Th17 Cells, Not IL-17+ γδ T Cells, Drive Arthritic Bone Destruction in Mice and Humans

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J Immunol published online 7 January 2011
http://www.jimmunol.org/content/early/2011/01/07/jimmunol.1003370

Supplementary Material
http://www.jimmunol.org/content/suppl/2011/01/07/jimmunol.1003370.DC1

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The mechanism whereby IL-17 drives rheumatoid arthritis remains incompletely understood. We demonstrate that anti–IL-17 therapy in collagen-induced arthritis ameliorates bone damage by reducing the number of osteoclasts in joints. We found equal numbers of CD4+ Th17 and IL-17 producing γδ T cells in the joints of arthritic mice, and in vitro, both populations similarly induced osteoclastogenesis. However, individual depletion and adoptive transfer studies revealed that in vivo, Th17 cells dominated with regard to bone destruction. Unlike γδ T cells, Th17 cells were found in apposition to tartrate-resistant acid phosphatase positive osteoclasts in subchondral areas of inflamed joints, a pattern reproduced in patient biopsies. This localization was caused by Ag-specific retention, because OVA-primed Th17 cells showed a γδ T cell-like diffuse distribution. Because IL-23, as produced by osteoclasts, enhanced T cell-mediated osteoclastogenesis, we propose that Ag-specific juxtaposition is key to foster the molecular cross talk of Th17 cells and osteoclasts, thus driving arthritic bone destruction.

**Materials and Methods**

**Mice**

Eight- to 10-wk-old DBA/1 male mice (Janvier) were used in accordance with Swiss Federal and Cantonal Authorities.

**Human samples**

Synovial fluid from three patients (60–68 y old) with RA based on American College of Rheumatology criteria were obtained from the Felix-Platter and University Hospital Basel. Duration of RA ranged from 16 to 000–000.

Reprinted from The Journal of Immunology, 2011, 186: 000–000.
20 y. Patients were undergoing cortisone treatment and one received abactocept. Informed consent from patients was obtained, and studies were conducted under guidance/agreement from the Basel Ethical Commission. Paraffin-embedded RA synovial tissue from a patient with chronic RA (53 y) treated with prednisolone, diclofenac, and leflunomide was purchased from Asterand. Activities of Asterand and their collaborators were conducted in accordance with applicable laws, regulations, and ordinances. Human metacarpal joints were obtained by informed consent from five RA patients (four women and one man) undergoing joint replacement surgery under the terms of the ethical approval granted by the combined office of human research ethics committee (COREC No. 07/Q0411/30). Patients were aged 44–65 y of age at the time of surgery.

**Th17 NOT IL-17**

Intracellular cytokines and surface staining

For intracellular cytokine staining, 1 × 10^6 cells/ml were cultured at 37°C for 4 h in medium containing 5 μg/ml brefeldin A, 50 ng/ml PMA, and 0.5 μg/ml ionomycin (Sigma-Aldrich). After activation, cells were stained for intracellular cytokines using an intracellular cytokine staining kit (BD Pharmingen). Abs were purchased from BD Pharmingen or eBioscience. Before staining, Fc-blockade (anti-CD16/32) was performed. For FACS, T cell populations were first gated on CD45 and subsequently on CD3^+CD4^+ and CD3^+CD8^+CD45R^-. For mouse, we employed the following Abs: FITC–IFN-γ, PE–IL-17, FITC–or PerCP–CD45, alloglycoprotein–CD3, biotinylated γΔTCR, Alexa 488– or PerCP–CD4, PE–CD8, CD11b–PerCP, FITC–KSI-67, alloglycoprotein–Gr1, streptavidin–PerCP, and streptavidin–alloglycoprotein. For human, we used the following Abs: FITC–IFN-γ, PE–γδTCR, PE–CD4, PerCP–CD3, and Alexa Fluor 647–IL-17A. Flow cytometry data were acquired on a FACS Calibur (BD Bioscience) and analyzed with FlowJo 6.42 software (Tree Star). Cell sorting was performed on a FACSAria II (BD Bioscience).

