Bone- and Cartilage-Protective Effects of a Monoclonal Antibody Against Colony-Stimulating Factor 1 Receptor in Experimental Arthritis

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Objective. Colony-stimulating factor 1 receptor (CSF-1R) essentially modulates monocyte proliferation, migration, and activation, which are considered important for the pathogenesis of rheumatoid arthritis (RA). We undertook this study to determine CSF-1R expression in human RA as well as the efficacy of a specific anti-CSF-1R monoclonal antibody (AFS98) in 2 different animal models of RA.

Methods. CSF-1R expression was examined in blood, synovium, and bone samples from RA patients, osteoarthritis (OA) patients, and healthy subjects. The efficacy of AFS98 was examined by clinical assessment, histology, and bone histomorphometry in collagen-induced arthritis (CIA) and serum-transfer arthritis.

Results. CSF-1R expression was increased in the synovium of RA patients compared to OA patients and healthy controls in fibroblast-like synoviocytes, follicular dendritic cells, macrophages, and osteoclasts. Circulating RA monocytes and neutrophils but not lymphocytes were CSF-1R+. In mice, blockade of CSF-1R abrogated cartilage damage, bone erosion, and systemic bone loss, and this was associated with the depletion of osteoclasts in both models. While blockade of CSF-1R did not affect inflammation in passive serum-transfer arthritis, it significantly reduced inflammation in CIA, and this was associated with the absence of synovial macrophages and reduced splenic CD11b+Gr-1− monocytes.

Conclusion. CSF-1R was broadly expressed in human RA. Blockade of CSF-1R protected against bone and cartilage destruction in both mouse models and also showed significant anti-inflammatory effects in the CIA model. These data provide evidence for CSF-1R as a therapeutic target in RA.

Joint destruction in rheumatoid arthritis (RA) is mediated by inflammatory synovial tissue and subsequent osteoclastic bone resorption (1). This process is guided by inflammatory cytokines, which induce the expression of key factors involved in osteoclast differentiation. While numerous studies have shown the importance of RANKL in osteoclast-mediated bone resorption in both experimental inflammatory arthritis and human RA, data have been very limited to date concerning the role of the second essential factor for osteoclast differentiation, colony-stimulating factor 1 (CSF-1).

The human CSF-1 receptor (CSF-1R; c-Fms) is a
transmembrane homodimeric type III receptor tyrosine kinase encoded by the c-Fms protooncogene, and it is part of the CSF-1R/platelet-derived growth factor receptor subfamily (2). CSF-1R is expressed in myelomonocytic lineage cells, including hemopoietic progenitors, tissue macrophages, immature dendritic cells (DCs), mature osteoclasts, and B cells, which are implicated in RA pathogenesis and bone destruction (2). Its ligand, CSF-1, is highly expressed in the synovial membrane of RA patients, and CSF-1 levels are increased in the serum and synovial fluid of RA patients and associated with disease activity (2,3). Similarly, interleukin-34 (IL-34), recently discovered as a second ligand for CSF-1R, is also elevated in the serum and synovial fluid of RA patients (4,5). One study demonstrated CSF-1R expression both systemically in RA peripheral blood mononuclear cells and in synovium using polyclonal antibodies against CSF-1R; however, the exact cellular targets were not well defined (6).

CSF-1R ligands, CSF-1 and more recently IL-34, have critical roles in canonical and noncanonical osteoclastogenesis and bone resorption, and they act independently of and in synergy with RANKL (7,8). CSF-1 has a pivotal role in regulating differentiation of monocytes and mobilizing them from the bone marrow, as well as in their activation and survival (2). CSF-1 and/or IL-34 are induced by and act downstream of the key proinflammatory cytokines tumor necrosis factor α (TNFα) and IL-1β, which are implicated in RA pathogenesis, and in turn they stimulate macrophage differentiation and growth as well as production of inflammatory mediators (5,9). Unlike TNFα and IL-1β, CSF-1 has a weak direct stimulatory effect but plays an important regulatory role by priming monocytes to respond to endotoxin by up-regulating CD14 (10,11). Furthermore, CSF-1 has potent chemotactic effects on monocytes, recruiting them to sites of inflammation, and regulates the differentiation and immune function of DCs (12–14). In RA, CSF-1 may promote the development of a subset of activated monocytes that express CD16 and Toll-like receptor 2 and secrete several proinflammatory cytokines (15).

