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IL-17 Promotes Bone Erosion in Murine Collagen-Induced Arthritis Through Loss of the Receptor Activator of NF-κB Ligand/Osteoprotegerin Balance¹

Erik Lubberts,²* Liduine van den Bersselaar,* Birgitte Oppers-Walgreen,* Paul Schwarzenberger,† Christina J. J. Coenen-de Roo,‡ Jay K. Kolls,† Leo A. B. Joosten,* and Wim B. van den Berg*

IL-17 is a T cell-derived proinflammatory cytokine in experimental arthritis and is a stimulator of osteoclastogenesis in vitro. In this study, we report the effects of IL-17 overexpression (AdIL-17) in the knee joint of type II collagen-immunized mice on bone erosion and synovial receptor activator of NF-κB ligand (RANKL)/receptor activator of NF-κB/osteoprotegerin (OPG) expression. Local IL-17 promoted osteoclastic bone destruction, which was accompanied with marked tartrate-resistant acid phosphatase activity at sites of bone erosion in cortical, subchondral, and trabecular bone. Accelerated expression of RANKL and its receptor, receptor activator of NF-κB, was found in the synovial infiltrate and at sites of focal bone erosion, using specific immunohistochemistry. Interestingly, AdIL-17 not only enhanced RANKL expression but also strongly up-regulated the RANKL/OPG ratio in the synovium. Comparison of arthritic mice from the AdIL-17 collagen-induced arthritis group with full-blown collagen-arthritis mice having similar clinical scores for joint inflammation revealed lower RANKL/OPG ratio and tartrate-resistant acid phosphatase activity in the latter group. Interestingly, systemic OPG treatment prevented joint damage induced by local AdIL-17 gene transfer in type II collagen-immunized mice. These findings suggest T cell IL-17 to be an important inducer of RANKL expression leading to loss of the RANKL/OPG balance, stimulating osteoclastogenesis and bone erosion in arthritis. The Journal of Immunology, 2003, 170: 2655–2662.

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3 Abbreviations used in this paper: RA, rheumatoid arthritis; RANKL, receptor activator of NF-κB ligand; RANK, receptor activator of NF-κB; sRANKL, soluble RANKL; OPG, osteoprotegerin; CII, type II collagen; CIA, collagen-induced arthritis; t.i.a., intra-articular; TRAP, tartrate-resistant acid phosphatase; RT, room temperature.
study, we examined the effects of local IL-17 overexpression on RANKL/OPG/RANK expression in the synovium of CII-immunized mice. We found that local IL-17 gene transfer strongly up-regulated the synovial RANKL/OPG ratio and enhanced the formation of osteoclast-like cells and bone erosion compared with the control groups. Accelerated expression of RANKL and RANK was found at sites of focal bone erosion, using specific immunohistochemistry. The IL-17-induced joint erosion was significantly suppressed by systemic OPG treatment, suggesting that the IL-17-induced acceleration of bone erosion in CII-immunized mice was strongly mediated by RANKL. Our data suggest T cell IL-17 to be a potent stimulator of positive regulators of osteoclastogenesis in arthritis, resulting in loss of the balance between RANKL-RANK signaling and the levels of OPG.

Materials and Methods

Animals

Male DBA-1/BOM mice were purchased from Bomholtgard (Ry, Denmark). The mice were housed in filter-top cages. The mice were used between 10 and 12 wk of age. Water and food were provided ad libitum.

Adenoviral vectors

AdML-17 was constructed as previously reported (8). Briefly, pACCMilL-17 vector was cotransfected into 911 cells with I-restricted AdCMVlacZ DNA using calcium-phosphate precipitation. AdMLL-17 (human adeno virus transfected with murine IL-17 cDNA) clones were screened by PCR, and protein production was confirmed by a murine IL-17 bioassay (8). All and protein production was confirmed by a murine IL-17 bioassay (8). All vector was cotransfected into 911 cells with the adenovirus (AdML-17) or the AdLuc vector, the recombinant replication-decient adenovirus AdLuc (AdControl) was used as a control vector throughout the study. All virus preparations had a PFU:particle ratio of <100:1 (8, 22).

