules inhibit osteoclast differentiation at early stages of osteoclastogenesis, such as generation of OCPs, and lose their inhibitory properties at later stages, at which point they augment RANKL-induced osteoclast differentiation. In chronic inflammatory conditions, TLR ligands and other inflammatory molecules predominately work as pro-osteoclastogenic molecules, despite their direct inhibitory effect on the osteoclastogenesis.

TLRs and IL-1R share a cytosolic domain, termed Toll–IL-1R, and common intracellular signaling molecules, such as MyD88, IL-1R-associated kinase, and TNFR-associated factor 6 (12), and the known stimulatory effects of IL-1 $\beta$  on the osteoclast differentiation are similar to the effects of TLR ligands. These findings suggest the

possibility that IL-1 $\beta$  also may regulate osteoclast differentiation by acting directly on OCPs, similarly to TLR ligands. Because little is known about the direct effect on human osteoclastogenesis by IL-1 $\beta$ , we examined the effects of IL-1 $\beta$  on osteoclastogenesis in primary human peripheral blood monocytes and RA synovial macrophages. We found that IL-1 $\beta$  induces shedding and thereby inactivation of c-Fms that drives RANK expression and makes early human OCPs refractory to RANK stimulation by downregulating expression of RANK, its costimulatory receptor, triggering receptor expressed on myeloid cells 2 (TREM-2), and downstream signaling molecules, such as B cell linker (BLNK). These findings identify a homeostatic function for a predominately inflammatory cytokine and suggest a new mechanism that can restrain osteoclastogenesis in inflammatory settings.

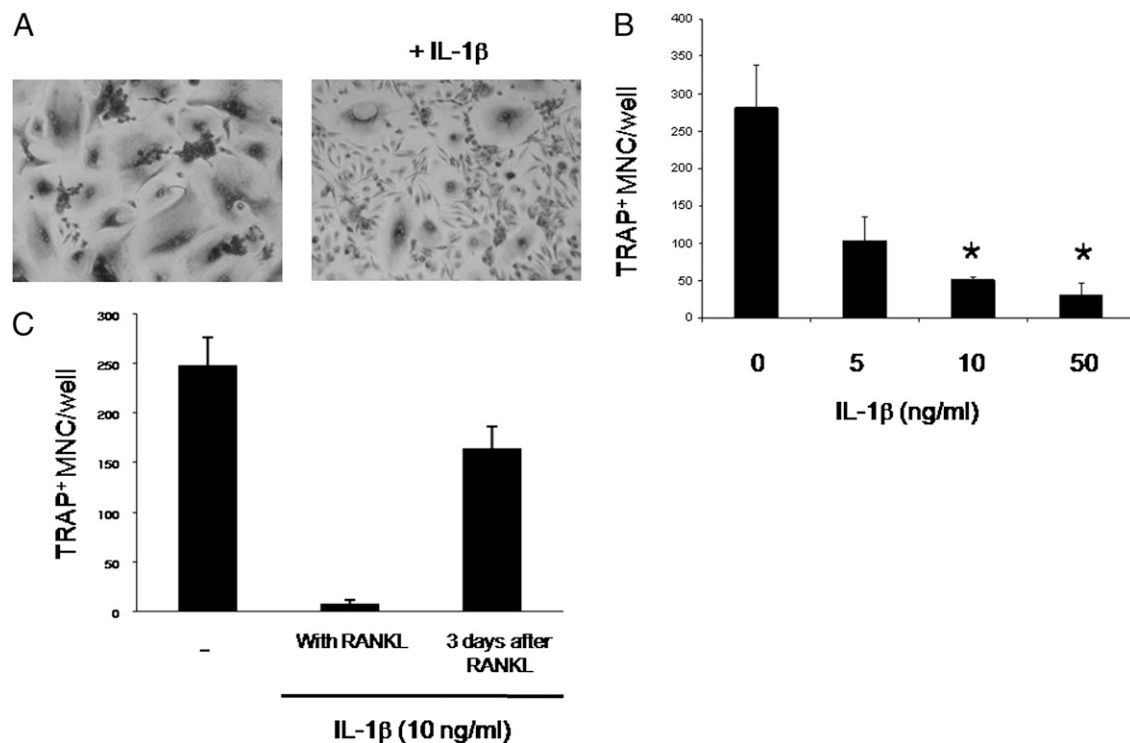
## Materials and Methods

### Materials

Recombinant human IL-1 $\beta$  was from R&D Systems (Minneapolis, MN), and human M-CSF and soluble RANKL were from PeproTech (Rocky Hill, NJ). SB203580, U0126, PD98059, GF 109203X, and TAPI-1 were purchased from Calbiochem (San Diego, CA). SB202190 was purchased from Sigma–Aldrich (St. Louis, MO). p38 Ab (catalog no. sc-535) was from Santa Cruz Biotechnology (Santa Cruz, CA), and STAT3 Ab (clone 84/Stat3) was from BD Transduction Laboratories (Franklin Lakes, NJ). M-CSFR Ab (catalog no. 3152) was from Cell Signaling Technology (Beverly, MA), and RANK Ab (clone 9A725) was from Alexis Biochemicals (San Diego, CA).

### Cell isolation and culture

PBMCs were obtained from normal blood donors or blood leukocyte preparations purchased from the New York Blood Center (New York, NY) by density gradient centrifugation with Ficoll (Invitrogen, Carlsbad, CA), using a protocol approved by the Hospital for Rheumatic Disease (Hanyang University, Seoul, Korea) and by the Hospital for Special Surgery (New York, NY) institutional review boards. Monocytes were obtained



**FIGURE 1.** IL-1 $\beta$  inhibits human osteoclastogenesis. Human monocytes were cultured with M-CSF (20 ng/ml) for 2 d, and then RANKL (40 ng/ml) was added for an additional 6 d. IL-1 $\beta$  (10 ng/ml) was added with RANKL or 3 d after RANKL treatment. Cells were stained for TRAP expression (original magnification  $\times 100$ ). TRAP-positive multinucleated (more than three nuclei/cell) cells were counted as osteoclasts. Data are shown as means  $\pm$  SD of triplicate determinants and are representative of more than three experiments. \* $p < 0.05$  versus control.

from PBMCs, using anti-CD14 magnetic beads, as recommended by the manufacturer (Miltenyi Biotec, Auburn, CA). Monocytes were cultured for 1 d in  $\alpha$ -MEM medium (Invitrogen) supplemented with 10% FBS (Hyclone, Logan, UT) with either M-CSF (20 ng/ml) in the presence or absence of IL-1 $\beta$ . Monocyte-derived preosteoclasts obtained after 2 d of culture with M-CSF were used unless otherwise noted in the figure legends, and the purity of monocytes/macrophages was >95%, as verified by flow cytometric analysis. RA synovial macrophages were obtained from RA synovial fluid mononuclear cells using anti-CD14 magnetic beads, as recommended by the manufacturer (Miltenyi Biotec). Thirteen patients were included in this study, and all patients fulfilled the revised American College of Rheumatology classification criteria for the diagnosis of RA. Except for one patient, the remaining 12 RA patients were treated with low-dose corticosteroids. Five patients were treated with methotrexate alone, two patients with methotrexate and sulfasalazine, two patients with methotrexate and leflunomide, one patient with methotrexate and tacrolimus, one patient with sulfasalazine, one patient with hydroxychloroquine, and one patient without antirheumatic drugs due to pregnancy. Twelve patients had active disease (erythrocyte sedimentation rate, 46–128 mm/h), and one patient had inactive disease (erythrocyte sedimentation rate, 9 mm/h). This study protocol was approved by the institutional review board of the Hospital for Rheumatic Disease, Hanyang University.