CSF-1 has been reported to have a role in experimental arthritis. Direct intraarticular or systemic injection of CSF-1 exacerbates experimental arthritis associated with increased levels of synovial macrophages (16–18). Similarly, overexpression of CSF-1 in CSF-1R–expressing cells leads to macrophage activation, osteoporosis, and premature death (19). Osteopetrotic mice deficient in CSF-1 (Csfop/Csfop) or CSF-1R (Csf1r−/−) have a marked reduction in numbers of synovial tissue macrophages, DCs, and osteoclasts (20,21). Neutralizing antibodies against CSF-1 inhibit ovarietomy-induced bone loss in mice (22). Furthermore, blockade of CSF-1 has antiarthritic effects in collagen-induced arthritis (CIA) (18). Nonspecific small-molecule CSF-1R tyrosine kinase inhibitors inhibit both synovial inflammation and inflammatory bone loss in CIA (23–27). In contrast to studies with nonspecific small-molecule CSF-1R tyrosine kinase inhibitors demonstrating antiarthritic effects, there are few reported studies of specific CSF-1R antagonism in experimental arthritis (9,28).

In this study, we first investigated CSF-1R expression in human RA using a newly generated monoclonal antibody (mAb) against human CSF-1R. We then determined the efficacy of specific anti–CSF-1R antibody in 2 different models of experimental arthritis.

**PATIENTS AND METHODS**

**Patients and healthy volunteers.** We studied 22 RA patients, 21 age- and sex-matched osteoarthritis (OA) patients, and 6 healthy control donors. RA patients fulfilled the American College of Rheumatology/European League Against Rheumatism classification criteria (29), and disease activity was measured by the Disease Activity Score in 28 joints (DAS28) (30,31). RA patients were predominantly women (87.5%) and had a mean age of 55 years. They had longstanding (mean duration 19 years) and active disease with a mean DAS28 of 5.1 (range 3.6–7.2), a mean erythrocyte sedimentation rate of 21 mm/hour (range 5–80), and a mean C-reactive protein level of 4 mg/dl (range 0.2–30.5). They were taking an average of 1.8 (range 0–3) disease-modifying antirheumatic drugs (DMARDs) including methotrexate, low-dose corticosteroids, and biologic agents (infliximab, adalimumab, abatacept, rituximab). Procedures were approved by the local ethics committee, and patients provided written informed consent.

**Detection of human CSF-1R expression.** Synovium/bone samples were obtained from RA patients, OA patients, or healthy controls undergoing routine joint surgery at the University of Erlangen–Nuremberg and from Tissue Solutions. Synovial cryosections (4 μm) were serially incubated with 10% goat serum, anti–human CSF-1R mAb (mouse IgG2a, clone CXIIAG6; Transgene) (32), dextran polymer–horseradish peroxidase (HRP)–conjugated goat anti-mouse IgG (Envision K4001; Dako), and diaminobenzidine chromogen (Dako), and then counterstained with hematoxylin. In negative control sections, a mouse IgG2a isotype control (clone C.1.18.4; BD Biosciences) was used. CSF-1R+ cells were quantified by counting positive cells in 10 consecutive high-power fields (hpf; 200× magnification). The number of positive cells/hpf was averaged, and results were expressed as the number of CSF-1R+ cells/mm² using Histolab microvision, version 5.9.2 (33). Histopathologic severity of synovial inflammation was graded as described (34).