Induction of CIA

Bovine CII was prepared as described (18) and diluted in 0.05 M acetic acid to a concentration of 2 mg/ml. This was emulsified in equal volumes of CFA (2 mg/ml Mycobacterium tuberculosis; strain H37Ra; Difco, Detroit, MI). To examine potential amplifying activity of IL-17, DBA-1 mice were immunized at the base of the tail with a lower dose of bovine CII (50 μg) dissolved in PBS, and normally arthritis onset occurs around day 25–28.

Assessment of arthritis

Mice were considered to have arthritis when significant changes in redness and/or swelling were noted in the digits or in other parts of the paws. Knee joint inflammation was scored visually after skin dissection, using a scale of noninflamed (0), mild (1), marked (1.5), or severe (2) inflammation. Scoring was done by two independent observers, without knowledge of the experimental groups.

Study protocol

CIA was induced in male DBA-1 mice as previously described. Just before expected onset of CIA (day 25), mice were scored visually for the appearance of arthritis. Mice without macroscopic signs of arthritis in the paws were selected. Just before expected onset of CIA (day 25), mice were anesthetized with ether, and a small aperture in the skin of the knee was performed for the intra-articular (i.a.) injection procedure. When absence of arthritis was confirmed in the knee joint, i.e. injections were performed with 10⁷ PFU/μl of either an IL-17-expressing (AdML-17) or a control vector, the recombinant replication-deficient adenovirus AdLuc). Previously, we showed that this dose of adenoviral vector did not induce an inflammatory response after i.a. injection of the viral vector, mice were sacrificed by cervical dislocation and the skin of the knee joint was removed. The appearance of arthritis in the injected knees was assessed, and severity score was recorded as previously described (18). Thereafter, knee joints were isolated and processed for light microscopy.

To examine whether the IL-17-induced bone erosion in the knee joints of CII-immunized mice was mediated by the induction of RANKL, the following experiments were performed. In addition to the experimental design as described above, mice were systemically treated with OPG (sOPG;Fc; kindly provided by Amgen, Thousand Oaks, CA) or BSA. Three systemic (i.p.) injections of OPG or BSA (4 mg/kg/injection) were given on alternate days, starting 2 h before AdML-17 or AdControl was locally (i.a.) injected in the knee joint of CII-immunized mice. Five days after the i.a. injection, the injected knee joints were scored for the severity of arthritis as previously described (18), and thereafter, knee joints were isolated for x-ray analysis.

Histology

Whole knee joints were removed and fixed for 4 days in 10% formalin. After decalcification in 5% formic acid, the specimens were processed for paraffin embedding (24). Tissue sections (7 μm) were stained with H&E or safranin O. Histopathological changes were scored using the following parameters. Infiltration of cells was scored on a scale of 0–3, depending on the amount of inflammatory cells in the synovial cavity (exudate) and synovial tissue (infiltrate). A characteristic parameter in CIA is the progressive loss of bone. This destruction was graded on a scale of 0–5, ranging from no damage to complete loss of the bone structure. Histopathological changes in the knee joints were scored in the patella and femur/tibia regions on five semiserial sections of the joint, spaced 70 μm apart. Scoring was performed by two observers without knowledge of the experimental group, as described earlier (18).

Tartrate-resistant acid phosphatase (TRAP) staining

At the end of the experiment, whole knee joints were fixed for 2 days in 10% formalin, followed by decalcification in 10% EDTA (Tritiplex III; Merck, Darmstadt, Germany) in 1 mM Tris-HCl (pH 7.4) for up to 4°C (18). Decalcified specimens were processed for parafin embedding (18). Staining of tissue sections (7 μm) for TRAP was performed by a leukocyte acid phosphatase kit, a cell-staining kit for the detection of TRAP from Sigma-Aldrich (St. Louis, MO).

