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Development of Proteoglycan-Induced Arthritis Is Independent of IL-17

Paul D. Doodes,* Yanxia Cao,‡ Keith M. Hamel,* Yumei Wang,‡ Balint Farkas,† Yoichiro Iwakura,§ and Alison Finnegan2**

IL-17 is the hallmark cytokine for the newly identified subset of Th cells, Th17. Th17 cells are important instigators of inflammation in several models of autoimmune disease; in particular, collagen induced arthritis (CIA) and experimental autoimmune encephalomyelitis (EAE), which were previously characterized as Th1-mediated diseases. Although high levels of IFN-γ are secreted in CIA and EAE, disease is exacerbated in IFN-γ or IFN-γ receptor-deficient mice due to the ability of IFN-γ to suppress IL-17 secretion. However, in proteoglycan-induced arthritis (PGIA), severe arthritis is dependent on the production of IFN-γ. We were therefore interested in determining the role of IL-17 in PGIA. We assessed the progression of arthritis in IL-17-deficient (IL-17−/−) mice and found the onset and severity of arthritis were equivalent in wild-type (WT) and IL-17−/− mice. Despite evidence that IL-17 is involved in neutrophil recruitment, synovial fluid from arthritic joints showed a comparable proportion of Gr1+ neutrophils in WT and IL-17−/− mice. IL-17 is also implicated in bone destruction in autoimmune arthritis, however, histological analysis of the arthritic joints from WT and IL-17−/− mice revealed a similar extent of joint cellularity, cartilage destruction, and bone erosion despite significantly reduced RANKL (receptor activator of NK-κB ligand) expression. There were only subtle differences between WT and IL-17−/− mice in proinflammatory cytokine expression, T cell proliferation, and autoantibody production. These data demonstrate that IL-17 is not absolutely required for autoimmune arthritis and that the production of other proinflammatory mediators is sufficient to compensate for the loss of IL-17 in PGIA. The Journal of Immunology, 2008, 181: 329–337.

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease of unknown etiology affecting ~1% of the world population. Disease is characterized by chronic inflammation of the synovial tissues of multiple joints leading to joint destruction and loss of function (1–3). Several induced animal models have been developed to aid our understanding of the disease process and for the development of therapeutic tools for intervention (4). Two such models are collagen-induced arthritis (CIA) and proteoglycan (PG)-induced arthritis (PGIA), where collagen and PG (aggrecan), respectively, are used as Ags. The histological, pathological, and some immune features of both CIA and PGIA closely resemble those of human disease and, as such, represent valuable models for the study of RA.

