Tumor Necrosis Factor–Inhibiting Therapy Preferentially Targets Bone Destruction but Not Synovial Inflammation in a Tumor Necrosis Factor–Driven Model of Rheumatoid Arthritis

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Objective. To investigate how tumor necrosis factor (TNF)–inhibiting therapy affects bone destruction and inflammation in a TNF-driven mouse model of rheumatoid arthritis.

Methods. In order to evaluate the influence of TNF on osteoclastogenesis in vitro, different concentrations of TNF were added to spleen cell–derived monocytes in the absence or presence of different concentrations of RANKL. In addition, the effects of TNF inhibition on osteoclast precursors as well as local bone destruction in vivo were assessed by treating TNF-transgenic mice with different doses of adalimumab.

Results. TNF stimulated osteoclastogenesis mainly by increasing the number of osteoclast precursor cells in vitro. This TNF effect was independent of the presence of RANKL. In the hTNF-transgenic mouse model of destructive arthritis, low-dose TNF-inhibiting therapy with adalimumab had no effect on synovial inflammation but significantly inhibited local bone destruction and the generation of osteoclasts. This inhibition was accompanied by a reduction in the number of c-Fms–positive osteoclast precursor cells in the bone marrow and a reduction of the osteoclast precursor pools in the blood and inflamed synovial membrane of hTNF-transgenic mice.

Conclusion. Low-dose TNF-inhibiting therapy significantly reduces bone erosions by reducing the number of circulating and joint-invading osteoclast precursors. This effect is uncoupled from its antiinflammatory action.

Proinflammatory cytokines play an important pathogenetic role in rheumatoid arthritis (RA). In particular, tumor necrosis factor (TNF) can elicit the whole inflammatory cascade, leading to the typical joint swelling and subsequent structural joint damage characteristic of the disease (1). Moreover, mice overexpressing TNF exhibit a severe inflammatory and rapidly destructive arthritis (2). Joint destruction involves both cartilage degradation and erosions of juxtaarticular bone. The latter is mediated by osteoclasts (OCs) (3,4), a cell population derived from the monocyte/macrophage lineage. Osteoclast formation requires the presence of macrophage colony-stimulating factor (M-CSF) in the early stages of development and involves the formation of mononuclear tartrate-resistant acid phosphatase (TRAP)–positive OC precursor cells (pre-OCs) as an intermediary step (5). Although OC differentiation, maturation, and activation are pivotally dependent on the availability of RANKL and its receptor RANK (5–7), TNF can amplify osteoclastogenesis in the presence (and possibly even in the absence) of RANKL (8–10).
The evolution of joint destruction in RA is highly related to the inflammatory process, as evidenced by the correlation of joint damage with swollen joint counts and levels of acute-phase reactants (the most direct surrogates of the inflammatory response) but also with overall disease activity as judged by composite measures (11–13). In line with these findings, recent observations suggest that damage occurs preferentially in joints that exhibit synovitis (14). Interestingly, however, this tight relationship is disrupted by inhibition of TNF, because progression of joint damage in RA is prevented, or highly retarded, by treatment with infliximab, a monoclonal antibody against TNFα, even if clinical signs and symptoms of inflammation are not affected (15). Similar observations have also been made for 2 other TNF inhibitors, etanercept and adalimumab (16,17). However, the mechanisms leading to this dissociation have not yet been clarified.

In the present study, we approached this question by first addressing the role of TNF in osteoclastogenesis in vitro and subsequently assessing the effects of TNF inhibition on inflammation and destruction in a TNF-driven in vivo model of experimental arthritis.

MATERIALS AND METHODS

Animals and clinical assessment of arthritis. The heterozygous hTNF-transgenic Tg197 mouse strain has been described previously (2). Briefly, mice transgenic for the human TNF gene construct were generated using an unmodified 5′ promoter region but a modified 3′ untranslated region, allowing constitutive expression of the human TNF gene in vivo. In these hTNF-transgenic mice, a chronic inflammatory and destructive polyarthritis starts to develop within 4–6 weeks after birth.

Arthritis was evaluated weekly in a blinded manner as described previously (3). Briefly, the grip strength of each paw was analyzed on a wire of 3-mm diameter using a semiquantitative score from 0 to −3 (0 = normal grip strength, −1 = mildly reduced grip strength, −2 = moderately reduced grip strength, −3 = severely reduced grip strength). In some experiments, hTNF-transgenic mice were treated intraperitoneally with different doses (0.1, 1, 10 mg/kg) of adalimumab (Abbott), a monoclonal antibody to human TNF, for 3 weeks starting at week 7, thus using a preventive rather than a therapeutic approach.