Radiology

At the end of the experiment, knee joints were isolated and used for x-ray analysis as a marker for joint destruction. X-ray photographs were carefully examined with a stereomicroscope, and joint destruction was scored on a scale from 0 to 5, ranging from no damage to complete destruction of the joint (18).

Immunohistochemistry of RANKL and RANK

Whole knee joints were fixed, decalcified, and paraffin embedded as previously described. Tissue sections (7 μm) were treated with 3% H₂O₂ for 10 min at room temperature (RT). Sections were incubated for 2 h with 10 mM citrate (pH 6.0) and thereafter incubated for 1 h with the primary Ab RANK (rabbit polyclonal Ab raised against the epitope corresponding to aa 46–317 of RANKL of human origin (FL-317)) or RANK (rabbit polyclonal Ab raised against the epitope corresponding to aa 317–616 mapping at the C-terminus of RANKL of human origin (H-300). Both Abs are from Santa Cruz Biotechnology, Inc. (Cruz, CA). Rabbit IgG Ab (X0936; DAKO, Carpinteria, CA) was used as a control. After rinsing, sections were incubated for 30 min with biotinylated goat anti-rabbit HRP (P0448; DAKO). Development of the peroxidase staining was done with 3,3′-diaminobenzidine (Sigma-Aldrich). Counterstaining was done with Mayer’s hematoxylin.

Determination of RANKL and OPG protein

To determine the levels of RANKL and OPG in patellar washouts, patellar tissue were isolated in a standardized manner from the knee joint, as previously described (25). Patellae were incubated in RPMI 1640 medium with 0.1% BSA, gentamicin (50 μg/ml), and l-glutamine (2 mM) (200 000 μl/patella) for 2 h at RT. After supernatant was harvested, the RANKL and OPG levels were measured by ELISA. Anti-murine RANKL Abs were purchased from R&D Systems (Abingdon, Oxon, U.K.; capture Ab: goat anti-mouse TRANCE mAb (AF462); detection Ab: goat anti-mouse TRANCE mAb biotin labeled (BAF462)). Briefly, ELISA plates were coated with the capture Ab (3 μg/ml) overnight incubation at 4°C in carbonate buffer (pH 9.6). Nonspecific binding sites were blocked by 1-h incubation at 37°C with 1% BSA in PBS/Tween. The supernatants from the patellar cultures were tested by 2-h incubation at RT. The plates were then incubated for 2 h at RT with the biotinylated secondary Ab, followed by a 30-min incubation at 37°C with streptavidin-polyperoxidase conjugate. Bound complexes were detected by reaction with orthophenylenediamine and H₂O₂. Absorbance was measured at 492 nm using an ELISA plate reader (Multiscan MCC/ 340; Titertek, Huntsville, AL). The cytokine concentration in the samples was calculated as picograms per milliliter using recombinant murine
RANKL as a standard (R&D Systems). To measure murine OPG levels, a commercial murine OPG ELISA kit was used (R&D Systems). The sensitivities of the RANKL and OPG ELISA kits are 20 and 4.5 pg/ml, respectively.

**Statistical analysis**

Differences between experimental groups were tested using the Mann-Whitney U test, unless stated otherwise.