CD4+ Th cells differentiate under the influence of APCs into effector cells specializing in cytokine secretion and function. Th1 cells produce IFN-γ and are important mediators of cell immune response to infectious agents in some autoimmune disease models (5–10). PGIA and CIA were originally designated as Th1-mediated autoimmune diseases based on the robust production of IFN-γ. The importance of IFN-γ in PGIA was confirmed by the observation that arthritis onset and severity are reduced under conditions where IFN-γ is neutralized or in mice deficient in IFN-γ (11). By contrast, in CIA, lack of the IFN-γ receptor exacerbates autoimmune disease. Similarly as in CIA, in another autoimmune model, experimental autoimmune encephalomyelitis (EAE), disease is enhanced in IFN-γ-deficient mice (12–14). Paradoxically, CIA and EAE were associated with Th1 responses based on studies in which disease is inhibited by treatment with neutralizing Abs to IL-12, a cytokine known to drive Th1 responses. The delineation of IL-12 into a family of cytokines including IL-23 (IL-12p40 and IL-12p19) and IL-27 (p28 and EB13) (15, 16) revealed the divergent roles for IL-12 and IL-23 in EAE and CIA (17–19). Reports showed that the induction of EAE and CIA is ablated in mice lacking only IL-23 (p19−/−) and in mice deficient in IL-12p40, which lack both IL-12 and IL-23. Conversely, mice deficient in IL-12 alone (IL-12p35−/−) remain susceptible (17–19), clearly establishing a requirement for IL-23 (20, 21). Evidence that IL-12 and IFN-γ are not responsible for inflammation in CIA or EAE implied the existence of a separate population of proinflammatory effector T cells. Studies revealed that mice deficient in IL-23 have reduced IL-17 secretion (22), demonstrating a link between IL-23 and IL-17 production. A role for IL-17 in the pathogenesis of CIA and EAE was confirmed in IL-17-deficient mice, by IL-17 overexpression, and neutralization studies (21, 23–26). These data lead to the characterization of the Th17 cell subset as a separate lineage of Th cells (27, 28). Importantly, it was found that IFN-γ regulates IL-17 activity (29) and thus provides an explanation for why a deficiency in IFN-γ or IFN-γ signaling in CIA and EAE leads to an increase in IL-17 and exacerbated disease.
There are several important functions of IL-17 that may contribute to its importance in models of arthritis and possibly RA. IL-17 acts on several cell types including macrophages, dendritic cells, T cells, endothelial cells, fibroblasts, and synovial cells to up-regulate chemokines, cell adhesion molecules, and importantly cytokines, in particular the proinflammatory cytokines IL-1β, TNF, and IL-6 (30). Neutrophils are the dominant cell population in the synovial fluid of RA patients and in autoimmune models of arthritis. IL-17 is involved in the accumulation and activation of neutrophils through the induction of CSFs and C-X-C chemokines (31–33). Overexpression of IL-17 in knee joints of naive and type II collagen-immunized mice results in neutrophil infiltration into the joint (24). Furthermore, an important role for IL-17 in osteoclactogenesis has been reported (24, 34–36). Both IL-1 and TNF II collagen-immunized mice results in neutrophil infiltration into rheumatoid arthritis joints. In addition there was no difference between WT and IL-17 generations and then intercrossed to obtain WT and IL-17-deficient mice on the BALB/c background (designated throughout as IL-17−/− or IL-17−/− mice). Our results demonstrated that the onset and severity of arthritis was similar in IL-17−/− and wild-type (WT) mice. Furthermore, WT and IL-17−/− mice exhibit comparable numbers of neutrophils in synovial fluid and equivalent bone erosion in arthritic joints. In addition there was no difference between WT and IL-17−/− mice in the systemic inflammatory responses dominated by IFN-γ, TNF, IL-1β, and IL-6. Our results demonstrate that any effect of IL-17 deficiency is compensated for by the production of other proinflammatory cytokines in PGIA.

Materials and Methods

Mice

IL-17-deficient mice on the BALB/c background (designated throughout as IL-17−/−) were generated as previously described (38). IL-17−/− mice were backcrossed to BALB/c for eight generations. The IL-17−/− mice were further backcrossed to BALB/c (Charles Rivers Laboratories) for two generations and then intercrossed to obtain WT and IL-17−/− litters. The BALB/c Charles Rivers (Kingston colony) is the most susceptible BALB/c subline for the induction of PGIA. Mice were genotyped using primers specific for IL-17A. WT and IL-17−/− litters were used in all experiments. IFN-γ−/−, IL-12p40−/−, and IL-12p35−/− mice were purchased from The Jackson Laboratory and maintained in the Rush University Medical Center facility (Chicago, IL). Female BALB/c age matched 12–14 wks of age were used in all experiments. All animal experiments were approved by the institutional Animal Care and Use Committee at Rush University Medical Center.