Paraffin-embedded sections of RA bone tissue were stained with tartrate-resistant acid phosphatase (TRAP) (386A-1KT; Sigma) and for CSF-1R using a polyclonal rabbit
anti–CSF-1R mAb (Clone C-20, sc692; Santa Cruz Biotechnology) or a rabbit IgG isotype control (PA1-27414; Thermo Fisher Scientific). CSF-1R double staining was performed by immunofluorescence. Briefly, synovial cytossections were incubated with anti–human CSF-1R mAb and then with antibodies against vimentin (mouse IgG1, clone V9; Dako), CD68 (mouse IgG2a, clone 514H12; AbD Serotec), CD1a (mouse IgG1, clone O10; Dako), CD3 (mouse IgG2a, clone PS1; Thermo Fisher Scientific), CD20 (mouse IgG2a, clone L26; Thermo Fisher Scientific), CD21 (mouse IgG1, clone 1F8; Dako), or CD208 (rat IgG2a, clone 1010E1.01; AbCys). In negative control sections, a mouse IgG2a isotype control (clone C.1.18.4; BD Biosciences or clone MG2a-53; BioLegend), rat or CD208 (rat IgG2a, clone O10; Dako), or CD208 (rat IgG2a, clone 1010E1.01; AbCys). In negative control sections, a mouse IgG2a isotype control (clone C.1.18.4; BD Biosciences or clone MG2a-53; BioLegend), rat IgG2a (clone R25-95; BD Biosciences), or mouse IgG1 (AbD Serotec) was used. Counterstaining was performed with DAPI (BD Biosciences). Double staining was evaluated by colocalization of fluorescence using an RGB Profiler (35).

Arthritis induction. CIA was induced in 7–9-week-old male DBA/1 mice (Janvier). Bovine type II collagen (BII)/H9262/H37Ra (4 mg/ml; Sigma). One hundred microliters tuberculin of fluorescence using an RGB Profiler (35). Arthritis incidence and severity were assessed macroscopically (15) daily by 2 independent observers (SP, BS) under blinded conditions. Arthritis was graded on a scale of 0–4 for each paw: 0 = no swelling; 1 = slight swelling and erythema with ≥0.1 mm increase in paw size; 2 = swelling and erythema with ≥0.2 mm increase in paw size; 3 = extensive swelling ≥0.3 mm and erythema with severe joint deformity or ankylosis; 4 = pronounced swelling ≥0.45 mm and joint deformity or ankylosis. Each limb was graded and scores were summed, yielding a maximum score of 16 per mouse. Serum-transfer arthritis was induced by intraperitoneal (IP) injection of 300 μl of serum from 6–12-week-old arthritic K/BxN mice (27). Arthritis incidence and severity were assessed macroscopically as described above.

Mice were injected IP with 25 mg/kg/day or 50 mg/kg/day of a murine anti–CSF-1R mAb (mouse IgG2a, clone AFS98; kindly provided by Dr. Shin-Ichi Nishikawa, RIKEN Center for Developmental Biology, Kobe, Japan), with isotype control (rat IgG; Rockland), or with phosphate buffered saline (PBS) for 7 or 14 days beginning at the onset of serum-transfer arthritis or CIA, respectively.

Histologic assessment of murine arthritis. In CIA, paraffin-embedded hind limb joints stained with hematoxylin (Sigma), TRAP, or toluidine blue (Sigma) were assessed for inflammation (0–3 scale), cartilage damage (0–3 scale), and bone destruction (0–3 scale). Numbers of TRAP-positive osteoclasts were quantified by 2 independent observers (SP, BS) using OsteoMeasure software (37).