**Results**

**Marked erosion of cortical, subchondral, and trabecular bone after local IL-17 gene transfer**

DBA-1 mice were immunized with suboptimal concentrations of CII, and shortly before expected onset (day 25), a single injection of AdIL-17 or control vector was given in the right knee joint. In line with our previous study (22), 5 days after viral injection, histologic analysis revealed a 100% incidence in the right knee joints of the AdIL-17 group compared with 41% incidence in the control vector group. All mice of the IL-17 group showed a maximal score for joint inflammation (Table I). In contrast, the mean score of joint inflammation in the control vector group was 0.6 ± 0.9, although the mice that developed arthritis had a mean score of 1.4 ± 1.0 (Table I). Five days after the viral injection, histologic analysis revealed that local IL-17 gene transfer induces hyperplastic synovial tissue, consisting of synoviocytes extending over the cortical bone surface of the patella and femur/tibia in all AdIL-17-injected knee joints. In addition, a significant increase in loss of cortical bone in the patella and femur/tibia region was noted compared with the control vector group (Fig. 1). Lateral and medial sites of the cortical bone of the patella and several parts of the femur/tibia were completely eroded 5 days after a single i.a. AdIL-17 injection in the knee joint of CII-immunized mice (Fig. 2).

**Table I. Effects of local IL-17 gene transfer on joint pathology during collagen arthritis**

<table>
<thead>
<tr>
<th>Incidence (%)</th>
<th>Inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>AdControl</td>
<td>41</td>
</tr>
<tr>
<td>AdIL-17</td>
<td>100</td>
</tr>
</tbody>
</table>

* DBA-1 mice were immunized and boosted with suboptimal concentrations of CII. Just before expected onset (day 25), mice were i.a. injected with 10⁷ PFU of AdIL-17 or the control vector (AdControl).

* Five days after viral injections, knee joints were histologically assessed for the appearance of arthritis in the injected joints.

* At the same time, the severity of knee joint inflammation in the arthritic mice only was histologically scored on a scale of 0–3. Results are the mean ± SD of two separate experiments with a total of at least 29 mice per group.

*, p < 0.001 vs control group, by Mann-Whitney U test.

**Figure 1.** AdIL-17 promotes bone destruction in CIA. DBA-1 mice were immunized and boosted with suboptimal concentrations of CII. Just before expected onset (day 25), mice were i.a. injected with 10⁷ PFU of AdIL-17 or control vector (AdControl). Five days after viral injection, histologic analysis revealed a 100% incidence in the right knee joints of AdIL-17 group compared with 41% incidence in the control vector group. All mice of the IL-17 group showed a maximal score for joint inflammation (Table I). In contrast, the mean score of joint inflammation in the control vector group was 0.6 ± 0.9, although the mice that developed arthritis had a mean score of 1.4 ± 1.0 (Table I). Five days after the viral injection, histologic analysis revealed that local IL-17 gene transfer induces hyperplastic synovial tissue, consisting of synoviocytes extending over the cortical bone surface of the patella and femur/tibia in all AdIL-17-injected knee joints. In addition, a significant increase in loss of cortical bone in the patella and femur/tibia region was noted compared with the control vector group (Fig. 1). Lateral and medial sites of the cortical bone of the patella and several parts of the femur/tibia were completely eroded 5 days after a single i.a. AdIL-17 injection in the knee joint of CII-immunized mice (Fig. 2).

**Figure 2.** Effects of IL-17 on bone erosion in CIA. A, Arthritic knee joint of a mouse 5 days after i.a. injection of 10⁷ PFU AdControl. Note the influx of inflammatory cells, but no bone erosion. B–C, Arthritic knee joint of a mouse 5 days after i.a. injection of 10⁷ PFU AdIL-17. Note the pronounced joint inflammation and (cortical) bone destruction in the patella (B) and at several places in the femur (C) (arrows). Original magnification, ×100. H&E was used. C, Cartilage; F, femur; JS, joint space; S, synovitis; Cb, cortical bone; P, patella.
FIGURE 3. AdIL-17 promotes TRAP activity in areas of subchondral, trabecular, and cortical bone erosions. A–C, Arthritic knee joint of a mouse 5 days after i.a. injection of 10⁷ PFU AdControl. Note the low TRAP staining in areas of subchondral (A), cortical (A), and trabecular (B) bone and in area of bone adjacent to the growth plate (C). D–F, Arthritic knee joint of a mouse 5 days after i.a. injection of 10⁷ PFU AdIL-17, showing TRAP activity in areas of cortical (D), subchondral (E), and trabecular (F) bone. G–H, Arthritic knee joint of a full-blown collagen-arthritis mouse with approximately the same degree of joint inflammation as the IL-17 group, showing TRAP activity in areas of trabecular (G) and cortical (H) bone. Note the enhanced TRAP activity induced by IL-17 compared with the control groups. Original magnification, ×100. C, Cartilage; S, synovitis; JS, joint space; SB, subchondral bone; TB, trabecular bone; CB, cortical bone; Bm, bone marrow; GP, growth plate.