Induction and assessment of arthritis

Human cartilage was obtained following joint replacement surgery and provided through the Orthopedic Tissue, Transplant, and Implant Repository of Rush University Medical Center with the approval of the Institutional Review Board of Rush University. PG was isolated as previously described (39). Female WT and IL-17−/− mice were immunized i.p. with 150 μg of human PG measured as protein in dimethylhexadecyl ammonium bromide (DDA) (Sigma-Aldrich) as described (40). Mice received booster immunizations at wk 3 and 6 with 100 μg of PG in DDA. Mice were monitored for arthritis twice weekly and scored in a blinded manner. Paw swelling was scored based on an established scoring system on a scale from one to four as follows: 0, normal; 1, mild erythema and swelling of several digits; 2, moderate erythema and swelling; 3, more diffuse erythema and swelling; and 4, severe erythema and swelling of complete paw with ankylosis. Incidence of arthritis denotes the percentage of mice that develop PGIA. Each animal received a cumulative score ranging from 0 to 16, based on individual paw scores of 0–4.

Detection of serum Ab titers by ELISA

Mice immunized with human PG were anesthetized and bled from the orbital plexus. Serum was obtained and examined for Abs against mouse and human PG by ELISA. EIA tissue culture “half-area” plates (Costar Coating) were coated overnight at 4°C with μg of chondroitinase ABC-digested PG or 0.75 μg of native mouse PG in carbonate buffer. Plates were serially diluted in PBS containing 0.5% Tween 20. Samples were incubated with the immobilized PG, and plate-bound human PG- or mouse PG-specific Ab was detected using peroxidase-conjugated rabbit IgG against mouse IgG1 and IgG2a (Zymed Laboratories), respectively, which was then detected with the substrate o-phenylenediamine. Samples were run in duplicate. Colorimetric change in each sample was measured with a spectrophotometer at 490 nm. Results were expressed as the mean ± SEM of Abs from 5–10 mice.

Assessment of T cell activation by proliferation

CD4+ T cells from the spleens of PG-immunized mice were purified by negative selection using CD4+ isolation kits and autoMACS automated separation (Milteny Biotech). Purified CD4+ T cells (2.5 × 106 cells/ml) were incubated in 24-well Falcon plates (Fisher Scientific) in 200 μl of serum-free IL-1 medium (Fisher Scientific) containing 100 μg/ml penicillin, 100 μg/ml streptomycin, and 2 mM l-glutamine (complete medium) in the presence or absence of PG (10 μg/ml). T cell cultures were incubated at 37°C in 1% CO2 for 5 days and pulsed with [3H]thymidine (0.5 μCi/well) for the last 18 h. The cells were harvested using a cell harvester (Tomtec) and the incorporated [3H]thymidine was measured using a scintillation counter (EC&G Wallac). Cells were cultured in triplicate.

Assessment of cytokines

Single cell suspensions of splenocytes harvested from PG-immunized mice were prepared as previously described (41). Splenocytes (2.0 × 106 cells/ml) were incubated in 24-well Falcon plates (Fisher Scientific) in RPMI 1640 complete medium in triplicate (41). CD4+ T cells were purified using CD4 isolation beads (Milteny Biotech) from spleens of immunized mice and cultured with naive irradiated (2500 rad) spleen cells. Cells were cultured in the absence or presence of PG (20 μg/ml). Cytokines were measured from supernatants harvested on day 4 by ELISA using the Opt EIA mouse IFN-γ, IL-4, IL-1β, TNF, and IL-6 sets (BD Pharmingen) and a mouse IL-17 ELISA kit (R&D Systems).

Assessment of splenic and synovial fluid cell populations

Spleen, lymph node, and synovial fluid cells were extracted at the time of sacrifice and analyzed by flow cytometry. Synovial fluid was collected from ankle joints of arthritic mice by repeated flushing of the open joint cavity with PBS followed by scraping of the synovial intima with a pipet tip. Approximately 3–5 × 106 cells were obtained from a single ankle joint with an arthritis score of 3–4. Cells were immunostained using fluorescent-conjugated Abs specific for GR1 or isotype control (BD Bioscience). Naive and PG-immune WT and IL-17−/− spleen and lymph node cells were stained with Abs specific for T cells (CD3, CD4, CD8, and B cells; CD19) (BD Bioscience). Regulatory T cells were stained with Abs specific for CD3, CD4, and CD25 (BD Biosciences) and intracellular Foxp3 using the Foxp3 staining kit (eBioscience). Stained cells were acquired using a FACSCanto II flow cytometry system (BD Pharmingen) and data were analyzed with FACSDiva software (BD Pharmingen).