#### Osteoclast differentiation

Human CD14<sup>+</sup> cells were incubated with 20 ng/ml M-CSF for 2 d to generate OCPs. OCPs were further incubated with 20 ng/ml M-CSF and 40 ng/ml human soluble RANKL for an additional 6 d in  $\alpha$ -MEM

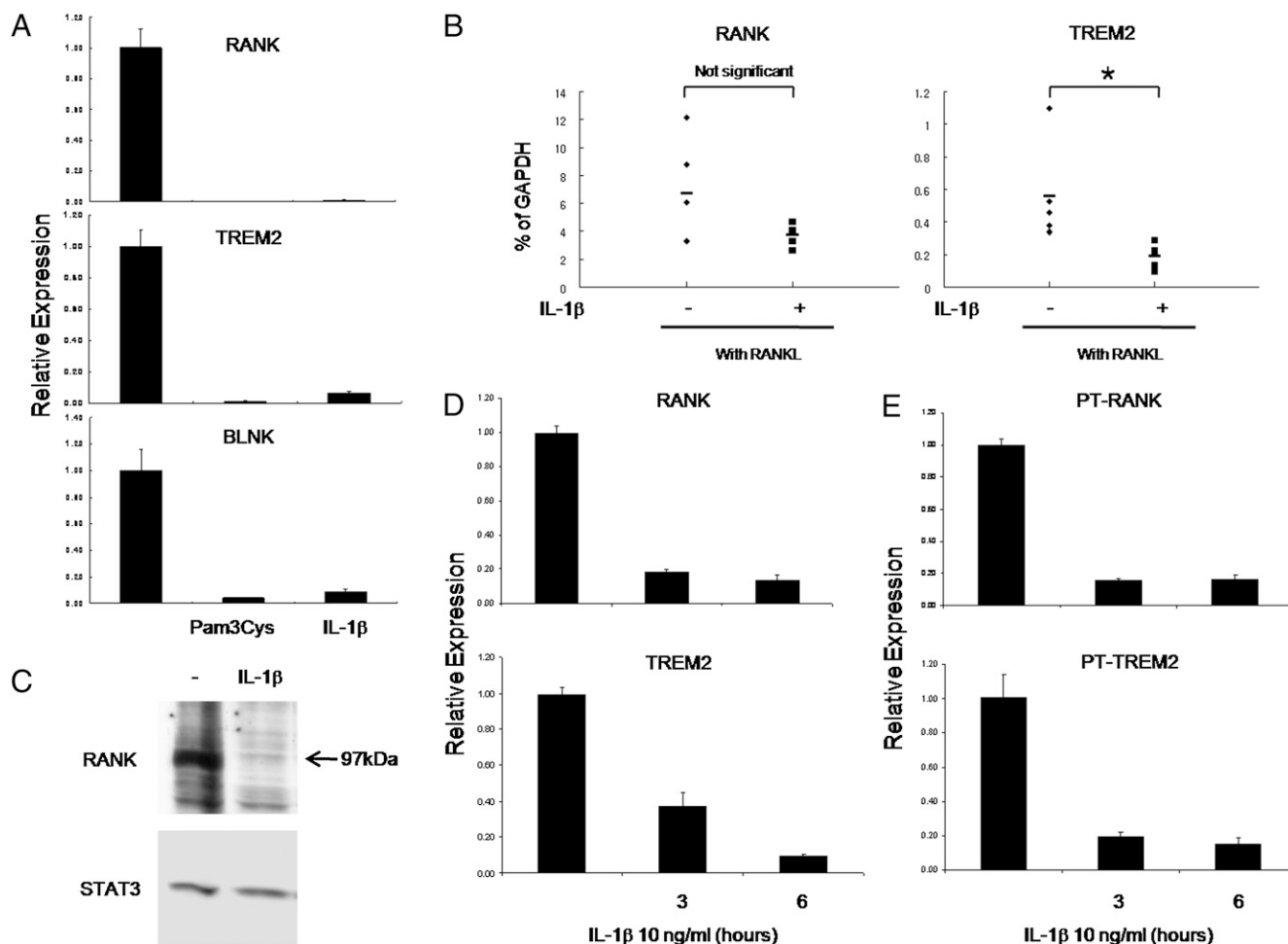
supplemented with 10% FBS. Cytokines were replenished every 3 d. On day 8, cells were fixed and stained for tartrate-resistant acid phosphatase (TRAP) using the Acid Phosphatase Leukocyte diagnostic kit (Sigma, San Diego, CA) as recommended by the manufacturer. Multinucleated (more than three nuclei) TRAP-positive osteoclasts were counted in triplicate wells.

#### Gene expression analysis

For real-time PCR, DNA-free RNA was obtained using the RNeasy Mini Kit (Qiagen, Hilden, Germany) or TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA) according to the manufacturer's instructions, and 1  $\mu$ g total RNA was reverse transcribed using a First Strand cDNA Synthesis kit (Fermentas, Hanover, MD). Real-time PCR was performed in triplicate using the iCycler iQ thermal cycler and detection system (Bio-Rad Laboratories, Hercules, CA) following the manufacturer's protocols. Expression of the tested gene was normalized relative to levels of GAPDH. Primary transcripts were measured using primers that amplified either exon-intron junctions or intronic sequences.

#### Immunoblotting

Whole-cell extracts were prepared by lysis in a buffer containing 20 mM HEPES (pH 7.0), 300 mM NaCl, 10 mM KCl, 1 mM MgCl<sub>2</sub>, 0.1% Triton X-100, 0.5 mM DTT, 20% glycerol, and 1 $\times$  proteinase inhibitor mixture (Roche, Basel, Switzerland). The protein concentration of extracts was quantified using a Bradford assay (Bio-Rad, Hercules, CA). For immunoblotting, 10  $\mu$ g cell lysates was fractionated on 7.5% polyacrylamide



**FIGURE 2.** IL-1 $\beta$  suppresses RANK and TREM-2 expression in human OCPs. *A*, Human monocytes were cultured with 20 ng/ml M-CSF in the presence or absence of IL-1 $\beta$  (10 ng/ml) or Pam3Cys (10 ng/ml) overnight, and mRNA was isolated and analyzed using real-time PCR. *B*, Human monocytes were cultured with 20 ng/ml M-CSF for 2 d, and then IL-1 $\beta$  (10 ng/ml) was added overnight with RANKL. RANK and TREM-2 mRNA expression ( $n = 5$  samples) was measured using real-time PCR, with results normalized to percentage of GAPDH. \* $p < 0.05$  versus control. *C*, Whole-cell lysates from OCPs were subjected to SDS-PAGE and immunoblotted with RANK and STAT3 Abs. *D*, Human monocytes were cultured with 20 ng/ml M-CSF overnight, and then IL-1 $\beta$  was added for the indicated times. mRNA levels were measured using real-time PCR and were normalized relative to the expression of GAPDH. *E*, Primary transcript levels of indicated genes were measured using real-time PCR and were normalized relative to the expression of GAPDH. Data are shown as means  $\pm$  SD of triplicate determinants and are representative of more than three experiments.

gels using SDS-PAGE, transferred to polyvinylidene fluoride membranes (Millipore, Billerica, MA), incubated with specific Abs, and ECL was used for detection.

*Flow cytometry*

Staining for cell surface expression of M-CSF receptor was performed using monoclonal anti-human M-CSF receptor (clone 61708; R&D Systems). A FACScan flow cytometer with CELLQuest software (Becton Dickinson, Franklin Lakes, NJ) was used.

*Statistical analysis*

Results are expressed as means ± SD. A Student *t* test was applied to evaluate group differences; a *p* value of <0.05 was considered significant.