Table II. Effects of local IL-17 overexpression on TRAP activity

<table>
<thead>
<tr>
<th>Joint Inflammation</th>
<th>Subchondral/trabecular bone</th>
<th>Cortical bone</th>
</tr>
</thead>
<tbody>
<tr>
<td>AdControl</td>
<td>0.6 ± 0.9</td>
<td>0.3 ± 0.4</td>
</tr>
<tr>
<td>AdIL-17</td>
<td>3.0 ± 0.1</td>
<td>2.5 ± 0.5***</td>
</tr>
<tr>
<td>Severe classic CIA</td>
<td>2.0 ± 0.4</td>
<td>1.1 ± 0.3</td>
</tr>
</tbody>
</table>

a DBA-1 mice were immunized and boosted with suboptimal concentrations of CII. Just before expected onset (day 25), mice were i.a. injected with 10⁷ PFU of AdIL-17 or the control vector (AdControl). Five days after viral injections, knee joints were taken for histology and analyzed for TRAP activity as described in Materials and Methods. In addition, mice with established collagen arthritis having approximately the same histologic joint inflammation scores (severe classic CIA) compared with the AdIL-17 group were analyzed for TRAP activity.

b The severity of arthritis in the injected joints and classic CIA was histologically assessed on a scale of 0–3.

c TRAP activity was scored in the patella/femur/tibia region using an arbitrary scale of 0–3. Results are the mean ± SD of at least 10 mice per group.

* p < 0.0001, vs control vector group; ** p = 0.0002, vs classic CIA; by Mann-Whitney U test.
Osteoclasts play a key role in bone resorption (18, 20, 21, 26, 27). To examine potential promotive osteoclastic activity after local IL-17 overexpression, TRAP staining was performed on paraffin-embedded knee joint sections. TRAP activity is a characteristic phenotypic marker of osteoclasts and osteoclast precursors and is expressed in osteoclast-like cells in mice with well-established collagen arthritis (18, 20). Mice of the control vector group revealed mild bone erosion and TRAP<sup>+</sup> osteoclast-like cells were only seen in mice that had developed marked arthritis. However, all mice of the IL-17 group showed marked TRAP<sup>+</sup> osteoclast-like cells at sites of erosion in subchondral, trabecular, and cortical bone of the patella and femur/tibia region (Table II; Fig. 3, A–F).

Local IL-17 overexpression promotes RANKL and RANK expression in the synovium and at sites of bone erosion

RANKL is a key mediator in osteoclastogenesis and bone resorption, and to be functionally active, RANKL needs binding to its unique RANK receptor (15, 16). Specific immunohistochemistry for RANKL and RANK was performed to examine the effect of IL-17 on local expression of RANKL and its receptor RANK in the synovium and at sites of bone erosion and whether the expression of RANK receptor colocalized with RANKL expression. Five days after the viral injection, local IL-17 overexpression in the synovium of CII-immunized mice results in accelerated local RANKL protein expression in the synovium compared with the control vector group (immunohistochemistry score for AdControl, 0.3 ± 0.1, vs AdIL-17, 2.0 ± 0.1) (Fig. 4, A and B). In addition, RANKL expression was detected at sites of focal bone erosion (Fig. 4D). Due to a higher degree of bone erosion, RANKL expression at sites of focal bone erosion was strongly elevated compared with the control vector group (AdControl, 0.3 ± 0.2, vs AdIL-17, 1.5 ± 0.4). Also more RANK-positive cells were found in the synovial infiltrate in the AdIL-17 group compared with the control vector group (AdControl, 0.2 ± 0.3, vs AdIL-17, 2.0 ± 0.3) (Fig. 4, E and F). Interestingly, the RANK receptor was expressed at sites of focal bone erosion (AdControl, 0.2 ± 0.3, vs