Quantitative RT-PCR (qRT-PCR)

Hind paws were minced and joint tissue RNA was isolated using TRI-Reagent (Molecular Research Center). Reverse transcription was performed with random hexamers for priming and SuperScript II reverse transcriptase (Invitrogen). Gene-specific amplification was performed using qRT SYBR Green Supermix (Bio-Rad) and normalized to β-actin levels for each sample. All samples were run in triplicate on a Bio-Rad iQ5 machine using Bio-Rad proprietary iQ5 software. To confirm that the same amount of RNA was added to each PCR, murine β-actin amplification was performed on each sample. Relative fold induction was calculated using the formula 2 ΔΔCt, where ΔΔCt(CT(treatment) − CT(control)) − ΔCt(CT(treatment) − CT(β-actin)) (where CT(treatment), CT(control), and CT(β-actin) is the cycle at which the threshold is crossed). PCR product quality was monitored using post-PCR melt curve analysis. Controls were from naive nonimmunized and target genes from PG-immunized WT and IL-17−/− joint tissue.
FIGURE 1. PGIA is dependent on IL-12p35, IL-12p40, and IFN-γ. Groups of age-matched female mice were immunized i.p. with human PG in adjuvant three times at 3-wk intervals and monitored for arthritis onset and severity by a blinded observer. WT (n = 10), IL-12p35−/− (n = 8), or IL-12p40−/− (n = 8) incidence (A) is expressed as the percentage of mice that developed arthritis. Disease severity (B) is the sum of paw inflammation scores divided by the number of arthritic mice. WT (n = 17) and IFN-γ−/− (n = 17) mice were immunized as described. Incidence (C) and severity (D) are shown. Results are shown as the mean scores ± SEM for week after the initial immunization. Asterisks (*) denote significant differences (p ≤ 0.05). Data are representative of two or three experiments performed.

Histology

Hind ankle joints of immunized mice were isolated at wk 13 after initial immunization. Joints were fixed in formalin, decalcified in 5% formic acid, and embedded in paraffin and H&E. Bone erosion was measured as follows. At least five nonarticulating bone surfaces 1 mm in length were measured at the distal tibia, calcaneus, and one or two metatarsal bones. Erosive changes were scored on three semiserial sections of the joint spaced 50 μm apart. Results were expressed as a percentage of the eroded length compared with the total length examined. Mean values were obtained for individual mice and then a mean was obtained for each group (n = 10). Data represent the mean ± SEM percentage of bone erosion. Cellular infiltration was measured on a scale of 0–4 by a blinded observer and values represent mean ± SEM of (n = 11) sections from two independent experiments.

Statistical analysis

The Mann-Whitney U test was used to compare nonparametric data for statistical significance. p < 0.05 was considered significant.

Results

IL-12p35, p40, and IFN-γ are essential for PGIA severity

The role of IL-12 in autoimmune disease models has been reexamined in light of the delineation of the IL-12 family of cytokines. In this study we used mice deficient in either IL-12p35 or IL-12p40 to examine the role of IL-12 in PGIA. IL-12p35−/− and IL-12p40−/− mice were immunized with PG and the arthritis onset and severity were compared with those in WT mice. The incidence of disease was not significantly reduced in IL-12p35−/− and IL-12p40−/− mice (Fig. 1A); however, a significant and sustained reduction in disease severity was observed in both the IL-12p35−/− and IL-12p40−/− mice in comparison to WT mice (Fig. 1B). The similarity in arthritis onset and severity in the IL-12p35−/− and IL-12p40−/− mice demonstrated that IL-12 is critical for the development of PGIA. However, we cannot eliminate a role for IL-23 in PGIA without investigating the development of arthritis in IL-23p19−/− mice.