**Results**

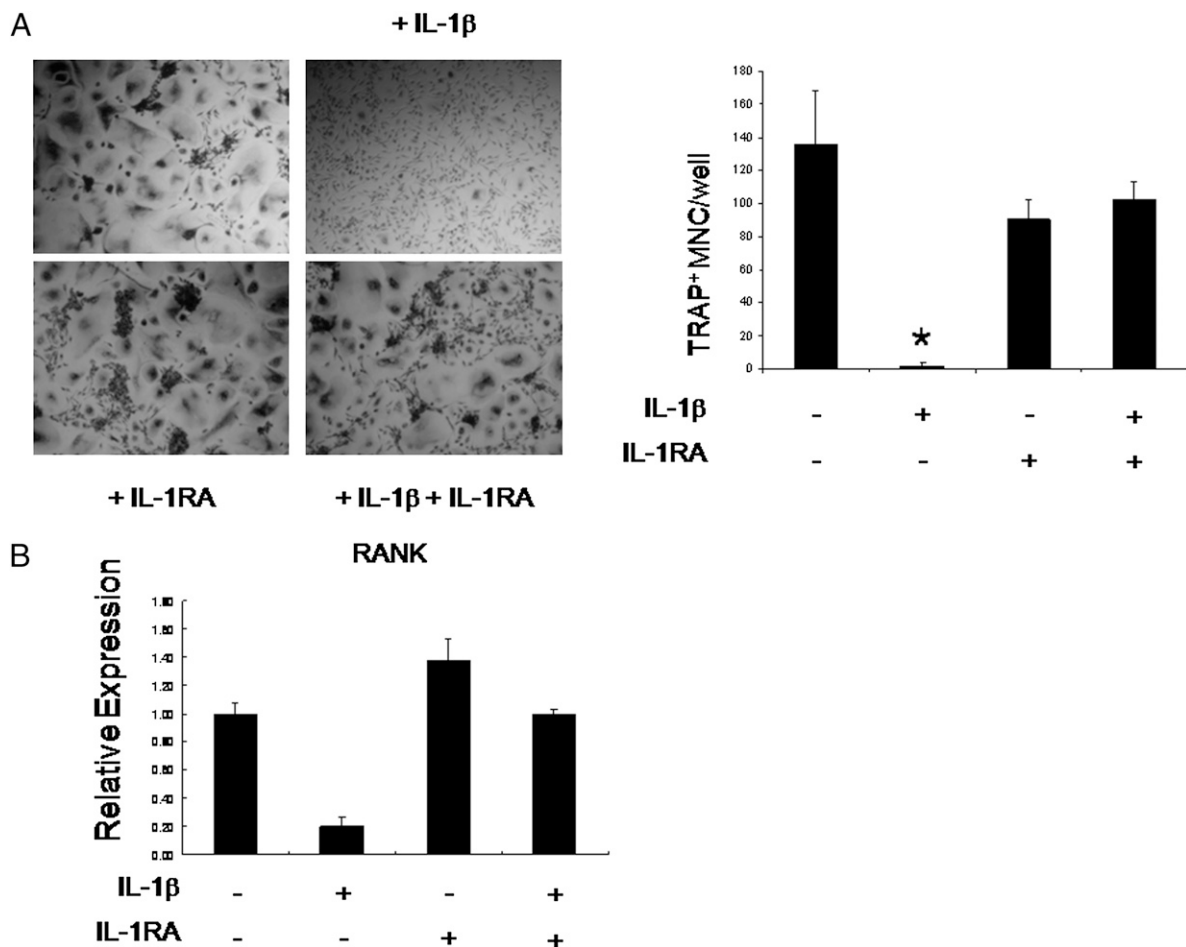
*IL-1β inhibits RANKL-induced human osteoclastogenesis*

We examined the effects of IL-1β on human osteoclastogenesis using a standard, validated system of human osteoclast differentiation (18). Treatment with M-CSF for 2 d induces the differentiation of primary human monocytes into RANKL-responsive OCPs via inducing RANK expression. OCPs are subsequently differentiated into mature osteoclasts in the presence of RANKL. Control cells cultured with M-CSF and RANKL efficiently differentiated into multinucleated (more than three nuclei/cell) TRAP-positive giant cells (Fig. 1A).

When added together with RANKL, treatment with IL-1β strongly inhibited RANKL-induced osteoclastogenesis (Fig. 1A). Osteoclastogenesis was inhibited by IL-1β in a dose-dependent fashion (Fig. 1B). IL-1β significantly inhibited osteoclastogenesis when added prior to addition of RANKL, although IL-1β-induced inhibition of osteoclastogenesis was less effective than when added together with RANKL (Supplemental Fig. 1). Also, when IL-1β was added several days after stimulation with RANKL, the IL-1β-induced inhibition of human osteoclastogenesis was significantly diminished (Fig. 1C). These results show a direct, but time-dependent, inhibitory effect of IL-1β on human osteoclast differentiation.

*IL-1β suppresses RANK, TREM-2, and BLNK expression in human OCPs*

To begin to investigate mechanisms by which IL-1β inhibits osteoclastogenesis, we examined the effects of IL-1β on RANK, TREM-2, and BLNK expression. Previously, we found that TLR ligands inhibit human osteoclast differentiation by suppressing RANK and TREM-2 expression (17). We compared the effects of IL-1β on RANK, TREM-2, and BLNK expression with the effects of Pam<sub>3</sub>CysSer(Lys)<sub>4</sub> (Pam3Cys; TLR2 ligand). TREM-2 is a DAP12-associated receptor that provides an essential ITAM-mediated costimulatory signal for RANKL-induced osteoclasto-



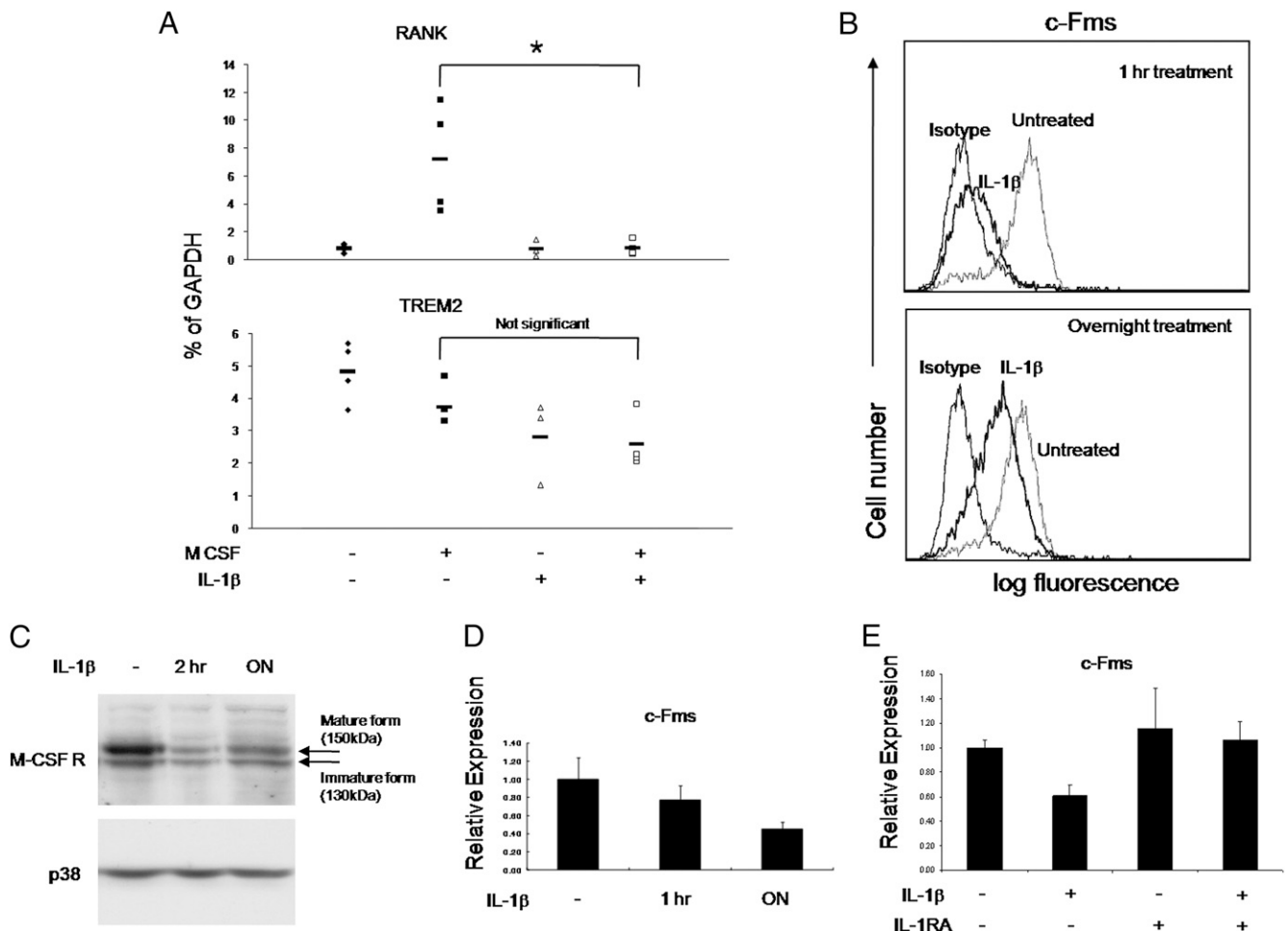
**FIGURE 3.** IL-1Ra reverses IL-1β-induced inhibition of human osteoclastogenesis and RANK gene expression. *A*, Human monocytes were cultured with M-CSF (20 ng/ml) for 2 d, and then RANKL (40 ng/ml) was added for an additional 6 d. IL-1β (10 ng/ml) and/or IL-1Ra (200 ng/ml) were added with RANKL. Cells were stained for TRAP expression (original magnification ×100). TRAP-positive multinucleated (more than three nuclei/cell) cells were counted as osteoclasts. Data are shown as means ± SD of triplicate determinants and are representative of more than three experiments. \**p* < 0.05 versus control. *B*, Human monocytes were cultured with 20 ng/ml M-CSF in the presence or absence of IL-1β (10 ng/ml) and/or IL-1Ra (200 ng/ml) overnight, and mRNA was isolated and analyzed using real-time PCR and was normalized relative to the expression of GAPDH. Data are shown as means ± SD of triplicate determinants and are representative of more than three experiments.