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Quantitative real-time PCR analysis of FACS-sorted T cells

**Quantitative real-time PCR analysis of FACS-sorted T cells**

FACS-sorted spleen, lymph nodes, or joint cells were placed in RNeasy Micro RNA Isolation Kit lysis buffer (Qiagen). eDNA was generated using the High Capacity CDNA RT Kit (Applied Biosystems). Quantitative real-time PCR (qRT-PCR) analyses were performed using a TaqMan Low Density Array (Applied Biosystems) with the following assays (assay ID): IL-17A (Mm00439618_m1), IL-17B (Mm00444686_m1), IL-17C (Mm00521397_m1), IL-17D (Mm01314722_m1), IL-17F (Mm00525142_m1), IL-23A (p19) (Mm00519894_m1), IL-23R (Mm00519943_m1), RANKL (Mm004419_08_m1), IL-1β (Mm01133618_m1), TNF-α (Mm00443258_m1), IL-6 (Mm00990641_m1), IL-21 (Mm00517640_m1), IL-22 (Mm00444241_m1), retinoic acid-related orphan receptor α (RORA) (Mm00443103_m1), ROY1 (Mm01264022_m1), CCR6 (Mm09999114_s1), and 18s RNA (Hs99999999_s1) for normalization.

**Adaptive transfer**

A total of 1 × 10^6 FACS-purified γδ and CD4^+ T cells from spleen and lymph nodes of CIA mice or CD4^+ T cells from spleen and lymph nodes of collagen type II or OVA-primed mice (day 10) were labeled with CellTracker CM-DIL (Invitrogen) and injected i.p. into CIA recipient mice. Forty-eight hours later, animals were sacrificed, paws snap-frozen, and 20-μm sections generated on a cryostat (Microm).

**In vitro induction of osteoclasts**

FACS-sorted γδ or CD4^+ T cells (5 × 10^5) isolated from organs of arthritic mice were cocultured with bone marrow cells (2 × 10^5) for 6–10 d. In one experiment, FACS-purified γδ or CD4^+ T cells were preincubated with 10 ng/ml recombinant mouse IL-23 for 24 h before coculture. Positive control was bone marrow cells incubated with M-CSF (10 μg/ml), RANKL (10 μg/ml), and IL-1α (5 μg/ml; R&D Systems). Tartrate-resistant acid phosphatase-positive cells were counted.

**Statistical analysis**

Unless otherwise noted, data are presented as means with the error bars representing SEM. Data were analyzed either by two sample tests (t test for normally distributed data, and rank sum test for data that was either not normally distributed or of unequal variance), or by ANOVA (one way for normally distributed data of equal variance or ANOVA on ranks when data were not normally distributed or of unequal variance). In addition, two-way ANOVA was used as appropriate (see figure legends). Post hoc tests for one-way ANOVA used Tukey’s test and for ANOVA on ranks used Dunn’s test, the latter was used because of unequal group sizes. Normality was detected by Shapiro–Wilk tests, and equal variance among groups was determined by an F-test. χ^2 test was used to determine the ratio of human T cells in proximity to bone. Global ρ value was set at 0.05. All tests were done using SigmaPlot 11 (Systat Software).

**Results**

Anti–IL-17A therapy protects bone destruction in CIA

IL-17 is known to be involved in the pathogenesis of arthritis. To understand the role of this cytokine with regard to bone destruction, we treated arthritic mice with two different neutralizing anti–IL-17A Abs (mAb 3-784-9 and mAb 421). Anti–IL-17A treatment before onset of clinical signs of disease potently inhibited disease severity (Fig. 1A). This was accompanied by a lower histopathological disease score with respect to inflammatory cell infiltrate, cartilage damage, and bone loss (Fig. 1B, 1C). Supplemental Fig.
Because osteoclast-mediated bone destruction is an integral component of CIA, TRAP+ cells were quantified in paw sections. As expected, osteoclast numbers were significantly reduced in joints from anti–IL-17A-treated animals (Fig. 1D). This confirmed the relevance of IL-17A for the induction of osteoclast-mediated bone destruction in arthritis.