All mice were bred and maintained on a CBA × C57BL/6 genetic background. When appropriate, wild-type (WT) littermates served as controls and were used for in vitro experiments. All mice were maintained under sterile conditions and fed a normal diet with water ad libitum. The institutional animal care and use committee approved all animal procedures. The hTNF-transgenic mice were kindly provided by Dr. George Kollias (Alexander Fleming Biomedical Center, Varkiza, Greece).

Histologic and immunohistochemical analyses. The hind paws of 10-week-old WT and hTNF-transgenic mice were fixed in 4.5% formalin for 6 hours and then decalcified in 14% EDTA (Sigma) at 4°C, with pH adjusted to 7.2 by the addition of ammonium hydroxide (Sigma), until the bones were pliable. After brief rinsing in water, the tissue was dehydrated with ethanol and embedded in paraffin at 56°C. Consecutive 2-μm sections were prepared and stained with either hematoxylin and eosin, toluidine blue, or TRAP. The extent of inflammation was determined as previously described (3), by determining the arithmetic mean of the areas of inflamed synovial tissue in a random section of each hind paw (expressed as mm²). The same type of calculation was performed to quantify the area of damaged cartilage (toluidine blue–stained sections) and erosions (TRAP-stained sections). The numbers of OCs at the site of bone erosion were determined by counting the numbers of TRAP-positive multinucleated cells in the immediate vicinity of erosions.

To determine the percentage of pre-OCs, which were previously defined as F4/80-positive cells (Serotec) (18), we counted the number of F4/80-positive and F4/80-negative cells in 4 high-power fields of each section and divided the total number of all cells by the number of F4/80-positive cells. Moreover, we stained paraffin sections of the hind paws with a monoclonal antibody against CD115 (LS-C117866; LifeSpan Biosciences) to determine the percentage of c-Fms-positive cells. Quantitative analysis of c-Fms expression was performed by tissue cytometry using HistoQuest (TissueGnostics) (19,20).

Enzyme-linked immunosorbent assay (ELISA). The expression of human TNF was determined in supernatants from in vitro OC formation assays using an ELISA. Tests were performed according to the manufacturer’s recommendations (R&D Systems). The lowest detectable concentration was 0.106 pg/ml.

In vitro OC generation. For in vitro OC formation, spleen-derived monocytes obtained from WT and hTNF-transgenic mice were used. To this end, splenocytes from WT or hTNF-transgenic mice were cultured in the presence of 30 ng/ml M-CSF for 24 hours. Then, nonadherent cells were harvested and subjected to OC differentiation by stimulating them with 30 ng/ml M-CSF and various concentrations of RANKL or in the absence of RANKL. All recombinant cytokines were purchased from R&D Systems. In experiments using splenocytes from hTNF-transgenic mice, a monoclonal antibody against hTNF, adalimumab (Abbott), was added at different concentrations (0–1,000 μg/ml). After 4 days under these osteoclastogenic conditions, TRAP staining was performed, and the numbers of OCs (TRAP positive and ≥3 nuclei) and pre-OCs (TRAP positive and mononuclear or binuclear) were determined.

Flow cytometry of peripheral blood pre-OCs. A FACSCanto II instrument (BD Biosciences) was used for flow cytometric analyses. Peripheral blood cells were stained for CD11b (AM1/70; BD Biosciences), Gr-1 (RB6-8C5; BD Biosciences), and CD115 (AFS98; eBioscience). Pre-OCs were characterized as CD11b<sup>high</sup> Gr-1<sup>low</sup> cells (21), and c-Fms<sup>+</sup>-positive pre-OCs were characterized as CD115<sup>-</sup>CD11b<sup>high</sup>.
Gr-1<sup>low</sup> cells. Isotype-matched control antibodies (all from BD Biosciences) served as negative controls.

**Messenger RNA (mRNA) expression and quantitative polymerase chain reaction (qPCR).** For qPCR analysis, we extracted mRNA from the tarsal joints and bones of 10-week-old mice, using an RNeasy kit (Qiagen). Briefly, the hind paws were cleaned of skin and tendon tissue. The metatarsal bones were cut close to the proximal joints, and the tarsal area was snap-frozen in liquid nitrogen and pulverized. Complementary DNA (cDNA) was prepared using a first-strand cDNA kit (Invitrogen), and qPCR analysis was performed on a LightCycler 480 system (Roche Applied Science) using previously described primers for various genes that are markers of inflammatory responses and osteoclasts (22). For the calculation of relative expression of mRNA, expression of the marker genes was normalized to that of GAPDH and compared with the mean control values. Error bars indicate the variance within the groups.