genesis in human cells (19–21). BLNK is an adaptor important for transducing RANK signals (22). Treatment of human OCPs with IL-1 $\beta$  resulted in a significant decrease of RANK, TREM-2, and BLNK mRNA, similar to treatment with Pam3Cys (Fig. 2A). Thus, pre-exposure to IL-1 $\beta$  very effectively suppresses expression of key molecules important for osteoclastogenesis, consistent with significant suppression of osteoclast differentiation when precursor cells were preincubated with IL-1 $\beta$  prior to addition of RANKL (Supplemental Fig. 1). Because IL-1 $\beta$  suppressed osteoclastogenesis when added together with RANKL (Fig. 1), we also tested the effects of simultaneous IL-1 $\beta$  and RANKL addition on RANK and TREM-2 expression. IL-1 $\beta$  significantly inhibited TREM-2 expression when added together with RANKL (Fig. 2B); inhibition of RANK was observed (Fig. 2B) but was less effective than in the absence of RANKL (Fig. 2A). These results suggest that downregulation of RANK contributes to the suppression of osteoclastogenesis when IL-1 $\beta$  and RANKL are added together, but that additional inhibitory mechanisms are engaged. Parallel to downregulation of RANK mRNA expression, expression of RANK protein was also suppressed (Fig. 2C). Also, RANK and TREM-2 mRNA decreased in a time-dependent man-

ner after IL-1 $\beta$  treatment (Fig. 2D). Inhibition of RANK, TREM-2, and BLNK expression was maintained for at least 2 d of IL-1 $\beta$  treatment (data not shown). These results suggest that IL-1 $\beta$  induces the unresponsiveness of OCPs to stimulation by RANKL via suppression of RANK, TREM-2, and BLNK expression in human OCPs.

We used primary transcript analysis (23) to determine the effects of IL-1 $\beta$  on transcription of the RANK and TREM-2 genes. The addition of IL-1 $\beta$  had a striking inhibitory effect on RANK and TREM-2 transcription (Fig. 2E).

Take et al. (24) found that PGE<sub>2</sub> inhibits osteoclast differentiation in human peripheral blood CD14<sup>+</sup> cells. IL-1 $\beta$  increases PGE<sub>2</sub> production via the induction and activation of cyclooxygenase-2 (COX-2). We wished to test whether the inhibitory effect of IL-1 $\beta$  on human osteoclastogenesis is mediated via the production of PGE<sub>2</sub> using NS398, a COX-2 inhibitor. However, addition of NS398 inhibited RANK expression and human osteoclastogenesis even in the absence of IL-1 $\beta$  (Supplemental Fig. 2), and thus the potential reversal of IL-1 $\beta$ -mediated inhibition of RANK expression and osteoclastogenesis by inhibition of PG production could not be addressed.



**FIGURE 4.** IL-1 $\beta$  inhibits M-CSF-induced RANK expression by downregulating cell surface c-Fms. *A*, Human monocytes were cultured overnight in the absence of M-CSF and then were stimulated with M-CSF  $\pm$  IL-1 $\beta$  (10 ng/ml) for 3 h. RANK and TREM-2 mRNA expression ( $n = 4$  samples) was measured using real-time PCR, with results normalized to percentage of GAPDH.  $*p < 0.05$  versus control. *B*, Human monocytes were stimulated with IL-1 $\beta$  for 1 h or overnight, and cell surface c-Fms levels were assessed by flow cytometry. *C*, Human monocytes were stimulated with IL-1 $\beta$  for the indicated time, and whole-cell lysates were analyzed by immunoblotting. The *upper arrow* marks mature cell surface c-Fms; the *lower arrow* marks the immature intracellular form. Data are representative of more than three experiments. *D*, Human monocytes were stimulated with IL-1 $\beta$  for the indicated time. mRNA levels were measured using real-time PCR and were normalized relative to the expression of GAPDH. *E*, Human monocytes were cultured with or without IL-1 $\beta$  or IL-1RA overnight. mRNA levels were measured using real-time PCR and were normalized relative to the expression of GAPDH. Data are shown as means  $\pm$  SD of triplicate determinants and are representative of more than three experiments.

We used IL-1Ra to block specifically IL-1 signaling and to confirm that the inhibitory effects observed were mediated by IL-1 $\beta$ . IL-1Ra significantly reversed the inhibition of human osteoclastogenesis by IL-1 $\beta$  (Fig. 3A). Also, the inhibition of RANK gene expression was completely reversed by IL-1Ra (Fig. 3B). These results demonstrated that human osteoclastogenesis is specifically suppressed by IL-1 $\beta$ .

*IL-1 $\beta$  downregulates c-Fms and inhibits M-CSF-induced RANK expression*

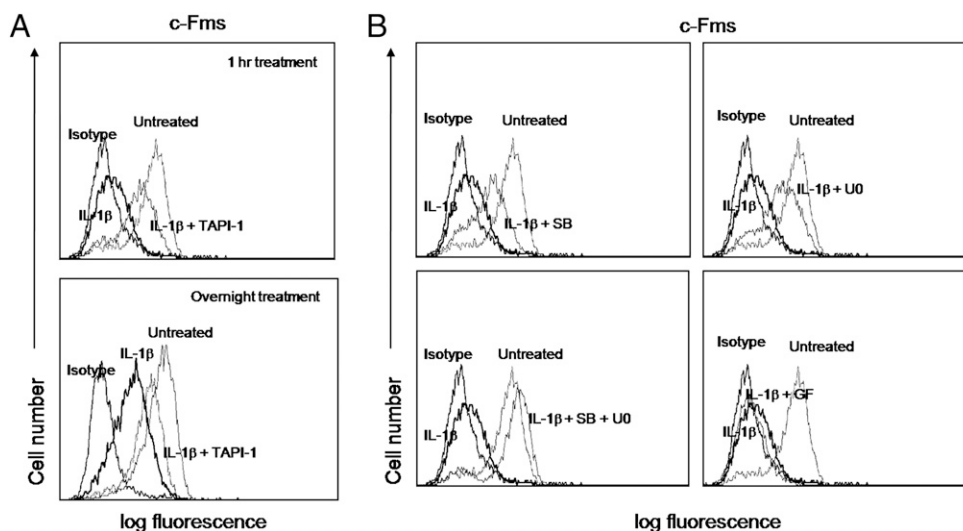
RANK expression is dependent on M-CSF, which binds to and signals via c-Fms (6). We tested whether IL-1 $\beta$  inhibits RANK expression by inhibiting the c-Fms-mediated induction. Isolated monocytes were cultured overnight in the absence of M-CSF to maintain low basal RANK expression, and then these cells were treated with M-CSF to induce the expression of RANK mRNA. M-CSF treatment rapidly induced expression of RANK mRNA, and this induction was significantly blocked by IL-1 $\beta$  treatment (Fig. 4A). In contrast with RANK expression, TREM-2 expression was not dependent on M-CSF (Fig. 4A).