Arthritic joint-derived γδ and CD4+ T cells take on Th17 characteristics, express pro-osteoclastogenic cytokines, and induce TRAP+ osteoclast formation

Next, we defined the source of IL-17 in arthritic joints. We observed that in CIA, IL-17 is produced solely by γδ and CD4+ T cells (data not shown). To define the net contribution of both T cell populations, we characterized these cells on isolation from different locations during CIA. This enabled us to track peripheral primed cells (in spleen and draining lymph nodes) with pathogenic effector T cells after invasion into arthritic joints. To evaluate any potential cross-contamination caused by preparative procedures when analyzing joint-derived lymphocytes, we determined the frequency of γδ and CD4+ T cells in purified bone marrow preparations. We found that the frequencies were low (≤0.4% γδ and ≤7.4% CD4+ T cells), and of these low percentages, ~7.6% and ~1.0%, respectively, were IL-17+ (data not shown).

Within the γδ T cell population, IL-17–producing cells were 3-fold enriched in arthritic joints as compared with the periphery. This enrichment was more pronounced for the CD4+ T cell fraction where a 14-fold greater accumulation of the percentage of IL-17+ cells was observed in the joint (Fig. 2A).

Although there were about 4-fold more CD4+ T cells than γδ T cells in the arthritic joints (Fig. 2B, left column), the number of IL-17–producing CD4+ and γδ T cells was almost equal (Fig. 2B, right column, 2C) because the latter population contained a greater proportion of IL-17+ cells compared with the CD4+ counterpart.

To gain insight into the effector mechanisms used by these two T cell types in CIA, we performed qRT-PCR analysis for expression patterns of Th17 lineage markers on FACS-sorted γδ and CD4+ T cells from the spleen, lymph nodes, and joints of CIA mice (Supplemental Fig. 2). γδ T cells in arthritic mice always displayed features of Th17 cells, most notably in inflamed joints. Both T cell populations when isolated from this tissue showed an upregulation of IL-17A, IL-17F, and IL-22, and transcription factors RORγt and RORα when compared with peripherally derived counterparts (Fig. 3A). Of note, both γδ and CD4+ T cells from arthritic joints expressed high and similar levels of IL-23R and the Th17-associated CCR6 (Fig. 3A).

Th17 cells display a pro-osteoclastogenic potential and enhance osteoclast activity via the induction of RANKL in osteoclastogenesis-supporting cells, thus linking T cell activation and bone destruction (29). However, the participation of IL-17–producing γδ T cells in this paradigm is not described. To compare γδ and CD4+ T cells for their potential to contribute to bone damage, they were isolated from the spleen, draining lymph nodes, or arthritic joints of CIA mice, and analyzed for the expression of cytokines known to be involved in osteoclast activation. In addition to IL-17, γδ and CD4+ T cells from the arthritic joints displayed a comparable expression pattern of other pro-osteoclastogenic factors such as RANKL, TNF-α, IL-1β, and IL-6 (Fig. 3B). To address the functional significance of these results, we assessed the bone pathogenic potential of γδ and CD4+ T cells. These cells were cocultured with bone marrow cells using TRAP staining to indicate de novo osteoclast formation. Because there is as yet no means to enrich for the IL-17+ subpopulations, we performed our studies with whole preparations of γδ and CD4+ T cells. Identical numbers of FACS-sorted γδ and CD4+ T cells isolated from the peripheral organs could induce some TRAP+ cells. However, in line with the previous molecular findings, both...
γδ and CD4+ T cells exerted a higher potential to induce osteoclasts when derived from arthritic joints (Fig. 3C). We saw an equipotent ability of γδ and CD4+ T cells from arthritis joints to induce osteoclast formation despite the knowledge that a greater percentage of γδ T cells produce IL-17 in this organ, highlighting the fact that IL-17 may not be the only driver of osteoclastogenesis.

Because of experimental procedures, we cannot exclude that a low percentage of contaminating non-T cells such as macrophages and fibroblasts were contributing to the pro-osteoclastogenic signature of joint-derived T cell populations (Supplemental Fig. 2). Nevertheless, these experiments demonstrate that γδ T cells analogous to CD4+ T cells exhibit an IL-17–biased and pro-osteoclastogenic signature especially on isolation from arthritic joints and have the potential to compromise bone integrity.