We have previously demonstrated a requirement for IFN-γ in PGIA (11). In the present study we confirm these findings to show that despite early resistance to disease, IFN-γ−/− mice eventually succumb to disease, albeit at a reduced incidence and severity in comparison to WT mice (Fig. 1, C and D). IFN-γ deficiency does not prevent the development of arthritis; however, it is an important factor in governing the severity of disease. The difference in disease onset and the reduction in the incidence of arthritis in IFN-γ−/− mice when compared with either IL-12p35−/− or IL-12p40−/− mice suggest that IFN-γ−/− and IL-12 contribute different signals in the induction of arthritis. Together, these results demonstrate that the development of PGIA is dependent upon IL-12 and IFN-γ, indicating that the Th1 pathway is operative in this autoimmune disease.

Systemic and local joint expression of inflammatory cytokines in PGIA

The emerging function of IL-17 in several models of inflammation (21, 42–46) suggested a possible role for IL-17 in PGIA. We first examined the concentration of IFN-γ and IL-17 and several known proinflammatory cytokines, TNF, IL-6, and IL-1β, in the synovial fluid from inflamed ankle joints of WT mice (Fig. 2A). Pooled samples of synovial fluid from arthritic mice were examined by ELISA and standardized to total protein. The T cell derived cytokines IFN-γ and IL-17 were present at low concentrations; conversely, TNF and particularly IL-6 and IL-1β were highly expressed.

We next assessed the level of IFN-γ and IL-17 transcripts in arthritic joint tissue. IFN-γ expression was modestly elevated in the joint tissue from arthritic mice while IL-17 transcripts were elevated >3-fold compared with control nonimmune joint tissue (Fig. 2B). The reduced levels of IL-17 protein in comparison to RNA transcripts may be due to the use of IL-17 protein by synovial tissue cells. To determine whether IL-17 was produced systemically in PGIA, spleen cells from arthritic mice were restimulated in vitro with PG and supernatants were examined for IFN-γ and IL-17 production by ELISA (Fig. 2C). IFN-γ and IL-17 were produced in spleen cell cultures; however, significantly more...
IFN-γ was produced in comparison to IL-17. The predominance of IFN-γ in the spleen and IL-17 mRNA in the joint suggested that both cytokines may play a role in the generation of a robust inflammatory response in PGIA.

**IL-17 is not required for the development of PGIA**

To assess the role of IL-17 in the development of PGIA, we used IL-17−/− mice. WT and IL-17−/− mice were immunized with PG. WT and IL-17−/− PG-specific T cells were tested for expression of IL-17 by ELISA, and the level of IL-17 protein in the supernatant from IL-17−/− T cells was below the limits of detection (Fig. 3A). IL-17−/− mice succumbed to disease with similar kinetics and degree of severity in comparison to those of WT mice (Fig. 3, C and D). These data demonstrate that IL-17 is not critical for the development of PGIA. Together with our previous data showing an important role for IFN-γ in PGIA, these data support an inflammatory role for IFN-γ (11) and not IL-17 in this model, indicating that in different models of arthritis IFN-γ and IL-17 may function differently.

**Joint tissue histology is similar in WT and IL-17−/− mice**

To determine whether the similarity in paw erythema and swelling in WT and IL-17−/− mice corresponded to comparable cellular infiltration and joint damage, we examined joint histology from hind limbs. The histological picture in WT and IL-17−/− mice was characteristic of acute arthritis (Fig. 4, A–D). The infiltration of mononuclear and polymorphonuclear cells in the synovial cavity and adjacent tissues, edema of the synovial and periarticular tissues, and synovial hyperplasia were similar in WT and IL-17−/− mice.