To investigate the mechanism underlying inhibition of M-CSF-induced RANK expression, we examined the effect of IL-1 $\beta$  on surface expression of c-Fms. Cell surface expression of c-Fms was nearly completely downregulated by a 1-h treatment with IL-1 $\beta$  (Fig. 4B, upper panel) and remained partially suppressed after overnight incubation with IL-1 $\beta$  (Fig. 4B, lower panel). M-CSF receptor expression was examined using immunoblotting. Consistent with the results of flow cytometry, the mature form of the M-CSF receptor that is expressed on the cell surface (Fig. 4C, upper band) was rapidly downregulated by IL-1 $\beta$ , and this downregulation was partially maintained after overnight incubation with IL-1 $\beta$ . In contrast, the immature intracellular form of c-Fms (Fig. 4C, lower band) was minimally affected by IL-1 treatment. We then tested whether rapid downregulation of surface c-Fms expression is caused by inhibiting expression of c-Fms mRNA. In contrast with strong and rapid downregulation of protein expression, expression of c-Fms mRNA was minimally affected after 1-h treatment with IL-1 $\beta$  (Fig. 4D) but evidenced a slow, time-dependent decrease after addition of IL-1 $\beta$  (Fig. 4D).

These results suggest that inhibition of c-Fms gene expression is not the major mechanism of the early phase of downregulation of c-Fms expression observed after 1 h of IL-1 $\beta$  treatment, although decreased c-Fms mRNA levels could contribute to the decreased total cellular pool of c-Fms protein observed at the overnight time point. Similar to the results obtained with RANK mRNA, IL-1Ra completely reversed the inhibition of c-Fms gene expression by IL-1 $\beta$  (Fig. 4E).

Ectodomain shedding contributes to rapid downregulation of cell surface c-Fms expression by cell surface a disintegrin and metalloproteinases (25–27). To examine the involvement of metalloproteinases in the downregulation of M-CSF receptor, cells were pretreated with the metalloproteinase inhibitor TAPI-1 for 1 h before treatment with IL-1 $\beta$ . TAPI-1 significantly reversed the nearly complete downregulation of c-Fms expression induced by a 1-h treatment with IL-1 $\beta$  (Fig. 5A, upper panel). Inhibition of metalloproteinases with TAPI-1 completely reversed the downregulation of c-Fms cell surface expression induced by overnight treatment of IL-1 $\beta$  (Fig. 5A, lower panel). These results suggest that metalloproteinase-mediated proteolysis is involved in the downregulation of c-Fms observed after IL-1 $\beta$  treatment.

Ectodomain shedding can be induced by many inflammatory stimuli via protein kinase C (PKC) or via ERK or p38 MAPKs, but pathways that mediate IL-1-induced shedding have not been well studied (28–30). Previous studies demonstrated that PKC and ERK are involved in downregulation of M-CSF receptor expression (25, 31). Therefore, kinase inhibitors were used to examine the involvement of PKC, p38, and ERK in IL-1 $\beta$ -induced downregulation of the M-CSF receptor. The p38 inhibitor SB203580 partially reversed IL-1 $\beta$ -induced inhibition of c-Fms expression when used alone (Fig. 5B, upper left panel). IL-1 $\beta$ -induced inhibition of c-Fms expression was also reversed by SB202190, another selective inhibitor of p38 MAPK (data not shown). The MEK-ERK inhibitor U0126 also partially reversed downregulation of c-Fms expression when used alone (Fig. 5B, upper right panel). Downregulation of c-Fms expression was also reversed by PD98059, a specific MEK inhibitor that is structurally unrelated to U0126 (data not shown). Combined inhibition of ERKs and p38 completely abrogated IL-1 $\beta$ -induced down-



**FIGURE 5.** IL-1 $\beta$ -induced downregulation of cell surface c-Fms is dependent on MMPs and ERK and p38 MAPKs. *A*, Human monocytes were cultured overnight in the absence of M-CSF. These cells were stimulated with IL-1 $\beta$  for 1 h or overnight in the presence or absence of the MMP inhibitor TAPI-1. *B*, Cells were cultured as in *A*, these cells were stimulated with IL-1 $\beta$  for 1 h, and GF109203X (1  $\mu$ M), U0126 (40  $\mu$ M), SB203580 (10  $\mu$ M), or SP600125 (10  $\mu$ M) were added 1 h prior to adding IL-1 $\beta$ . Cell surface c-Fms levels were assessed by flow cytometry. Data are representative of more than three experiments.

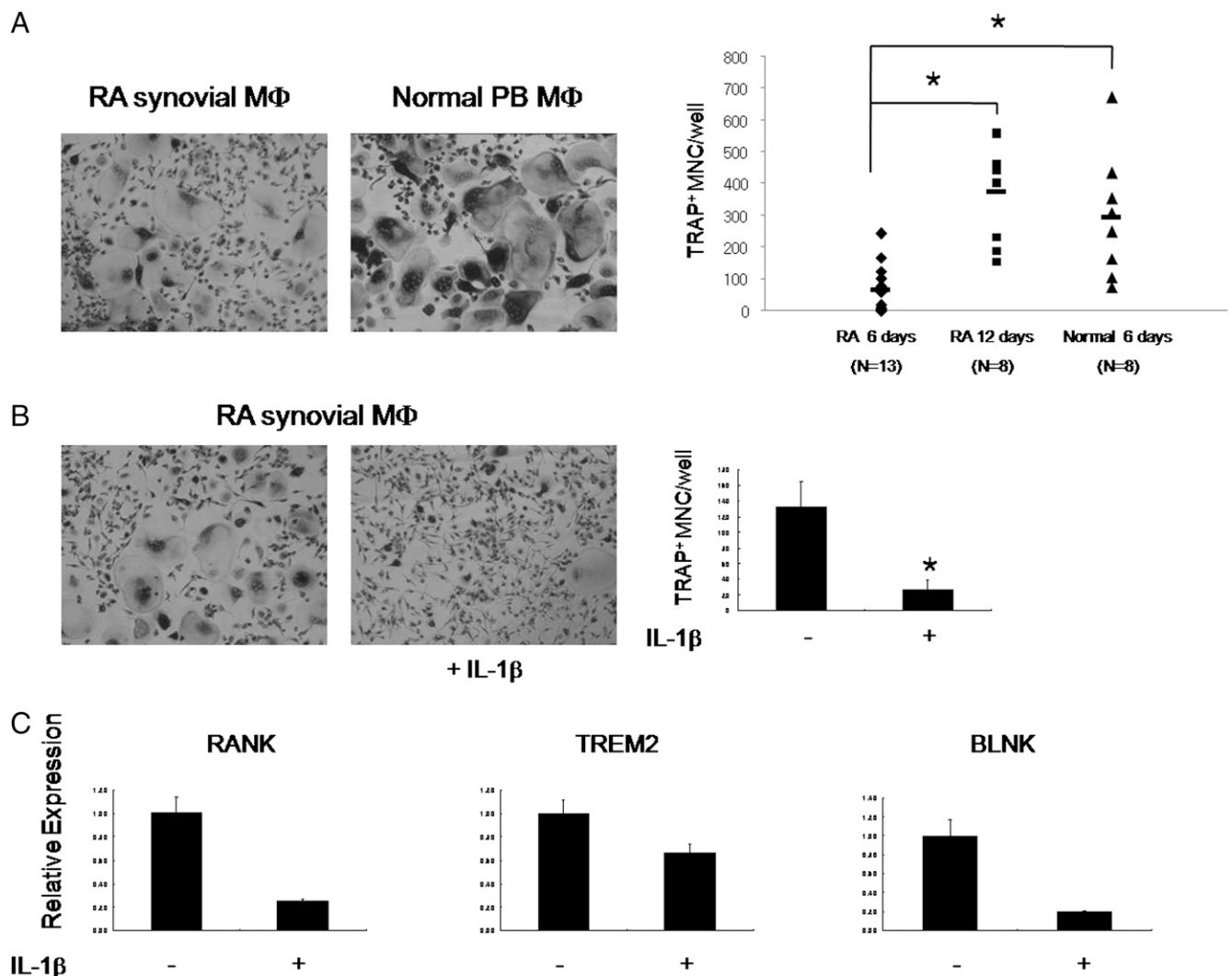
regulation of cell surface c-Fms expression (Fig. 5B, lower left panel). In contrast, inhibition of PKC had no detectable effect on IL-1 $\beta$ -induced downregulation of c-Fms (Fig. 5B, lower right panel).