**FIGURE 4.** Effect of AdIL-17 on RANKL and RANK expression in the synovium and at sites of focal bone erosion. A, C, E, and G Arthritic knee joint of a mouse 5 days after i.a. injection of 10<sup>7</sup> PFU of AdControl, showing RANKL (A and C) and RANK (E and G) in the synovium. B, D, F, and H, Arthritic knee joint of a mouse 5 days after i.a. injection of 10<sup>7</sup> PFU AdIL-17, showing RANKL (B and D) and RANK (F and H) in the synovium and at sites of early focal bone erosion (RANKL (D) and RANK (H)). Note the increase of RANKL and RANK expression in the synovium and the colocalization of RANKL and RANK expression at site of early focal bone erosion after IL-17 overexpression. A, B, E, and F Original magnification, ×100; C, D, G, and H, Original magnification, ×200. No staining was found using a control antirabbit Ab (data not shown). C, cartilage; F, femur; Cb, cortical bone; S, synovitis; Bm, bone marrow.
AdIL-17, 1.8 ± 0.2) (Fig. 4H). In addition, sites of expression of RANK receptor correlated well with sites of RANKL expression under IL-17-overexpressed conditions (Fig. 4, D and H). These data indicate that IL-17 accelerates and enhances the local expression of RANKL and its receptor RANK.

**Increase in synovial RANKL/OPG ratio by AdIL-17**

The balance between RANKL-RANK signaling and the levels of the decoy receptor OPG regulate development and activation of osteoclasts and bone metabolism (15, 16). Therefore, we examined the effect of AdIL-17 on soluble RANKL (sRANKL) and OPG protein levels in the synovium using specific ELISAs. IL-17-induced joint inflammation in CIA results in a 3-fold increase in sRANKL levels compared with the control vector group (p = 0.0013). However, no significant difference in OPG levels were found between these groups (Fig. 5A). Interestingly, the sRANKL/OPG ratio was strongly enhanced by IL-17 (AdControl, 0.33, vs AdIL-17, 2.27) (Fig. 5B), implying that the IL-17-induced promotion of osteoclastic bone erosion is mediated by loss of the local sRANKL/OPG balance in the synovium.

**Comparison of AdIL-17 with full-blown collagen-arthritic mice**

To exclude the possibility that only a higher degree of joint inflammation is responsible for the increased TRAP activity after IL-17 overexpression, we compared arthritic mice from the AdIL-17 CIA group with control full-blown collagen-arthritic mice (classic CIA) having a comparable degree of joint inflammation (Table II). Full-blown classic collagen-arthritic mice revealed lower TRAP activity in areas of subchondral, trabecular, and cortical bone compared with the AdIL-17 group (Fig. 3, D–H, and Table II). These data indicate that overexpression of IL-17 promotes the formation of osteoclast-like cells in the bone marrow as well as in the synovium at sites of focal bone loss.