We further characterized the synovial infiltrating cells, because IL-17 is known to play an important role in the recruitment of neutrophils (24, 47). Neutrophils were necessary to maintain chronic inflammation in PGIA, because the depletion of neutrophils with anti-Gr1 mAb suppressed chronic inflammation (data not shown). If IL-17 was necessary for neutrophil recruitment in PGIA, we would anticipate that IL-17 deficiency would lead to a reduction of the neutrophils recruited to the synovial cavity. We isolated the synovial fluid from ankle joints of WT and IL-17−/− arthritic mice and stained for Gr1+ cells (Fig. 4F). Gr1+ cells comprised 80–90% of the cell population in the synovial fluid. There was no significant difference in the proportion of Gr1+ neutrophils or the number (data not shown) in the synovial fluid of WT and IL-17−/− arthritic mice. These data demonstrate that in PGIA, IL-17 deficiency does not affect inflammation or the infiltration of neutrophils.

![Figure 2](http://www.jimmunol.org/)

**FIGURE 2.** Inflammatory cytokines are expressed systemically and in joints of arthritic mice. A, IFN-γ, IL-17, IL-4, TNF, IL-6, and IL-1β cytokine concentrations in synovial fluid of arthritic WT mice standardized to total protein levels (n = 8). B, RNA was harvested from inflamed joint tissues and analyzed by qRT-PCR. The relative increase in RNA expression to nonimmune joint tissue was determined. Asterisk (*p ≤ 0.05) denotes significant differences between IFN-γ and IL-17 transcripts. C, Spleen cells from immunized WT mice were cultured in the presence and absence of PG for 4 days (n = 5). Supernatants were harvested and assayed by ELISA for IFN and IL-17. Values represent the mean ± SEM of (quadruplicate cultures of individual mice). Results are representative of at least two experiments. Asterisk (*) denotes significant differences (p ≤ 0.05) between IFN-γ and IL-17 cytokine production.

![Figure 3](http://www.jimmunol.org/)

**FIGURE 3.** IL-17−/− mice succumb to PGIA with similar onset and severity as WT mice. WT (n = 7) and IL-17−/− (n = 17) age-matched female mice were immunized i.p. with human PG in adjuvant three times at 3-wk intervals and monitored for arthritis onset and severity by a blinded observer. A, Splenocytes were stimulated in the presence and absence of PG. B, Incidence is expressed as the percentage of mice that developed arthritis. C, Disease severity is the sum of paw inflammation scores for each mouse divided by the number of arthritic mice. Results are shown as the mean scores ± SEM for the week after the initial immunization. Data are representative of three experiments performed.
Expression of RANKL transcripts does not correlate with bone erosion in IL-17−/− mice

IL-17 induces the expression of RANKL in osteoblasts and RANKL is an important regulator of osteoclastogenesis (37, 48). To investigate the possibility that RANKL expression may be reduced as consequence of ablated IL-17, we compared the expression of RANKL transcripts in inflamed joint tissues in WT and IL-17−/− mice (Fig. 5A). We observed a reduction in RANKL expression of >50% in IL-17−/− mice. To determine whether the reduction in RANKL expression resulted in decreased bone erosion, we examined the degree of bone erosion in WT and IL-17−/− arthritic mice. Despite the substantial reduction in RANKL expression in the joint tissues, we observed similar degrees of bone erosion in WT and IL-17−/− mice visualized as cellular infiltration into nonarticular bone surface areas (Fig. 5B), suggesting that the level of RANKL expression was sufficient for osteoclast activation and bone erosion. Thus, the extent of cellular infiltration, the similarity in the neutrophil composition, and the level of bone erosion in WT and IL-17−/− mice suggest that, in the absence of IL-17, a redundant pathway exists for inflammation.