Double staining for CSF-1R/F4/80+ macrophages was performed by immunofluorescence. Briefly, sections were serially incubated with rabbit anti–CSF-1R mAb or isotype control (Affinity BioReagents), dextran polymer–HRP–conjugated goat anti-rabbit IgG, and fluorescein isothiocyanate (FITC)–conjugated cyclosporin A (CSA) (PerkinElmer). Sections were serially incubated with rat anti-mouse F4/80 (Caltag) or IgG2a isotype control (clone R25-95; BD Pharmingen), biotinylated rabbit anti-rat IgG (Vector), streptavidin–HRP (SouthernBiotech), and Cy3-conjugated CSA (PerkinElmer), and then counterstained with DAPI. Double staining was evaluated by colocalization of fluorescence using an RGB Profiler.

Flow cytometry. Red blood cells in whole blood obtained from patients were lysed (BD Pharm Lyse; BD Biosciences), and cells were incubated serially with goat anti-human IgG (Fc (Rockland), anti–human CSF-1R mAb or mouse IgG2a isotype control (Rockland), phycoerythrin (PE)–conjugated anti-mouse IgG, PE–Cy7–conjugated anti-CD45 (IgG1x), allophtocyanin (APC)–conjugated anti-CD14 (mouse IgG2aec), and FITC–conjugated anti-CD3 (mouse IgG1x) or PerCP–Cy5.5–conjugated anti-CD20 (mouse IgG1; BD Biosciences), or with isotype-matched controls, and analyzed by flow cytometry (FACS Calibur; BD Biosciences). Neutrophils were identified by forward and side scatter characteristics and by the absence of CD14, CD3, or CD20 staining.

In mice, cells from bone marrow or spleen were counted (Countess cell counter; Invitrogen). FC receptors were blocked by a mouse anti-CD16/32 purified antibody (clone FCR-4G8; Invitrogen), and cells were stained for CD11b (PE–conjugated anti-mouse CD11b, clone M1/70; BioLegend), Gr-1 (APC–conjugated anti-Gr-1, clone RB6-8C5; Miltenyi Biotec), or CD3 (FITC–conjugated anti-mouse CD3, clone 17A2; BioLegend), or with isotype-matched controls (BD Biosciences), and then analyzed by flow cytometry (Epics XL-MCL; Beckman Coulter). The total cell count was calculated by multiplication of frequencies of each respective subpopulation by the cell counts obtained from total spleen or bone marrow.

Enzyme-linked immunosorbent assay (ELISA). Serum was analyzed for TRAP-5b (Mouse TRAP Assay; IDS) and CSF-1 (R&D Systems) by ELISA.

Bone histomorphometry. Methylmethacrylate-embedded tibial bones were stained with Goldner’s trichrome, and quantification was performed using an OsteoMeasure program, version 2.2, assessing bone volume/total volume (BV/TV), trabecular number (ThBn), trabecular thickness (ThTb), osteoclast surface/bone surface (OcS/BS), osteoclast number/bone surface (OcN/BS), and osteoblast number/bone surface (ObN/BS) (38).

Statistical analysis. Student’s 2-tailed t-test, Mann-Whitney test, or one-way analysis of variance were used as appropriate for group comparisons, and results are expressed as the mean ± SEM. P values less than 0.05 were considered significant.

RESULTS

High expression of CSF-1R in human RA synovium. Levels of CSF-1R expression were significantly higher in RA patients compared to OA patients (P < 0.05) and healthy individuals (Figures 1A and B). In healthy individuals, no CSF-1R expression was observed.
Patients with synovial hyperplasia (>3–7 cell layers thick) and severe synovitis regardless of disease status (RA or OA) had significantly more CSF-1R expression compared to patients with minimal synovitis ($P < 0.05$) (Figure 1B). CSF-1R+ cells were observed in the synovial lining but were more abundant in the sublining, including in follicle-like structures when these were present and in cells invading cartilage and bone (Figures 1A and C). There was no correlation of synovial CSF-1R expression with the DAS28 score or with rheumatoid factor or anti–citrullinated protein seropositivity (data not shown).