**Statistical analysis.** Data are presented as the mean ± SEM. Group means were compared by Student's unpaired 2-tailed t-test or one-way analysis of variance with Bonferroni correction for multiple testing, as appropriate, using GraphPad Prism software. P values less than 0.05 were considered significant.

**RESULTS**

**TNF activates pre-OCs but not OCs in the absence of RANKL.** It has been demonstrated in vivo that TNF cannot compensate for RANK in osteoclastogenesis, although RANK is not required for TNF-mediated pre-OC induction (23). To confirm these data in vitro, we stimulated spleen-derived monocytes with increasing amounts of TNF and RANKL. We did not detect any effects of TNF on OC numbers in the absence of RANKL. However, at higher RANKL concentrations, we observed a costimulatory effect of TNF on OC numbers. In contrast, stimulation with TNF alone, in the absence of RANKL, enhanced pre-OC numbers in a dose-dependent manner (data not shown).

Having confirmed that TNF acts mainly via induction of pre-OCs, we next performed an OC formation assay using spleen cells of hTNF-transgenic mice. In supernatants from unstimulated hTNF-transgenic cells, we detected human TNF at a concentration of 4.7 pg/ml. As expected, we did not observe human TNF in supernatants from the WT littermates (Figure 1A). Next, we stimulated both hTNF-transgenic and WT spleen cells with M-CSF and RANKL to induce osteoclastogenesis (Figure 1B). Cell cultures derived from hTNF-transgenic mice exhibited a 53% increase in the number of OCs compared with cultures derived from their WT littermates (Figure 1C). Strikingly, the number of pre-OCs was enhanced >2-fold in cell cultures derived from transgenic mice, again indicating a pronounced effect of TNF on pre-OCs (Figure 1D).

**TNF inhibition reduces the number of TNF-induced pre-OCs but not OCs ex vivo.** We next sought to determine how TNF inhibition affects RANKL-induced osteoclastogenesis ex vivo. We cultured spleen cells obtained from hTNF-transgenic mice with M-CSF and RANKL and added different concentrations of adalimumab, a monoclonal antibody to human TNF. Interestingly, we could not detect any effects of adalimumab on OC numbers (Figure 2A). However, we observed that TNF inhibition considerably reduced pre-OC numbers, in a dose-dependent manner (Figure 2B). Moreover, TNF inhibition was effective in reducing pre-OC numbers not only in the absence but even in the presence of RANKL (Figures 2C and D). Taken together, these data suggested that TNF inhibition mainly affects pre-OC differentiation rather than the development of mature OCs, at least under these conditions.

**Low-dose TNF-inhibiting therapy affects osteoclastogenesis and bone destruction but not synovial inflammation in vivo.** We next investigated the effects of TNF inhibition on osteoclastogenesis in vivo. To this end, we treated hTNF-transgenic mice with different
doses (0.1 mg/kg, 1 mg/kg, or 10 mg/kg) of adalimumab or placebo. Wild-type mice served as controls.

At week 7, when therapy was started, hTNF-transgenic mice already exhibited clinical signs of arthritis, such as a significant ($P < 0.001$) loss of grip strength when compared with their WT littermates (additional information is available on the Arthritis & Rheumatism web site at http://onlinelibrary.wiley.com/doi/10.1002/art.37797/abstract). After 3 weeks of therapy, hTNF-transgenic mice receiving placebo demonstrated further clinical deterioration. In contrast, hTNF-transgenic mice receiving the highest concentration of adalimumab (10 mg/kg) showed dramatically improved grip strength. Lower concentrations of adalimumab, however, did not significantly reduce arthritis symptoms (additional information is available on the Arthritis & Rheumatism web site at http://onlinelibrary.wiley.com/doi/10.1002/art.37797/abstract).

Figure 2. Effects of TNF-inhibiting therapy on osteoclastogenesis ex vivo. Spleen cells from hTNF-transgenic mice were stimulated with M-CSF and RANKL. Adalimumab was added at different concentrations. A and B, Numbers of OCs (A) and pre-OCs (TRAP-positive mononuclear cells) (B) following stimulation with adalimumab. C and D, Effect of TNF inhibition on pre-OCs in the absence (C) and presence (D) of RANKL. All data are presented as the percentage of maximal osteoclastogenesis (hTNF-transgenic cells stimulated with 30 ng/ml M-CSF and 100 ng/ml RANKL = 100%). Bars in A, C, and D show the mean ± SD. * = $P < 0.05$; ** = $P < 0.01$, versus no adalimumab. See Figure 1 for definitions.