To further examine the potential differences in RANKL and OPG expression under these conditions, OPG and sRANKL protein levels were measured in the synovium of mice with severe CIA and compared with those of the AdIL-17-treated group. As shown in Fig. 5A, relatively high amounts of OPG were already detected in synovial washouts of naive mice. An increase in OPG levels (factor of 1.6 higher) was noted in synovial washouts of mice which were immunized with CII but without showing macroscopic signs of arthritis. No significant difference in OPG levels was detected in synovial washouts of mice with mild and severe collagen arthritis compared with the nonarthritic CII-immunized mice (factor of 3 higher in mild and severe CIA compared with naive mice), although high variation was found between individual mice. In contrast to the relatively high OPG expression in nonarthritic mice, RANKL was hardly detectable under these conditions (Fig. 5A). However, high levels of sRANKL were found in mice with severe collagen arthritis (factor of 28 higher compared with nonarthritic CIA mice, CIA severe vs CIA nonarthritics, p = 0.0007, by Mann-Whitney U test). Interestingly, even higher sRANKL levels were found in the AdIL-17 group compared with...
severe collagen-arthritic mice (factor of 2 higher), although the same maximal clinical score for joint inflammation was noted between these groups (p = 0.0016) (Fig. 5A). No significant difference was found for OPG levels (Fig. 5A), which resulted in a higher sRANKL/OPG ratio in the AdIL-17 group compared with that of the severe CIA group (Fig. 5B).

**Systemic OPG treatment prevented IL-17-induced bone erosion in CIA**

To further examine whether the IL-17-induced acceleration of bone erosion in CIA is due to RANKL up-regulation, CIA-immunized mice were, in addition to a local IL-17 gene transfer approach, also systemically treated with OPG to block RANKL activity in vivo. Five days after viral injections, no difference was observed in the arthritis incidence and severity of arthritis in the AdIL-17-injected knee joints of mice systemically treated with OPG or control BSA protein (data not shown). This indicates that systemic OPG treatment has no effect on the IL-17-induced promotion of joint inflammation in CIA. Radiologic analysis revealed marked joint erosion in the knee joints of CIA-immunized mice locally injected with AdIL-17 and systemically treated with the control protein (Fig. 6). In contrast, mice systemically treated with OPG showed only a mild degree of joint damage in the knee joints locally injected with AdIL-17 (p = 0.0002) (Fig. 6). These data strongly indicate that the IL-17-induced acceleration of bone erosion in CIA is strongly mediated by RANKL.

**Discussion**

In this study, we clearly demonstrated the potential of IL-17 to increase the sRANKL/OPG ratio in the synovium, promoting osteoclastic bone erosion in murine collagen arthritis. In addition, IL-17 accelerated the expression of RANKL and its receptor RANK in the synovial infiltrate and at sites of focal bone erosion. Furthermore, systemic OPG treatment in CIA-immunized mice prevented IL-17-induced bone erosion. This implies T cell IL-17 to be a potent stimulator of positive regulators of osteoclastogenesis and bone erosion in arthritis.

RA is considered a Th1-associated disease (28). Th1, but not Th2 cell clones isolated from RA synovium expressed IL-17. IL-17 may play an important role in the early stage of RA and during relapsing flares. It has been shown that IL-17 induces the key proinflammatory cytokines IL-1 and TNF-α (13, 22). In addition, in the present study, we showed that IL-17 is an potent inducer of RANKL in vivo. IL-17 can synergize with these cytokines (IL-1, TNF-α, and RANKL) but probably has direct pathogenic activity as well (7, 14, 22).

T cell cytokines are important regulators of osteoclastogenesis and bone erosion (29). Activated human T cells directly induced osteoclastogenesis in a coculture system using human cells (30). Activated T cells may influence bone metabolism through RANKL and RANK, although other T cell mediators could have a role as well. Recently, it was shown that T cell production of IFN-γ suppresses osteoclastogenesis by interfering with the RANKL/RANK signaling pathway (31). Furthermore, we reported that gene therapy with the Th2 cytokine IL-4 in collagen arthritis suppresses synovial IL-17 and RANKL and prevents osteoclastogenesis and bone erosion (18). The expression of IFN-γ and IL-4 in RA synovium is scant compared with relatively high synovial IL-17 expression (5). The local balance of these T cell cytokines may favor the induction of RANKL. Therefore, the presence of Th1 cells in the synovial infiltrate of RA patients may have major consequences on joint destruction, in which IL-17 is an important cytokine that has direct effect on joint damage and, in addition, can induce and synergize with IL-1 and RANKL signaling pathways (5, 22). In the present study, no differences were found in mRNA expression of IFN-γ or -β in the synovium of mice locally injected with AdIL-17 or AdControl.