**CSF-1R–expressing cells in RA synovium and bone.** In the RA synovial lining, CSF-1R+ cells were predominantly CSF-1R+CD68+ macrophages, but a small proportion were CSF-1R+vimentin+ fibroblast-like synoviocytes (FLS) (Figure 1C). In the synovial sublining of RA patients, CSF-1R+ cells were mainly CD68+ macrophages, but there were also a few CD21+ follicular DCs in follicle-like structures. CSF-1R was not
expressed in CD3+ T cells, CD20+ B cells, CD1a+ immature DCs, or CD208+ mature DCs. Of the RA bone sections examined, only 12 of 23 patients (52%) had detectable TRAP+ multinucleated osteoclasts; however, 100% of these 12 patients had detectable CSF-1R+TRAP+ osteoclasts (Figure 1D). CSF-1R was not expressed in human osteoblasts, which were identified by their cuboid morphology and location along trabecular bone.

Circulating RA monocytes and neutrophils but not lymphocytes express CSF-1R. Mild-to-moderate expression of CSF-1R was detected in RA peripheral blood CD14+ monocytes and neutrophils but not on CD3+ lymphocytes (Figure 2A). There was no significant difference in CSF-1R expression in circulating CD14+ monocytes and neutrophils in RA patients compared to healthy controls (Figure 2B).

Specific CSF-1R blockade inhibits inflammatory bone and cartilage loss in CIA. We next examined effects of a neutralizing anti–CSF-1R mAb in CIA. CSF-1R neutralization did not lower the incidence of arthritis compared to isotype control or PBS treatment (Figure 3A), but CSF-1R neutralization (anti–CSF-1R mAb at 50 mg/kg/day) significantly inhibited arthritis...
severity on day 14 after arthritis onset compared to isotype control treatment ($P < 0.05$) and on days 12–14 after arthritis onset compared to PBS treatment ($P < 0.05$) (Figure 3B). This effect was associated with inhibition of synovial inflammation, a marked reduction in cartilage destruction and bone erosion, and an absence of detectable TRAP$^+$ osteoclasts ($P < 0.05$ versus isotype control treatment) (Figure 3C). Anti–CSF-1R mAb at 25 mg/kg/day also significantly inhibited arthritis scores on day 14 compared to PBS treatment ($P < 0.05$) but not compared to isotype control treatment (Figure 3B). This was associated with a reduction in bone erosions ($P < 0.05$) and TRAP$^+$ osteoclast numbers ($P < 0.05$), but there were no effects on cartilage destruction or synovial inflammation scores (Figure 3C).

**CSF-1R antagonism reduces synovial joint macrophage infiltrates and splenic monocyte recruitment.** We next investigated whether the antiinflammatory effects of anti–CSF-1R mAb treatment were associated with reduced macrophage infiltration in the synovial tissue and the spleen. In synovial joints, the antiinflammatory effects of anti–CSF-1R mAb at 50 mg/kg/day were accompanied by a complete absence of synovial CSF-1R$^+$ cells and F4/80$^+$ macrophage infiltrates compared to isotype control treatment (Figure 4A). Treatment with anti–CSF-1R mAb at 25 mg/kg/day was associated with a lack of synovial CSF-1R$^+$F4/80$^+$ macrophage infiltrates compared to isotype control treatment, except in 1 of 9 mice, in which sparse double-positive cells could be identified (Figure 4A).
In the spleen, anti–CSF-1R mAb at 50 mg/kg/day led to a reduction in CD11b+/Gr-1− monocyte recruitment compared to isotype control treatment (P < 0.05) (Figure 4B), but there was no effect on splenic CD11b+/Gr-1+ granulocytes or CD3+ lymphocytes (Figure 4B). CSF-1R inhibition did not reduce bone marrow CD11b+/Gr-1− cell numbers (Figure 4B). Analysis of serum CSF-1 demonstrated a significant increase in CSF-1 after treatment with anti–CSF-1R mAb at either dosage compared to isotype control treatment (P < 0.05) (Figure 4C).