Consistent with the results of the clinical assessment, histologic analyses of the hind paws revealed severe synovitis with an accumulation of inflammatory cells in the synovial membrane of hTNF-transgenic mice receiving placebo. However, when hTNF-transgenic mice were treated with the highest concentration of adalimumab (10 mg/kg), we observed a dramatic reduction ($-83\%$) in synovial inflammation; hTNF-transgenic mice treated with 1 mg/kg adalimumab also experienced significantly ($P < 0.001$) reduced inflammation ($-58\%$). In contrast, hTNF-transgenic mice treated with the lowest dose of TNF inhibitor (0.1 mg/kg adalimumab) showed no significant reduction in synovitis (Figure 3A).

We also performed staining for TRAP-positive OCs and quantified the area of bone resorption. Placebo-treated hTNF-transgenic mice exhibited a large area of bone erosion. Treatment with the highest dose of adalimumab (10 mg/kg) resulted in a striking reduction in the area of bone erosion. Surprisingly, even the lowest dose of adalimumab (0.1 mg/kg), which had no significant antiinflammatory effects, reduced bone destruction to an extent similar to that induced by the highest dose of adalimumab (10 mg/kg) (Figure 3B). This suggested that very low concentrations of TNF-inhibiting therapy uncouple TNF-driven bone damage from inflammation. Accordingly, OC numbers were increased in the inflamed synovial membrane of placebo-treated mice, while mice receiving even the lowest dose of adalimumab showed a significant reduction in OC numbers, despite ongoing inflammation (Figure 3C).

We also assessed the effects of TNF-inhibiting therapy on articular cartilage. Human TNF–transgenic
mice receiving placebo demonstrated significant destruction of articular cartilage, whereas mice receiving 10 mg/kg of adalimumab were protected against cartilage loss. Lower concentrations of adalimumab (0.1 mg/kg and 1 mg/kg), however, did not significantly alter the amount of cartilage damage when compared with placebo (Figure 3D). This suggests that cartilage loss is similar to the overall inflammatory response.

**Low-dose TNF-inhibiting therapy affects osteoclast-associated but not inflammation-associated gene expression.** To further elucidate the mechanisms leading to the uncoupling of bone destruction from synovial inflammation in hTNF-transgenic mice after treatment with low concentrations of anti-TNF, we used qPCR to assess paw extracts for gene expression. In extracts derived from placebo-treated hTNF-transgenic mice, mRNA levels of the inflammatory process markers interleukin-1 (IL-1), matrix metalloproteinase 3 (MMP-3), and MMP-13 were significantly increased. Interestingly, a reduction in these increased levels was detected only in hTNF-transgenic mice treated with a high concentration of anti-TNF (10 mg/kg adalimumab), whereas treatment with a low concentration (0.1 mg/kg adalimumab) had no significant effect (Figures 4A–C). Messenger RNA expression of the OC-associated genes NF-ATc1 and cathepsin K was also highly up-regulated in placebo-treated hTNF-transgenic mice compared with their WT littermates (Figures 4D and E). Likewise, mRNA expression of c-Fms, the receptor for M-CSF, was up-regulated in placebo-treated hTNF-transgenic mice (Figure 4F). In contrast to what was observed for proinflammatory genes, however, up-regulation of OC-associated genes was abolished even at the lowest dose of anti-TNF (0.1 mg/kg adalimumab) (Figures 4D–F).

Taken together, these data not only provided functional evidence for the anticipated antiresorptive

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**Figure 3.** Histologic analysis of the tarsal area of the hind paws of WT and hTNF-transgenic mice after 3 weeks of treatment with different concentrations of adalimumab. A, Left, Representative hematoxylin and eosin–stained sections showing dense cellular infiltrates. Right, Quantification of synovial inflammation. B, Left, Representative TRAP-stained sections showing red-stained OCs at the sites of bone erosion. Right, Quantification of area of bone erosion. C, TRAP-positive multinucleated cells representing OCs within the sections. D, Cartilage degradation in hTNF-transgenic mice treated with different concentrations of adalimumab. Bars show the mean ± SD. * = P < 0.05; ** = P < 0.01; *** = P < 0.001, versus WT mice. See Figure 1 for definitions.
effects of TNF inhibitor treatment but also revealed that the antiresorptive action was dissociated from the anti-inflammatory action when using low doses of the TNF inhibitor.