Inflammatory cytokine expression in spleen and joint

We have seen robust inflammation in IL-17−/− mice similar to that in WT mice (Fig. 3, A and B), demonstrating effective homing of inflammatory cells to the joint. To determine whether there were any differences in the downstream effects of IL-17 deficiency, we measured the level of ex vivo spleen cells from arthritic WT and IL-17−/− mice to generate cytokines associated with inflammation. Concentrations of TNF, IL-1β, and IL-6 were similar in WT and IL-17−/− splenocyte culture supernatants (Fig. 6, A–C). We also examined the expression of cytokine transcripts from joint tissues of arthritic mice (Fig. 6, D–F). Similar to the results from spleen cultures, TNF expression in WT and IL-17−/− was comparable. However, IL-1β was expressed significantly more in IL-17−/− joints while, conversely, IL-6 expression was significantly less in IL-17−/− mice compared with WT mice. Despite differences in mRNA expression, inflammation was indistinguishable between IL-17−/− and WT mice (Fig. 3, A and B) demonstrating the complex and redundant pathways used in mediating inflammation.

PG-specific T cell and B cell responses were similar in WT and IL-17−/− mice

The demonstrated inflammatory nature of both IFN-γ and IL-17 in autoimmune diseases suggests that both cytokines may contribute to inflammation in PGIA. IL-17 production is regulated by IFN-γ (29), and some data suggest that IL-17 may regulate IFN-γ expression (49). To determine whether IFN-γ was up-regulated in IL-17−/− mice, we cultured CD4+ T cells from arthritic WT and IL-17−/− mice in the presence and absence of PG. There was no significant difference in IFN-γ production by CD4+ T cells or whole spleen cultures from WT and IL-17−/− mice (Fig. 7A and data not shown).

We have shown that IL-4 functions to suppress the severity of PGIA via STAT6 (11, 41, 50). Similarly IL-4 inhibits the IL-17-induced inhibition of chondrocyte PG synthesis in intact murine articular cartilage (34). To examine the possible role of IL-4 in IL-17−/− mice, we assessed IL-4 production from arthritic WT and IL-17−/− mice (Fig. 7B). In accord with our earlier studies, we found minimal IL-4 production in WT or IL-17−/− mice. We were unable to identify an inhibitory role for IL-17 in IL-4 production in PGIA.

To determine whether CD4+ T cells were similarly activated, we examined the Ag-specific proliferation of CD4+ T cells from immunized WT and IL-17−/− mice. We found proliferation to be significantly higher in the IL-17-deficient CD4+ T cells compared with WT cells (Fig. 7C). This is in accord with a previously described antiproliferative role for IL-17 (51). However, we were unable to identify a significant difference in either the total number of...
of lymphocytes in the spleen and lymph nodes or the percentages of CD3, CD4, CD8, regulatory T cells, and CD19 cells in WT and IL-17/H11002/H11002/H11002 mice (data not shown).

We have previously shown PG-specific T cells and B cells to be critical for the development of PGIA (52, 53). Recently, IL-17 deficiency has been shown to inhibit IgG2a production in CIA (42) and to play a role in germinal center development in autoimmune mice (54). To determine whether B cell responses were intact in IL-17/H11002/H11002/H11002 mice, we examined PG-specific Ab isotype concentrations from the serum of immunized arthritic mice. We found anti-mouse and anti-human PG IgG1 and IgG2a Ab isotypes to be comparable in WT and IL-17/H11002/H11002/H11002 mice (Fig. 7, D and E).

Discussion

PGIA is critically dependent on CD4+ T cells and their production of IFN-γ (41, 55). The recent identification of the new members of the IL-12 family and the discovery of the pathogenic Th17 subset (15, 26, 28, 56) suggested the possibility that IL-17 may be involved in the pathogenesis of PGIA. We first determined that IL-12 (p40/p35)-deficient mice developed significantly less severe arthritis than WT mice. Although we cannot rule out a role for IL-23 (p40/p19) or IL-35 (p35/EbI3), the reduction in arthritis correlates with a similar suppression of arthritis in IFN-γ−/− and STAT4−/− mice, further establishing PGIA as a Th1-mediated