#### IL-1 $\beta$ inhibits osteoclast differentiation from RA synovial fluid macrophages

To determine whether RA synovial fluid macrophages are capable of osteoclast differentiation and to compare the efficiency of osteoclastogenesis between RA synovial fluid macrophages and normal peripheral blood monocytes, macrophages were isolated from synovial fluids of RA patients and cultured with M-CSF and RANKL for 6 d. Compared with normal peripheral blood monocytes, RA synovial fluid macrophages exhibited decreased osteoclastogenesis when cultured with M-CSF and RANKL for 6 d (Fig. 6A). However, culture of synovial fluid macrophages for 12 d in the presence of M-CSF and RANKL resulted in comparable numbers of osteoclasts as in culture of normal peripheral blood

monocytes for 6 d. These results demonstrate that RA synovial fluid macrophages are capable of osteoclast differentiation but are less efficient in osteoclast formation than normal peripheral blood monocytes. Potential explanations for this result include that synovial monocytes have an intrinsically lower ability to form osteoclasts than blood monocytes, that the inflammatory synovial environment contains molecules that suppress monocyte potential to differentiate subsequently into osteoclasts, and that inflammatory synovial monocytes on ex vivo culture produce factors (such as IL-1 $\beta$ ) that act to restrain osteoclast differentiation. The latter possibility was tested using IL-1Ra to examine the involvement of IL-1 $\beta$  in the delayed osteoclast differentiation of RA synovial fluid macrophages. IL-1Ra did not affect the osteoclastogenesis of RA synovial OCPs in ex vivo cultures (Supplemental Fig. 3).

To examine the converse possibility, whether IL-1 $\beta$  can inhibit osteoclastogenesis in RA synovial fluid macrophages, we treated RA synovial fluid macrophages with IL-1 $\beta$  in the presence of



**FIGURE 6.** IL-1 $\beta$  inhibits osteoclast differentiation from RA synovial fluid macrophages and suppresses expression of RANK, TREM-2, and BLNK. **A**, Human monocytes and RA synovial macrophages were cultured with M-CSF (20 ng/ml) for 2 d, and then RANKL (40 ng/ml) was added for an additional 6 or 12 d. Cells were stained for TRAP expression (original magnification  $\times 100$ ). TRAP-positive multinucleated (more than three nuclei/cell) cells were counted as osteoclasts.  $*p < 0.05$  versus RA 6 d. **B**, RA synovial macrophages were cultured with M-CSF (20 ng/ml) and RANKL (40 ng/ml) for 6 d. IL-1 $\beta$  (10 ng/ml) was added with RANKL. Cells were stained for TRAP expression (original magnification  $\times 100$ ). TRAP-positive multinucleated (more than three nuclei/cell) cells were counted as osteoclasts.  $*p < 0.05$  versus control. **C**, RA synovial macrophages were cultured in the presence or absence of IL-1 $\beta$  (10 ng/ml) for 3 h, and mRNA was isolated and analyzed using real-time PCR and were normalized relative to the expression of GAPDH. Data are shown as means  $\pm$  SD of triplicate determinants and are representative of more than three experiments.



M-CSF and RANKL. Similar to normal peripheral blood monocytes, addition of IL-1 $\beta$  strongly inhibited RANKL-induced osteoclastogenesis in RA synovial fluid macrophages (Fig. 6B). To investigate the mechanisms by which IL-1 $\beta$  inhibits osteoclastogenesis in RA synovial fluid macrophages, we tested the effects of IL-1 $\beta$  on RANK, TREM-2, and BLNK expression. Addition of IL-1 $\beta$  to RA synovial fluid macrophages resulted in a decrease of RANK, TREM-2, and BLNK mRNA expression (Fig. 6C). Collectively, these results suggest that inflammatory molecules, such as IL-1 $\beta$ , can restrain osteoclastogenesis in inflammatory diseases, and this inhibition works as a homeostatic mechanism to prevent excessive bone destruction in inflammatory diseases, such as RA.

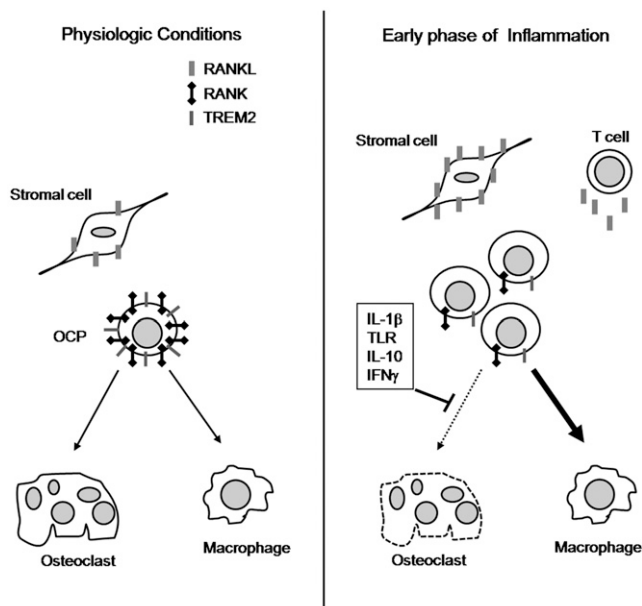
## Discussion

In this study, we have shown that IL-1 $\beta$  renders early human OCPs and RA synovial macrophages resistant to osteoclast differentiation in response to subsequent stimulation with RANKL. The mechanism of IL-1 $\beta$ -mediated inhibition of osteoclastogenesis involved coordinate inhibition of key signaling molecules required for cellular responses to RANKL–RANK, TREM-2, and BLNK. IL-1 downregulated RANK expression by inducing shedding and thereby inactivation of cell surface c-Fms, whose signaling is required to maintain RANK expression. Our findings identify a new homeostatic function for IL-1 $\beta$ , a cytokine best known for its inflammatory functions, including promoting bone resorption by inducing RANKL expression on stromal cells and cooperating with RANKL at later stages of osteoclast differentiation. A homeostatic role for IL-1 in tempering osteoclastogenesis is consistent with the emerging notions that many if not most inflammatory cytokines also activate feedback inhibition loops to restrain the amount of inflammation and associated toxicity and tissue damage. The findings also suggest that the extent of bone destruction in chronic inflammatory disease may be determined by the balance between inflammatory factors that promote osteoclastogenesis and the relative potency of homeostatic mechanisms described in this study and by us and others in previous studies (17, 32).

The effects of IL-1 $\beta$  on osteoclastogenesis are strikingly time-dependent. As demonstrated in this study using human OCPs and as suggested in previous work with murine cells (33), exposure to IL-1 $\beta$  prior to, or simultaneously with, RANKL suppresses osteoclastogenesis. On the contrary, exposure to IL-1 $\beta$  after RANKL stimulation has the opposite effect—promotion of osteoclast differentiation and resorptive function. In addition, IL-1 $\beta$  potently induces RANKL expression. Substantial evidence from both murine models, and from studies of patients treated with IL-1 blockers, such as anakinra (IL-1ra) (34–40), indicates that on balance IL-1 is a proresorptive cytokine in many disease settings. Indeed, IL-1 blockade in disease models and in clinical trials with human patients have shown that IL-1 blockade suppresses bone resorption, thus indicating that on balance in many settings *in vivo* the effects of IL-1 are predominately proresorptive. However, previous work has also found that IL-1 $\beta$  can suppress bone resorption in selective *in vivo* models (41, 42). In this context, our findings suggest that suppressive functions of IL-1 $\beta$  on osteoclastogenesis may become apparent and biologically important in limiting the extent of bone resorption under conditions where exposure to IL-1 precedes initial steps of differentiation in response to RANKL. Our experiments with RA synovial macrophages suggest that such conditions may exist at least in part in RA synovium. Although our results do not definitively resolve why RA synovial monocytes differentiate into osteoclasts less effectively than blood monocytes, the results show that exogenous IL-1 $\beta$  can suppress osteoclast differentiation of RA synovial monocytes, similar to suppression of blood monocytes. These results suggest that IL-1 $\beta$  that is present in the RA synovial environment may restrain and partially limit osteoclastogenesis, although this homeostatic mechanism is insufficient to fully prevent bone resorption. Overall, the results support the notion that IL-1 $\beta$  engages inhibitory feedback mechanisms to limit the extent of osteoclastogenesis, but that under most conditions the homeostatic functions of IL-1 $\beta$  are not enough to overcome the stimulatory effects on bone erosion. Similar dual effects have been reported for the inflammatory cytokines GM-CSF and IFN- $\gamma$ , although on balance these cytokines are more protective and IL-1 $\beta$  is more destructive.