Depletion of CD4+ T cells protects bone damage in CIA

The in vitro data could not entirely exclude pro-osteoclastogenic effects from contaminating cells. Thus, to unequivocally define the individual relevance of the pathogenic capability of γδ and CD4+ T cells in vivo, cell type-specific depletion studies were performed on early onset of disease in arthritic mice. These experiments revealed a strikingly distinct role for these T cell populations. Whereas ablation of γδ T cells delayed disease, albeit nonsignificantly, depletion of CD4+ T cells suppressed arthritis progression (Fig. 4A, Supplemental Fig. 3A, 3B). This was reflected in the histopathological and microcomputerized tomography analysis where anti-CD4+ T cell treatment led to a strong protection of bone integrity (Fig. 4B–D). These dichotomous in vivo results were unexpected because our previous experiments revealed comparable numbers of IL-17–producing CD4+ and γδ T cells.
T cells in the arthritic joints (Fig. 2B) with in vitro capacities to affect bone integrity (Fig. 3C). In histopathological joint sections, TRAP + osteoclasts were prominent within areas of high bone turnover in control and anti-gd T cell-treated animals. However, ablation of CD4+ T cells led to a drastic reduction in osteoclast number (Fig. 4E). This indicates that presence of CD4+ but not gd T cells is an essential component for osteoclast activity in murine arthritis.

Differential localization of gd and CD4+ T cells within the arthritic joint

To define the mechanism behind these in vivo findings, we investigated whether there may be a differential localization of gd and CD4+ T cells within the inflamed tissue. Labeled gd or CD4+ T cells from collagen-primed animals were adoptively transferred into arthritic recipients to assess their migration patterns. Although both cell types possessed comparable capacities to migrate to the spleen (Supplemental Fig. 4A,4B), there were markedly less total gd T cells infiltrating the joint (Fig. 5A), in line with our findings in Fig. 2B. These adoptive transfer studies revealed major differences in the localization of gd or CD4+ T cells within the inflamed joint. Although about half of the CD4+ T cell population was found in close proximity (within 100 μm) to bone, only 10% of the gd T cells could be identified in this area (Fig. 5B). Histopathological analysis of serial sections revealed that the labeled gd T cells were predominantly positioned near soft tissue structures such as tendons where osteoclasts and other bone-associated cells were absent. Thus, although gd T cells were found in areas of IL-17 staining, they were not associated with TRAP+ osteoclasts (Fig. 5C, left column, Supplemental Fig. 4C). In contrast, many fluorescently labeled CD4+ T cells (confirmed by IHC analysis in Supplemental Fig. 4D) could be found adjacent to TRAP+ osteoclasts in areas of IL-17 positivity (Fig. 5C, middle and right columns). However, we assume that not all of the CD4+ T cells were positive for this cytokine.

One major difference between gd and CD4+ T cell subsets is their responsiveness to the priming Ag collagen type II, with only the latter mounting an Ag-specific response (30, 31). Proliferation (thymidine incorporation) assays with CD4+ T cells were performed to monitor the collagen-specific recall response using splenocytes and lymphocytes from collagen-immunized DBA/1 animals. In both organs, an Ag-specific response with a stimulation index between 2 and 3 was routinely measured on incubation with 50 μg/ml collagen II over baseline (data not shown). To dissect whether Ag-specific retention of CD4+ T cells is an
underlying cause for the observed phenotype, we performed adoptive transfer studies using CD4\(^+\) T cells from donors primed with collagen II or OVA in adjuvant. Although both Ag-primed CD4\(^+\) T cells had an equal capacity to migrate into the arthritic joints (data not shown), only the collagen type II-specific T cells associated with bone. In contrast, OVA-primed CD4\(^+\) T cells exhibited a similar localization pattern as the innate γδ T cells (Fig. 5D).

Because we have previously shown that IL-17-producing cells were enriched in joint versus peripheral CD4\(^+\) T cell fractions (Fig. 2), we also wanted to determine how many of the adoptively transferred collagen II- and OVA-primed peripheral CD4\(^+\) T cells became IL-17\(^+\) on invasion into arthritic joints. Although we managed to recover the transferred CFSE-labeled cells, their numbers were so low that a solid determination of IL-17 positivity was not possible.