Specific CSF-1R inhibition blocks systemic bone loss by blockade of osteoclast differentiation. We next determined whether anti–CSF-1R mAb treatment inhibited systemic bone loss. PBS- and isotype control–treated groups demonstrated significant bone loss, as evident by reductions in BV/TV and TbN and by an increase in OcN/BS (P < 0.05) (Figure 5A), while TbTh remained unchanged. Compared to isotype control treatment, anti–CSF-1R mAb at 50 mg/kg/day significantly increased BV/TV and TbN (P < 0.05 for both), which was accompanied by decreased OcN/BS and OcS/BS (P < 0.05 for both) but no reduction in ObN/BS (Figure 5A). Similarly, serum TRAP-5b levels were significantly reduced following CSF-1R inhibition (P < 0.05) (Figure 5B).

CSF-1R inhibition protects against bone and cartilage erosion independent of effects on inflammation in serum-transfer arthritis. Anti–CSF-1R mAb at both concentrations did not affect the clinical or histologic parameters of joint inflammation, as previously reported (9), and was not associated with a reduction in macrophage infiltrates or CSF-1R staining (Figures 6A and B and data not shown). In contrast, anti–CSF-1R mAb at 50 mg/kg/day significantly inhibited cartilage erosion (P < 0.05), and anti–CSF-1R mAb dose-dependently inhibited bone erosion, TRAP+ osteoclast numbers, and serum TRAP-5b levels (P < 0.05 for all) (Figures 6B and C).

DISCUSSION

Targeting macrophages and osteoclasts is considered an effective approach to inhibit inflammation and structural damage in RA, and this notion is supported by the clinical benefit of therapies which inhibit macrophage-derived TNF (anti-TNF biologic agents), reduce macrophage infiltration (DMARDs), or inhibit osteoclastogenesis (anti-RANKL mAb), and by the correlation of synovial macrophage levels with radiographic destruction and response to biologic agents. To our knowledge, this is the first report describing abundant CSF-1R expression in RA synovium/bone as assessed by a specific mAb against human CSF-1R. CSF-1R expression was increased in RA patients compared to OA patients or healthy controls, and particularly in patients with highly active disease. Elevated CSF-1R expression has previously been reported in RA synovial CD68+ macrophages as assessed by a less specific polyclonal rabbit anti-human CSF-1R antibody (6). We found that RA synovial lining and sublining macrophages, follicular DCs, and bone TRAP+ osteoclasts were CSF-1R+.
CSF-1R expression in follicular DCs in follicular-like structures and mature osteoclasts in human RA has not previously been reported, although expression in multiple other DC subtypes and osteoclasts has been observed in other models (14,39). Alongside CSF-1R+ macrophages, follicular DCs may contribute to T cell antigen presentation and activation in RA inflammation. The lack of CSF-1R expression on osteoblasts, T and B cells, and immature DCs suggests that these would not be cellular targets of a specific CSF-1R–neutralizing mAb in RA. This finding is in contrast to reports of CSF-1R expression in osteoblasts, B cells, and immature DCs in nonarthritic diseases (2).

Interestingly, CSF-1R expression was also detected on RA FLS. RA FLS, together with RA synovial endothelial cells, activated monocytes, osteoblasts, human chondrocytes, stromal cells, and smooth muscle cells, produce abundant CSF-1 (2,3,40). Similarly, IL-34 is secreted by RA FLS and macrophages (5). The concomitant expression of CSF-1R and its ligand(s) on RA FLS and macrophages in close contact may contribute to amplifying autocrine and paracrine synovial inflammation and bone destruction.

Circulating RA monocytes and, to a lesser extent, neutrophils but not lymphocytes expressed CSF-1R. Human neutrophils have been reported to express CSF-1R messenger RNA and surface protein in the presence of activating stimuli (41). This may be related to the plasticity of neutrophils capable of transdifferentiating into macrophages in the presence of CSF-1 and other inflammatory signals (42). Clinical trials using recombinant CSF-1 in patients following bone marrow transplantation or myeloablative therapy demonstrated improved circulating granulocyte recovery times and function (43,44). Circulating blood monocytes and neutrophils are highly activated in RA, which may be based in part on high levels of CSF-1. Circulating CSF-1R–expressing activated monocytes and neutrophils may migrate to the joint, contributing to a continual source of synovial CSF-1R–expressing cells and to ongoing chronic joint inflammation and destruction.