**Low-dose TNF-inhibiting therapy blocks TNF-induced up-regulation of c-Fms in the bone marrow and reduces the pool of pre-OCs in the blood and synovial membrane.** It has been previously demonstrated that TNF increases c-Fms expression on pre-OCs in the bone marrow of hTNF-transgenic mice (24). Because we showed that TNF inhibition significantly reduced pre-OCs numbers ex vivo and down-regulated OC-associated genes (among them c-fms, the gene coding for the M-CSF receptor), we next investigated the effects of TNF-inhibiting therapy on pre-OCs and c-Fms in hTNF-transgenic mice. We examined the bone marrow, peripheral blood, and synovial membranes of 10-week-old WT, placebo-treated, and anti-TNF–treated hTNF-transgenic mice for the presence of CD11b<sup>high</sup> Gr-1<sup>−/low</sup> cells, previously defined as pre-OCs (21), and for the expression of c-Fms.

The analysis of bone marrow revealed, in line with a previous study (24), a small (+6%) but significant ($P < 0.05$) increase in the numbers of pre-OCs in placebo-treated hTNF-transgenic mice compared with their WT littermates (Figure 5A). However, when we determined the percentage of c-Fms–positive pre-OCs, we observed a significant ($P < 0.05$) and marked increase (+57%) in the relative numbers of c-Fms–positive pre-OCs. Of note, this increase could be completely inhibited even with the lowest dose of anti-TNF treatment (0.1 mg/kg adalimumab) (Figure 5B).

An examination of peripheral blood revealed higher pre-OC numbers (+139%) in placebo-treated hTNF-transgenic mice compared with WT mice (Figure 5C). However, in contrast to our findings in bone marrow, the percentage of c-Fms–positive pre-OCs was not significantly different between placebo-treated hTNF-transgenic mice and WT mice (Figure 5D). Treatment with anti-TNF almost completely abolished the marked increase in pre-OC numbers in the blood. Again, even low-dose anti-TNF treatment (0.1 mg/kg adalimumab) was sufficient to achieve this effect (Figure 5C).

When we examined histologic sections of the
joints, we observed markedly (+30%) increased numbers of F4/80+ cells in the inflamed synovial membrane of placebo-treated hTNF-transgenic mice compared with WT mice. Again, TNF-inhibiting therapy, even at low concentrations (0.1 mg/kg adalimumab), resulted in a reduction in the number of pre-OCs (−50%) (Figure 5E). Similar to what was observed in the peripheral blood, the percentage of c-Fms+ pre-OCs was not significantly different between placebo-treated hTNF-transgenic mice and hTNF-transgenic mice treated with either high-dose (10 mg/kg) or low-dose (0.1 mg/kg) adalimumab (Figure 5F).

Taken together, our data showed that TNF in the bone marrow mainly increased the number of c-Fms+ pre-OCs. This increase was accompanied by an increase in the pool of pre-OCs in the peripheral blood and synovial membrane of hTNF-transgenic mice. Of note, the relative expression of c-Fms in the blood or inflamed synovial membrane was not altered by TNF. Treatment with anti-TNF, even at low concentrations that lack any effect on the extent of synovial inflammation, completely abrogated the up-regulation of c-Fms in the bone marrow and in turn significantly reduced the pool of pre-OCs in the blood and synovial membrane.

DISCUSSION

In this study, we analyzed the effects of TNF and anti-TNF therapy on osteoclastogenesis, in vitro, ex vivo, and in vivo, to advance understanding of the destructive processes in RA joints. We observed that spleen monocytes readily differentiate into pre-OCs also in the absence of RANKL at higher TNF concentrations. This suggests that high doses of TNF are needed for the induction of pre-OCs. Of note, treatment of hTNF-transgenic mice with adalimumab, a monoclonal antibody to human TNF that also is used for the treatment of RA (25), revealed differential dose-dependent effects on inflammation, cartilage damage, and bone erosions. At a high dose of anti-TNF, both inflammation and joint damage were inhibited. However, at a low dose of anti-TNF, only bone destruction was significantly inhibited; this therapy did not inhibit inflammation clinically and histologically, or the levels of proinflammatory genes.
We also observed that TNF affects pre-OCs differently in distinct hematopoietic and peripheral compartments. In bone marrow, TNF increased predominantly the pool of c-Fms–positive pre-OCs and also the number of pre-OCs, but only to a very limited extent. In the peripheral blood, the situation was reversed. Whereas the pool of pre-OCs was markedly increased under the constant influence of TNF, the relative numbers of c-Fms–positive pre-OCs remained unchanged. The picture was similar in the synovial membrane; again the numbers of pre-OCs were highly increased, whereas the relative numbers of c-Fms–positive pre-OCs remained almost unaffected. Of note, this whole cascade of TNF-induced pre-OC activation leading to OC-mediated bone erosion can be blocked even with low-dose anti-TNF therapy, which has no influence on synovial inflammation. See Figure 1 for definitions.