These data suggest that γδ and CD4\(^+\) T cells have different functions in the pathogenesis of arthritis. Collagen-specific CD4\(^+\) T cells have, by nature of their localization, a greater capacity to interact with osteoclasts in vivo and thus accentuate joint pathology.

**CD4\(^+\) T cells in close apposition to osteoclasts in human RA**

To assess whether our findings translate to human RA, we analyzed γδ and CD4\(^+\) T cells in synovium and synovial fluid of RA patients. Analogous to studies by Ito et al. (18), we found that in the synovial fluid, the percentage of IFN-γ-positive γδ or CD4\(^+\) T cells far outnumbered those producing IL-17 (Supplemental Fig. 5). To study the localization of γδ and CD4\(^+\) T cell populations, we performed IHC on biopsy sections of RA synovial tissue showing bone destruction, vessel-rich regions, and inflammatory cell infiltrates. Similar to the CIA mouse model, there were less total numbers of infiltrating γδ versus CD4\(^+\) T cells in the inflamed tissue (Figs. 2B, 6A). Many CD4\(^+\) T cells were localized in perivascular cuffs in blood vessel-rich areas, whereas the γδ T cells were more disperse within similar regions (Fig. 6A). In addition, and in striking analogy to the mouse system, we found ~55% of CD4\(^+\) T cells in close proximity to bone (Fig. 6B), often in clusters surrounding multinucleated osteoclasts (Fig. 6C). γδ T cells were more diffusely distributed with only ~35% of the cells close to bone surfaces. Similar to the CIA model, IL-17 immunoreactivity was detected in proximity of the bone (Fig. 6D), highlighting the potential for bone-resorbing osteoclasts to respond to this secreted proinflammatory cytokine. These findings again point toward a differential contribution of γδ and CD4\(^+\) T cells to bone pathology in RA patients in analogy to the CIA model.

**IL-23 produced by osteoclasts enhances the osteoclastogenic profile of T cells**

Increasing evidence points toward a bidirectional cytokine communication between cells of the immune system and osteoclasts. In human RA, the relevance of the IL-23/IL-17 axis has been
FIGURE 5. Differential localization of γδ and Ag-specific CD4+ T cells within the arthritic joint. A, Quantification of total infiltrating CM-DIL+ γδ and CD4+ T cells in CIA joints after adoptive transfer. Statistical analysis was a rank sum test. B, Percentage of bone-associated (within 100 μm) γδ and CD4+ T cells in CIA joints after adoptive transfer. A and B, Data are average ± SEM. Blinded scoring of three midsections per inflamed paw with n = 3–6 animals per adoptive transfer group. Pooled data from two separate experiments. C, Histopathological analysis of joints from CIA recipient mice after adoptive transfer of γδ T cells (left column) or CD4+ T cells (middle and right columns). Photomicrograph showing H&E-stained frozen sections. Boxes in first row indicate areas displayed in rows 3–5. Influx of fluorescently labeled CM-DIL γδ T cells or CD4+ T cells. Dashed lines show outline of bone/tendon. Asterisk shows autofluorescence in the lower power CM-DIL images. IHC for IL-17 (faint staining in γδ T cell section [arrows]). Asterisk shows nonspecific staining of tendon with anti–IL-17 Ab. TRAP staining for presence of active osteoclasts. Scale bars, 50 μm. D, Percentage of bone-associated (within 100 μm) collagen II- or OVA-primed CD4+ T cells in CIA joints after adoptive transfer. Data are average ± SEM. Blinded scoring of a midsection per inflamed paw with n = 6–7 paws per group. Statistical analysis for B and D was a two-way ANOVA using Tukey’s test for pairwise comparisons. B, bone; T, tendon.
described to be associated with the T cell-mediated bone destruction phase of the disease (26, 27). Studies with IL-23–deficient mice further support the requirement for this cytokine toward development of arthritis (20). We found that IL-23p19 mRNA was highly expressed in the joints of arthritic mice, whereas it was only marginally present in the periphery of arthritic animals and joints of naive mice (Fig. 7A). By RT-PCR, we found expression of IL-23p19 mRNA on FACS-sorted cells from arthritic joints. Monocytic cells (Gr1med CD11bhigh) and neutrophils (Gr1high CD11bhigh) were positive for this cytokine (Fig. 7B).