CSF-1R neutralization completely abrogated bone erosion and reduced systemic bone loss accompanied by the complete absence of detectable TRAP+ osteoclasts and markedly reduced serum TRAP in 2 arthritis models. In contrast, we did not observe detrimental effects on osteoblasts, a finding supported by the absence of CSF-1R expression in human RA osteoblasts, the lack of inhibitory effects of CSF-1R antagonism on osteoblast numbers, and preserved bone volume in murine arthritis. Potent bone-protective effects of anti-CSF-1R mAb may be due to indirect and direct inhibition of osteoclast differentiation and osteoclast bone

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**Figure 6.** Effects of anti–CSF-1R monoclonal antibody (AFS98) treatment in serum-transfer arthritis. Mice were administered phosphate buffered saline (PBS), AFS98, or isotype control at the indicated doses for 7 days starting on day 1 of arthritis induction. A, Arthritis score. B and C, Histologic parameters of inflammation area, erosion area, cartilage erosion, and TRAP+ osteoclast numbers (B) as well as serum TRAP-5b levels (C) (n = 10 mice per group). Values are the mean ± SEM. * = P < 0.05 versus isotype control at the same dose. See Figure 1 for other definitions.
resorption, respectively (7,32,39). Taken together, these data suggest that specific anti–CSF-1R mAb may mediate selective inhibition of osteoclast-mediated bone destruction in the absence of deleterious effects on bone formation in RA.

An unexpected and interesting finding was marked protection against cartilage destruction in both arthritis models. This could be attributed at least in part to indirect effects of inhibition of joint inflammation in the CIA model, or possibly to direct effects on the cartilage. Along with RA FLS, macrophages, and osteoblasts, chondrocytes have been reported to coexpress CSF-1R and its ligand (45). CSF-1 has a reported role in the proliferation and differentiation of chondrocytes and osteoblasts in vitro (45). We were unable to detect CSF-1R expression in human RA or murine chondrocytes (data not shown). Overall, although CSF-1R has been reported to be widely expressed on cells implicated in both cartilage and bone formation and/or destruction, expression data in human RA and net effects in murine arthritis models suggest an overall protective effect of specific CSF-1R inhibition on cartilage and bone.

CSF-1R neutralization also showed significant antiinflammatory effects in CIA both macroscopically and histologically, due to a relatively specific reduction in monocyte/macrophage tissue infiltration in the spleen and the complete absence of this infiltration in the synovium. However, we were unable to demonstrate effects on splenic interferon-γ or other Th1/Th2/Th17 cytokines or on proliferation of splenic T cells (data not shown), which are known to be implicated in priming of arthritis in this model. Similarly, mice deficient in CSF-1 (Csfo/−/−) or CSF-1R (Csf1r−/−) had markedly reduced macrophage infiltration into synovial and other tissue without impairment in T cell numbers (20,21). Antiinflammatory effects of the same CSF-1R mAb (AFS98) at doses similar to those in this study, associated with reduced tissue macrophage infiltrates, have been demonstrated elsewhere in several other experimental inflammation models such as renal tubular inflammation, lipopolysaccharide-induced peritonitis, atherosclerosis, renal lupus, and mouse tumors (2,9,46–48). However, previous studies and the present study showed that CSF-1R neutralization with AFS98 protected against bone and cartilage destruction but did not inhibit inflammation in serum-transfer arthritis and methylated bovine serum albumin–induced arthritis (9,28). In those studies, small single doses of AFS98 were used, and arthritis was acute and short in duration.