In this study, we show that the expression of RANK in OCPs is driven by M-CSF, and M-CSF-dependent induction of the RANK gene was greatly suppressed by IL-1 $\beta$  through induced shedding of the M-CSF receptor. Activation of Erk1/2 and p38 played a significant role in IL-1 $\beta$ -induced downregulation of M-CSF receptor. Previous work has shown that TACE, which can be activated by either p38 or ERKs, participates in the cleavage of M-CSF receptor (27, 31, 43), and our results show that IL-1 $\beta$ -induced shedding of the M-CSF receptor is at least in part dependent on matrix metalloproteinases (MMPs). Based on these previous reports and our data, we suggest that MMPs, such as TACE, are involved in IL-1 $\beta$ -induced downregulation of the M-CSF receptor. Notably, expression of the MMP sheddases TACE and a disintegrin and metalloprotease 10 is elevated in RA synovial macrophages (44, 45), suggesting that increased shedding of macrophage cell surface receptors may contribute to the delayed kinetics of osteoclast differentiation we observed with these cells.

In this study, we examined whether PGE<sub>2</sub> endogenously produced by IL-1 $\beta$  is involved in the IL-1 $\beta$ -induced inhibition of RANK expression and osteoclastogenesis by using COX-2 inhibitor. However, we could not address this question due to the direct inhibitory effect of NS398 on human osteoclastogenesis. Recently, Kawashima et al. (46) reported that celecoxib and indomethacin, other COX inhibitors, directly inhibit human osteo-



**FIGURE 7.** Model of the effects of inflammatory stimuli on osteoclastogenesis during inflammation.

clast differentiation. Therefore, additional studies are needed to test whether PGE<sub>2</sub> is involved in the IL-1 $\beta$ -induced inhibition of human osteoclastogenesis.

Our results and previous work on the effects of inflammatory factors on osteoclastogenesis support a model (depicted schematically in Fig. 7) where during early phases of inflammation or early after entry of OCPs into inflamed tissues, inflammatory molecules, such as IL-1 $\beta$  and TLR ligands, downregulate expression of M-CSF receptors and RANK, TREM-2, and BLNK. This downregulation counterbalances the augmentation of RANKL expression in inflammatory conditions by making cells less responsive to RANKL and thus restrains the extent of osteoclastogenesis. However, as inflammation progresses, such inhibition can be overcome by high RANKL expression, expansion of OCP pools (47), and changes in inflammatory factor expression such that the cell microenvironment less effectively suppresses RANK expression (e.g., decreased expression of TLR ligands as infection is cleared). Under conditions of high and sustained RANKL expression, IL-1 $\beta$  will directly promote osteoclast differentiation and function in cooperation with RANKL, and thus the overall effect of IL-1 $\beta$  is to promote osteoclast formation and bone destruction.

## Disclosures

The authors have no financial conflicts of interest.