There is ample evidence that joint inflammation and destruction can be uncoupled (32). The balance of destructive and protective mediators determines the relative erosive nature of a given arthritis, rather than the bulk of the inflammatory mass (18, 33). IL-1 and TNF are key cytokines in inflammatory joint disease. IL-1 exerts direct effects on osteoclast bone resorption via enhancement of osteoclast activity (26, 34). TNF-α appears to act principally on induction of osteoclast differentiation (34). Intriguingly, the present study showed relatively high OPG levels under normal conditions which increased during inflammation. This suggests a buffer condition preventing direct induction of bone erosion after joint inflammation. The balance between RANKL-RANK and OPG will ultimately determine the degree of bone destruction. Recently, we reported the proinflammatory and joint destructive potential of IL-17 in CIA (22). Elevated levels of synovial IL-1β were induced by AdIL-17. Blocking IL-1 using anti-IL-1α/β Abs, had no effect on the IL-17-induced acceleration of arthritis in the knee. Interestingly, radiologic analysis showed no difference in IL-17-induced joint damage between the anti-IL-1-treated group and the control group, indicating an IL-1-independent role for IL-17 in joint destruction. No reduction of RANKL and RANK expression was noted by specific immunohistochemistry after blocking IL-1 (data not shown). Data from the present study imply that the IL-17-induced bone destruction is strongly mediated by loss of the local RANKL/OPG balance.

IL-17 overexpression in the synovium of CIA-immunized mice enhanced osteoclast-like activity in the synovium and bone marrow. Synovial AdIL-17 gene transfer may result in influx of IL-17 in the marrow through bone canals. IL-17 in the marrow can activate bone lining cells promoting RANKL expression by these cells which may contribute to the enhanced TRAP activity found in the marrow (11). In the present study, we showed enhanced TRAP activity in the marrow at sites of subchondral and trabecular bone erosions after local IL-17 gene transfer. Apart from the effects in the marrow, high TRAP activity was detected at sites of focal bone erosion of the cortical bone, suggesting active osteoclastogenesis in the synovium. From this study, it is not clear whether there is a correlation between osteoclastogenesis in the marrow and synovium or osteoclastogenesis takes place/starts independently in the marrow as well as in the synovium. Interestingly, RA synovial macrophages are capable of differentiation into osteoclastic bone-resorbing cells in the presence of RANKL and M-CSF (35). Further characterization of osteoclast differentiation in the synovium under IL-17-induced inflammatory conditions is at present under investigation.

T cell IL-17 is expressed by a restricted set of cells (1–4). However, its receptor is ubiquitously expressed on many different cells. IL-17-induced RANKL expression has been shown in cultures of osteoblasts (11). In this study, we reported RANKL expression in synoviocytes (fibroblast-like and macrophage-like cells) in the arthritic synovium. Also, chondrocytes and T cells expressed RANKL (21, 29, 36–39). This indicates that IL-17 may induce RANKL expression in different cell types which can contribute to the stimulation of osteoclastogenesis leading to erosive lesions in affected joints.

In conclusion, the present study revealed clear increase of RANKL and RANK expression in the synovium by IL-17 in collagen-induced arthritis. Importantly, IL-17 up-regulates the synovial RANKL/OPG ratio, and systemic OPG treatment in CIA-immunized mice prevented IL-17-induced bone erosion. This loss of RANKL/OPG balance is likely the major cause for the enhanced
osteoclastic bone erosion induced by local IL-17 gene transfer. These data suggest that IL-17 is an important mediator in the activation of positive regulators of osteoclastogenesis and bone erosion, making this T cell cytokine an important target to prevent joint destruction in RA.

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References