Serum-transfer arthritis in particular is a passive and much more rapid inflammatory process, mainly driven by immune complex–mediated influx of inflammatory cells, and macrophages may play only a minor role in the acute inflammation in this model in contrast to CIA. Consequently, reduction in macrophage numbers by CSF-1 inhibition may come too late to influence the inflammatory process. In CIA, the inflammatory process develops somewhat more slowly, and macrophages may play a more important role. Therefore, reduction of macrophage numbers in the joints may become relevant. The data from experiments using this model demonstrated antiinflammatory effects with amelioration of the clinical score at later time points. Thus, variable effects on synovial inflammation could be attributed to differences in arthritis models and drug mechanisms including selectivity of CSF-1R inhibition. In contrast to AFS98 (a mouse IgG2a antibody), another CSF-1R–specific mAb (M279, rat IgG1) did not demonstrate antiinflammatory effects in other nonarthritis models of inflammation; however, this may have been due to differences in the antibodies used and different modes of action (49). Taken together, it is likely that anti–CSF-1R mAb treatment will be more useful in established chronic inflammation than in priming of arthritis or early-stage disease.

We observed reduced numbers of CD11b+Gr-1− splenic cells associated with complete depletion of synovial CSF-1R+/F4/80+ macrophages in CIA. The mechanism of action behind targeted effects on tissue macrophages by specific CSF-1R neutralization in murine CIA may be related to indirect inhibition of macrophage differentiation in the blood (49,50). This hypothesis is supported by the absence of an effect on bone marrow CD11b+Gr-1− monocytes (Figure 4B). The latter finding is consistent with the lack of effect on bone marrow precursors in other models of inflammation (49,51). In addition, although we did not directly determine effects on blood monocyte counts as we did not have sufficient blood samples to perform flow analyses in the CIA model. However, in other reported studies of experimentally induced inflammation (such as lung inflammation, peritonitis, and graft-versus-host disease) in which there are no effects on bone precursors, the reduction in tissue macrophage numbers after treatment with the same anti–CSF-1R antibody (AFS98) was linked to a specific decrease in differentiation from Ly-6ChighGr-1+ macrophages to more mature Ly-6Clow Gr-1− macrophages in the blood, but not to a decrease in neutrophils or lymphocytes (49,50).
Interestingly, PD-0360324, a specific anti–CSF-1 antibody, also depleted circulating Ly-6C<sup>low</sup> monocytes in RA (51). Consistent with this, we observed a reduction in splenic monocytes but not in neutrophils or lymphocytes (Figure 4B). We might anticipate peripheral blood monocyteopenia in the absence of effects on bone marrow precursors or other peripheral blood cell types during CSF-1R blockade, but we would need to evaluate this in clinical trials.

In conclusion, CSF-1R was broadly expressed in RA FLS, macrophages, follicular DCs, mature TRAP<sup>+</sup> osteoclasts, and circulating monocytes and neutrophils. Specific anti–CSF-1R mAb neutralization abrogated cartilage damage, bone erosion, and systemic bone loss, and this was associated with depletion of TRAP<sup>+</sup> osteoclasts in 2 murine arthritis models. Antiinflammatory effects of CSF-1R inhibition were associated with complete absence of synovial macrophage infiltrates in CIA. CSF-1 and CSF-1R are current therapeutic targets, and inhibitors are in preclinical and clinical development for the treatment of RA, other autoimmune diseases, and cancer. However, while few specific mAb against CSF-1R are in clinical development for treating RA, these mAb may be useful for attenuating cartilage destruction and aggressive local and/or systemic bone loss, possibly in addition to or following control of inflammation by current biologic agents.

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**AUTHOR CONTRIBUTIONS**

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Toh had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Transgene SA had no role in the collection, analysis, or interpretation of the data. Transgene SA was involved in the study design, the writing of the manuscript, and the decision to submit the manuscript for publication. Publication of this article was contingent upon approval by Transgene SA.

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