## References

- Takayanagi, H. 2007. Osteoimmunology: shared mechanisms and crosstalk between the immune and bone systems. *Nat. Rev. Immunol.* 7: 292–304.
- Teitelbaum, S. L. 2006. Osteoclasts; culprits in inflammatory osteolysis. *Arthritis Res. Ther.* 8: 201.
- Walsh, N. C., T. N. Crotti, S. R. Goldring, and E. M. Gravallese. 2005. Rheumatic diseases: the effects of inflammation on bone. *Immunol. Rev.* 208: 228–251.
- Bartold, P. M., R. I. Marshall, and D. R. Haynes. 2005. Periodontitis and rheumatoid arthritis: a review. *J. Periodontol.* 76(11, Suppl): 2066–2074.
- McInnes, I. B., and G. Schett. 2007. Cytokines in the pathogenesis of rheumatoid arthritis. *Nat. Rev. Immunol.* 7: 429–442.
- Arai, F., T. Miyamoto, O. Ohneda, T. Inada, T. Sudo, K. Brasel, T. Miyata, D. M. Anderson, and T. Suda. 1999. Commitment and differentiation of osteoclast precursor cells by the sequential expression of c-Fms and receptor activator of nuclear factor kappaB (RANK) receptors. *J. Exp. Med.* 190: 1741–1754.
- Boyce, B. F., P. Li, Z. Yao, Q. Zhang, I. R. Badell, E. M. Schwarz, R. J. O'Keefe, and L. Xing. 2005. TNF-alpha and pathologic bone resorption. *Keio J. Med.* 54: 127–131.
- Dinarello, C. A. 1994. The interleukin-1 family: 10 years of discovery. *FASEB J.* 8: 1314–1325.
- Gowen, M., D. D. Wood, E. J. Ihrle, M. K. McGuire, and R. G. Russell. 1983. An interleukin 1 like factor stimulates bone resorption in vitro. *Nature* 306: 378–380.
- Nakamura, I., and E. Jimi. 2006. Regulation of osteoclast differentiation and function by interleukin-1. *Vitam. Horm.* 74: 357–370.
- Wei, S., H. Kitaura, P. Zhou, F. P. Ross, and S. L. Teitelbaum. 2005. IL-1 mediates TNF-induced osteoclastogenesis. *J. Clin. Invest.* 115: 282–290.
- Dunne, A., and L. A. O'Neill. 2003. The interleukin-1 receptor/Toll-like receptor superfamily: signal transduction during inflammation and host defense. *Sci. STKE* 2003: re3.
- Ha, H., J. H. Lee, H. N. Kim, H. B. Kwak, H. M. Kim, S. E. Lee, J. H. Rhee, H. H. Kim, and Z. H. Lee. 2008. Stimulation by TLR5 modulates osteoclast differentiation through STAT1/IFN-beta. *J. Immunol.* 180: 1382–1389.
- Ishii, J., R. Kitazawa, K. Mori, K. P. McHugh, E. Morii, T. Kondo, and S. Kitazawa. 2008. Lipopolysaccharide suppresses RANK gene expression in macrophages by down-regulating PU.1 and MITF. *J. Cell. Biochem.* 105: 896–904.
- Takami, M., N. Kim, J. Rho, and Y. Choi. 2002. Stimulation by toll-like receptors inhibits osteoclast differentiation. *J. Immunol.* 169: 1516–1523.
- Zou, W., and Z. Bar-Shavit. 2002. Dual modulation of osteoclast differentiation by lipopolysaccharide. *J. Bone Miner. Res.* 17: 1211–1218.
- Ji, J. D., K. H. Park-Min, Z. Shen, R. J. Fajardo, S. R. Goldring, K. P. McHugh, and L. B. Ivashkiv. 2009. Inhibition of RANK expression and osteoclastogenesis by TLRs and IFN-gamma in human osteoclast precursors. *J. Immunol.* 183: 7223–7233.
- Sørensen, M. G., K. Henriksen, S. Schaller, D. B. Henriksen, F. C. Nielsen, M. H. Dziegiel, and M. A. Karsdal. 2007. Characterization of osteoclasts derived from CD14+ monocytes isolated from peripheral blood. *J. Bone Miner. Metab.* 25: 36–45.
- Cella, M., C. Buonsanti, C. Strader, T. Kondo, A. Salmaggi, and M. Colonna. 2003. Impaired differentiation of osteoclasts in TREM-2-deficient individuals. *J. Exp. Med.* 198: 645–651.
- Colonna, M., I. Turnbull, and J. Klesney-Tait. 2007. The enigmatic function of TREM-2 in osteoclastogenesis. *Adv. Exp. Med. Biol.* 602: 97–105.
- Park-Min, K. H., J. D. Ji, T. Antoniv, A. C. Reid, R. B. Silver, M. B. Humphrey, M. Nakamura, and L. B. Ivashkiv. 2009. IL-10 suppresses calcium-mediated costimulation of receptor activator NF-kappa B signaling during human osteoclast differentiation by inhibiting TREM-2 expression. *J. Immunol.* 183: 2444–2455.
- Shinohara, M., T. Koga, K. Okamoto, S. Sakaguchi, K. Arai, H. Yasuda, T. Takai, T. Kodama, T. Morio, R. S. Geha, et al. 2008. Tyrosine kinases Btk and Tec regulate osteoclast differentiation by linking RANK and ITAM signals. *Cell* 132: 794–806.
- Murray, P. J. 2005. The primary mechanism of the IL-10-regulated anti-inflammatory response is to selectively inhibit transcription. *Proc. Natl. Acad. Sci. USA* 102: 8686–8691.
- Take, I., Y. Kobayashi, Y. Yamamoto, H. Tsuboi, T. Ochi, S. Uematsu, N. Okafuji, S. Kurihara, N. Udagawa, and N. Takahashi. 2005. Prostaglandin E2 strongly inhibits human osteoclast formation. *Endocrinology* 146: 5204–5214.
- Baccarini, M., P. Dello Sbarba, D. Buscher, A. Bartocci, and E. R. Stanley. 1992. IFN-gamma/lipopolysaccharide activation of macrophages is associated with protein kinase C-dependent down-modulation of the colony-stimulating factor-1 receptor. *J. Immunol.* 149: 2656–2661.
- Rettenmier, C. W., M. F. Roussel, R. A. Ashmun, P. Ralph, K. Price, and C. J. Sherr. 1987. Synthesis of membrane-bound colony-stimulating factor 1 (CSF-1) and downmodulation of CSF-1 receptors in NIH 3T3 cells transformed by cotransfection of the human CSF-1 and c-fms (CSF-1 receptor) genes. *Mol. Cell. Biol.* 7: 2378–2387.
- Rovida, E., A. Paccagnini, M. Del Rosso, J. Peschon, and P. Dello Sbarba. 2001. TNF-alpha-converting enzyme cleaves the macrophage colony-stimulating factor receptor in macrophages undergoing activation. *J. Immunol.* 166: 1583–1589.
- Garton, K. J., P. J. Gough, and E. W. Raines. 2006. Emerging roles for ectodomain shedding in the regulation of inflammatory responses. *J. Leukoc. Biol.* 79: 1105–1116.
- Takenobu, H., A. Yamazaki, M. Hirata, T. Umata, and E. Mekada. 2003. The stress- and inflammatory cytokine-induced ectodomain shedding of heparin-binding epidermal growth factor-like growth factor is mediated by p38 MAPK, distinct from the 12-O-tetradecanoylphorbol-13-acetate- and lysophosphatidic acid-induced signaling cascades. *J. Biol. Chem.* 278: 17255–17262.
- Xu, K. P., D. Zoukhri, J. D. Zieske, D. A. Dartt, C. Sergheraert, E. Loing, and F. S. Yu. 2001. A role for MAP kinase in regulating ectodomain shedding of APLP2 in corneal epithelial cells. *Am. J. Physiol. Cell Physiol.* 281: C603–C614.
- Glenn, G., and P. van der Geer. 2008. Toll-like receptors stimulate regulated intramembrane proteolysis of the CSF-1 receptor through Erk activation. *FEBS Lett.* 582: 911–915.
- Lorenzo, J., M. Horowitz, and Y. Choi. 2008. Osteoimmunology: interactions of the bone and immune system. *Endocr. Rev.* 29: 403–440.
- Kim, J. H., H. M. Jin, K. Kim, I. Song, B. U. Youn, K. Matsuo, and N. Kim. 2009. The mechanism of osteoclast differentiation induced by IL-1. *J. Immunol.* 183: 1862–1870.
- Aksentijevich, I., S. L. Masters, P. J. Ferguson, P. Dancy, J. Frenkel, A. van Royen-Kerkhoff, R. Laxer, U. Tedgård, E. W. Cowen, T. H. Pham, et al. 2009. An autoinflammatory disease with deficiency of the interleukin-1-receptor antagonist. *N. Engl. J. Med.* 360: 2426–2437.
- Dinarello, C. A. 2010. Anti-inflammatory agents: present and future. *Cell* 140: 935–950.
- Gabay, C., C. Lamacchia, and G. Palmer. 2010. IL-1 pathways in inflammation and human diseases. *Nat. Rev. Rheumatol.* 6: 232–241.
- Horai, R., S. Saijo, H. Tanioka, S. Nakae, K. Sudo, A. Okahara, T. Ikuse, M. Asano, and Y. Iwakura. 2000. Development of chronic inflammatory arthropathy resembling rheumatoid arthritis in interleukin 1 receptor antagonist-deficient mice. *J. Exp. Med.* 191: 313–320.
- Jiang, Y., H. K. Genant, I. Watt, M. Cobby, B. Bresnihan, R. Aitchison, and D. McCabe. 2000. A multicenter, double-blind, dose-ranging, randomized, placebo-controlled study of recombinant human interleukin-1 receptor antagonist in patients with rheumatoid arthritis: radiologic progression and correlation of Genant and Larsen scores. *Arthritis Rheum.* 43: 1001–1009.
- Reddy, S., S. Jia, R. Geoffrey, R. Lorier, M. Suchi, U. Broeckel, M. J. Hessner, and J. Verbsky. 2009. An autoinflammatory disease due to homozygous deletion of the IL1RN locus. *N. Engl. J. Med.* 360: 2438–2444.
- Sims, J. E., and D. E. Smith. 2010. The IL-1 family: regulators of immunity. *Nat. Rev. Immunol.* 10: 89–102.
- Bajayo, A., I. Goshen, S. Feldman, V. Csernus, K. Iverfeldt, E. Shohami, R. Yirmiya, and I. Bab. 2005. Central IL-1 receptor signaling regulates bone growth and mass. *Proc. Natl. Acad. Sci. USA* 102: 12956–12961.
- Vargas, S. J., A. Naprta, M. Glaccum, S. K. Lee, J. Kalinowski, and J. A. Lorenzo. 1996. Interleukin-6 expression and histomorphometry of bones from mice deficient in receptors for interleukin-1 or tumor necrosis factor. *J. Bone Miner. Res.* 11: 1736–1744.
- Xu, P., and R. Derynck. 2010. Direct activation of TACE-mediated ectodomain shedding by p38 MAP kinase regulates EGF receptor-dependent cell proliferation. *Mol. Cell* 37: 551–566.
- Ohta, S., M. Harigai, M. Tanaka, Y. Kawaguchi, T. Sugiura, K. Takagi, C. Fukasawa, M. Hara, and N. Kamatani. 2001. Tumor necrosis factor-alpha (TNF-alpha) converting enzyme contributes to production of TNF-alpha in synovial tissues from patients with rheumatoid arthritis. *J. Rheumatol.* 28: 1756–1763.
- van der Voort, R., A. W. van Lieshout, L. W. Toonen, A. W. Slöetjes, W. B. van den Berg, C. G. Figdor, T. R. Radstake, and G. J. Adema. 2005. Elevated CXCL16 expression by synovial macrophages recruits memory T cells into rheumatoid joints. *Arthritis Rheum.* 52: 1381–1391.
- Kawahima, M., Y. Fujikawa, I. Itonaga, C. Takita, and H. Tsumura. 2009. The effect of selective cyclooxygenase-2 inhibitor on human osteoclast precursors to influence osteoclastogenesis in vitro. *Mod. Rheumatol.* 19: 192–198.
- Schett, G. 2007. Erosive arthritis. *Arthritis Res. Ther.* 9(Suppl 1): S2.