Because osteoclasts are derived from the monocytic lineage, we were interested to investigate whether IL-23 is also produced by the mature bone-resorbing cells. A mixture of IL-1β, RANKL, and M-CSF induced de novo generation of TRAP+ osteoclasts, which was accompanied by a significant upregulation of IL-23p19 mRNA as the cells matured in culture (Fig. 7C).

It is known that IL-23 is essential to generate pathogenic IL-17+ effector T cells (19). Indeed, conditioning peripheral CD4+ T cells with recombinant mouse IL-23 overnight led to a significant increase in IL-17 production (data not shown) and an enhancement of TRAP+ osteoclasts on coculture with bone marrow cells (Fig. 7D). We conclude that there is a cross talk between IL-17+ Ag-specific CD4+ T cells and IL-23–producing osteoclasts that leads to aggravated bone erosion (Fig. 8).

Discussion

We demonstrate that in arthritic joints, collagen II-specific Th17 cells rather than IL-17+ γδ T cells are the main effectors that trigger osteoclast-mediated joint destruction in an IL-23–dependent manner.

Pathogenic bone erosion in RA is mediated by the mobilization and subsequent differentiation of osteoclast precursors within the
inflamed synovial tissue. Active osteoclasts localize at the boundary between synovium and articular bone, leading to erosion (32). These cells require complex cytokine signals from surrounding stroma and immune cells, to generate and maintain osteoclast activity (2). In RA patients, pro-osteoclastogenic cytokines such as RANKL, IL-23, and IL-17 are increased in synovial fluid and synovium compared with osteoarthritic and normal counterparts (11, 26, 27, 33, 34), and are predictive for bone destruction (35).

We confirmed that blockade of endogenous IL-17A significantly reduced bone erosion in arthritic mice (28, 36, 37), and that gd and CD4+ T cells are responsible for production of this cytokine. Furthermore, both T cell populations, when derived from the arthritic joint, displayed a remarkably similar in vitro profile with respect to Th17 gene signatures; however, because our study inhibited only IL-17A, we cannot exclude an additive effect by blocking other Th17-associated cytokines.

Both joint-derived CD4+ and γδ T cells similarly upregulated pro-osteoclastogenic genes including RANKL, IL-23, and IL-17 are increased in synovial fluid and synovium compared with osteoarthritic and normal counterparts (11, 26, 27, 33, 34), and are predictive for bone destruction (35).

We confirmed that blockade of endogenous IL-17A significantly reduced bone erosion in arthritic mice (28, 36, 37), and that γδ and CD4+ T cells are responsible for production of this cytokine. Furthermore, both T cell populations, when derived from the arthritic joint, displayed a remarkably similar in vitro profile with respect to Th17 gene signatures; however, because our study inhibited only IL-17A, we cannot exclude an additive effect by blocking other Th17-associated cytokines.

Both joint-derived CD4+ and γδ T cells similarly upregulated pro-osteoclastogenic genes including RANKL and induced osteoclastogenesis in in vitro cocultures, revealing a comparable potential to destroy bone. IL-17 can exert its osteoclastogenic effects via RANKL (11), but the cellular source thereof is under debate (3). Sato et al. (29) used in vitro polarized Th17 cells and showed that these induced osteoclastogenesis only in the presence of osteoblasts. In our studies, we used cell populations isolated from arthritic animals to show ex vivo osteoclastogenic capabilities of CD4+ and γδ T cells, which may highlight a direct function for T cells in the disease situation, in line with a role of IL-17 in osteoclastogenesis (12). Interestingly, the mRNA of IL-1β, although rarely recognized as a product of T cells, was present in low and high levels in the spleen and joint-derived T cell populations, respectively. However, studies in other disease settings have indicated that IL-1 may be produced by T cells (38–40). Therefore, we cannot eliminate the possibility that Ag-specific T cells are producing this cytokine in the CIA model.