Figure 6. Effect of TNF on pre-OCs in distinct hematopoietic and peripheral compartments. In the bone marrow, TNF markedly increases the pool of c-Fms–positive pre-OCs. This leads to an egress of c-Fms–positive pre-OCs in the peripheral blood and synovial membrane, increasing the pool of these cells. The whole cascade of TNF-induced pre-OC activation leading to OC-mediated bone erosion can be blocked even with low-dose anti-TNF therapy, which has no influence on synovial inflammation. See Figure 1 for definitions.

Taken together, these data reveal that pre-OCs require higher TNF concentrations to become activated than are needed to elicit an inflammatory response, and in turn, that small doses of a TNF inhibitor that presumably lead to small reductions in TNF expression interfere with osteoclastogenesis, while inflammation is affected only when higher doses are used.

Relating these findings to observations in humans, it is well established that TNF is highly overexpressed in RA (26) and more so than in other inflammatory joint diseases, such as psoriatic arthritis (PsA), let alone degenerative joint disease (27). Likewise, levels of circulating and tissue-expressed RANKL are highly increased in patients with RA when compared with healthy individuals and patients with other joint diseases (28,29). Consistent with these findings and our observations in hTNF-transgenic mice, joint destruction and especially bone erosions are more severe in RA than in any other inflammatory joint disorder, and this effect is mediated by a dramatic increase in and up-regulation of OCs originating in the synovial membrane (30). Furthermore, levels of circulating pre-OCs have been observed to be highly increased in PsA (31). Finally, it is of particular interest that a disassociation of the usually tight link between the extent of inflammation and joint damage has been repeatedly observed in patients with
RA receiving TNF-inhibiting therapy (15). It has been postulated that a different threshold for the inflammatory compared with the bone damage–activating effects of TNF may exist (32). The present findings now provide evidence supporting this hypothesis.

Interestingly, although this finding was not reported for other biologic agents, inhibition of IL-6 also appears to disassociate the link between inflammation and joint destruction in RA (33). Because IL-6 also constitutes an OC-promoting cytokine (34), this further supports the present findings. The effects of IL-6 inhibition could not be studied in the present model, because it is driven mainly by TNF, and IL-6 inhibition has little if any effect on the inflammatory response in hTNF-transgenic animals (35). In contrast, IL-1 appears to be importantly involved in mediating joint destruction in hTNF-transgenic mice, especially with respect to cartilage damage (36,37). In support of these findings, the observations made in the present study reveal that cartilage damage is not disassociated from the inflammatory response, because it is inhibited only by high doses of adalimumab. Indeed, when the gene expression of inflammation-associated markers was assessed, IL-1 was among those that were suppressed only at high doses of adalimumab, contrasting with OC-associated gene expression. Thus, at least in this model, cartilage damage relates more strongly to the inflammatory response than to bone damage. Although this aspect has not been sufficiently studied in patients with RA, it is noteworthy that in inflammatory joint diseases with much fewer (if any) bone erosions, such as reactive arthritis, cartilage damage may be significant, and levels of circulating cartilage breakdown products can by far exceed those seen in RA (38).

Taken together, the data presented provide evidence for a differential effect of TNF on osteoclastogenesis and inflammation that is dose dependent. It appears that low-dose TNF–inhibiting treatment is insufficient to reduce the signs and symptoms of joint inflammation but interferes with pre-OC generation and thus joint damage in RA. These findings may exemplify a mechanism that could also be used by other pleomorphic cytokines that act in other cell and organ systems.

**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. Redlich 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.

**Study conception and design.** Binder, Puchner, Hayer, Blüml, Smolen, Redlich.

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**ROLE OF THE STUDY SPONSOR**

Abbott had no role in the study design or in the collection, analysis, or interpretation of the data, the writing of the manuscript, or the decision to submit the manuscript for publication. Publication of this article was not contingent upon approval by Abbott.

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