Despite the many aforementioned similarities of the IL-17–producing CD4+ and γδ T cells, our studies highlighted the particular importance of CD4+ T cells over γδ T cells for bone pathology. Ag-specific retention appeared to account for the
accumulation of CD4⁺ T cells to the collagenous surfaces of bone and cartilage in the CIA mouse because CD4⁺ T cells primed for an irrelevant epitope were less frequently found near bone. Indeed, osteoclasts may serve as APCs: they express MHC class I and II, CD80, CD86, and CD40, and have the capacity to present soluble Ags and activate T cells (41). We speculate that in CIA, osteoclasts, among other APCs, present peptides to CD4⁺ T cells leading to their subsequent activation and local retention on formation of a stable immunological synapse. This preferential retention of Ag-specific Th17 cells in the arthritic joint may explain the enrichment of IL-17⁺ CD4⁺ T cells in arthritic joints and in proximity to the bone.

In human RA, the close proximity of CD4⁺ T cells and osteoclasts in areas of IL-17 production within the rheumatoid synovium closely resembled our observations in the CIA model. This would imply that targeting CD4⁺ T cells might be a successful therapeutic strategy for RA. However, clinical RA trials with depleting anti-CD4 Abs were not successful (42, 43). This has been attributed to inadequate dosing, duration of treatment, or because the anti-CD4 Abs did not reach the synovium (44). Nevertheless, the therapeutic principle of interference with the biological synapse has been validated clinically by the successful use of CTLA4-Fc in RA patients (45). Whether formation of the biological synapse is blocked in the joint or in the lymphoid organs is not known. In two clinical trials, however, structural joint changes were significantly slowed down in patients treated with abatacept versus placebo controls (46, 47), pointing toward a potential effect of the inhibitor at the T cell–osteoclast interface. The relevant pathogenic Ag(s) in RA remain under debate (48). However, our data and the results from the abatacept trials suggest that bone-derived Ags are involved in pathology, at least within the joint, and that bone-associated CD4⁺ T cells may be key contributors toward bone erosion in RA.

Although under nonpathological conditions IL-23 has been described to have a protective effect on bone mass via its inhibitory effect on osteoclastogenesis (49), a pathogenic role for the IL-17/IL-23 axis is well established in autoimmune diseases, including arthritis. In a pathogenic setting, IL-23 is known to enhance the effector functions of Th17 cells (19, 20, 50, 51), as well as IL-23R⁺ γδ T cells (18, 22, 52). Our data are in line with studies by Yago et al., who demonstrated that IL-23 stimulates human osteoclast differentiation indirectly via Th17 cell activation (53). We demonstrated that the osteoclastogenic potential of T cells from the periphery was enhanced when cells were either precultured with IL-23 or derived from IL-23-rich arthritic joints.

Besides monocytes and neutrophils, we identified osteoclasts as the main effectors of IL-23 in CIA joints. This suggested the existence of a self-perpetuating feedback loop between these cells and IL-17–producing T cells. We propose that the joint-destructive aspect of IL-17 mostly stems from the IL-17⁺ CD4⁺ T cells that localize to bone and activate/differentiate osteoclasts. IL-23–expressing osteoclasts, in turn, capture Th17 cells and enhance their expression of RANKL and IL-17, and thus, their pathogenic capacity.

We therefore speculate that in CIA, as well as RA, IL-17 produced by CD4⁺ T cells plays the dominant role with respect to osteoclast function and bone destruction. It is still too early to evaluate the prevention of structural damage with anti–IL-17 therapies in clinical RA trials; however, our findings would suggest that a bone-protective mechanism is involved.

Acknowledgments
We thank Rita Nagele, Grażyna Wieczorek, Nadja Mamber, Melanie Ceci, Catherine Huck, and Kathrin Wagner for technical assistance and Dr. Daniele Benz for provision of human samples. We also thank Dr. José Carballo for fruitful scientific discussions.

Disclosures
B.P., T.J., B.M., C.A., S.B., R.K., F.R., F.D.P., T.O.R., D.D.P., and A.L.-E. are or were employees of Novartis Institutes for BioMedical Research and/or own Novartis shares.

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