FIGURE 6. Systemic inflammatory cytokines were unaffected by IL-17 deficiency, whereas joint tissue mRNA transcripts were altered. Spleen cells from immunized mice (n = 5) were cultured in the presence or absence of PG for 4 days. Supernatants were harvested and assayed by ELISA for TNF (A), IL-1β (B), and IL-6 (C). Values represent the mean ± SEM. TNF (D), IL-1β (E), and IL-6 (F) transcripts were harvested from inflamed ankle joints of arthritic and WT and IL-17−/− mice and analyzed by qRT-PCR. Each sample was standardized to β-actin expression and run in triplicate. Data represents cytokine transcript expression in IL-17−/− joint tissue relative to WT. Values are the mean ± SEM. Asterisks (*) denote significant differences (p ≤ 0.05). Results are representative of two experiments.

FIGURE 7. Cytokines and PG-specific CD4+ T cell proliferation were enhanced and PG-specific Ab isotypes were minimally affected by IL-17 deficiency. Spleen cells from PG-immunized WT (n = 8) and IL-17−/− mice (n = 8). CD4+ T cells were isolated and stimulated in the presence or absence of PG with irradiated naive spleen cells. Supernatants were harvested and assayed by ELISA for IFN-γ (A) or IL-4 (B). For proliferation, T cells were pulsed with [3H]thymidine (C). Serum concentrations of anti-human (h) and anti-mouse (m) PG IgG1 (D) and IgG2a (E). Values are the mean ± SEM. Asterisk (*) denotes significant differences (p ≤ 0.05).
disease. We next assessed whether IL-17 was produced in PGIA. We found IL-17 transcripts to be expressed in arthritic joint tissues but only minimally in the spleen, the converse of the IFN-γ expression pattern. To demonstrate a contribution of IL-17 in the development of PGIA, we used IL-17-deficient mice. There was no apparent consequence of a loss of IL-17 expression on the onset or the severity of arthritis. Assessment of the inflamed joints histologically showed that cellular infiltration, cartilage destruction, and bone erosion were similar in IL-17−/− and WT mice.

In PGIA, the synovial fluid is comprised mainly of neutrophils. The role of IL-17 in granulopoiesis and the recruitment of neutrophils (23) suggested that the primary cell population infiltrating the inflamed joint may be altered in IL-17−/− mice. However, the numbers and percentages of Gr1+ neutrophils in the synovial fluid of arthritic joints were similar in WT and IL-17−/− mice. These data reveal a redundant pathway for neutrophil recruitment in PGIA. It is likely that IFN-γ or downstream effector cytokines such as TNF, IL-1β, and IL-6, which are present in IL-17−/− mice, mediate the recruitment of neutrophils in the absence of IL-17. Sun et al. demonstrated that IL-12 is capable of promoting IFN-γ-dependent recruitment of neutrophils (57) and augmentation of TNF, which is itself capable of recruiting neutrophils (58). In addition, IL-6 has been shown in a model of rheumatoid synovitis to directly mediate neutrophil recruitment (59). Thus, the ability of IL-17 to augment neutrophil recruitment in the presence of other proinflammatory cytokines is not required in PGIA.

In several models of arthritis, IL-17 plays a role in stimulating osteoclastogenesis. We quantified the degree of bone erosion in serial sections of arthritic hind limbs from WT and IL-17−/− mice. We were unable to identify any difference in the extent of bone erosion between WT and IL-17−/− mice. IL-17 induces fibroblasts to produce TNF and IL-6 and promotes bone erosion in CIA via RANKL and IL-1β (47). However, our data indicate that IFN-γ, TNF, IL-1β, and IL-6 were sufficient to induce bone erosion in the absence of IL-17. We observed an ~50% reduction in RANKL expression in IL-17−/− mice, confirming the importance of IL-17 in RANKL expression. Nevertheless, the degree of inhibition of RANKL was insufficient to prevent the destruction of bone. Studies looking at bone erosion in three separate models, ovariectomized mice, LPS stimulation, and inflammation, reveal that IFN-γ stimulates osteoclast activation indirectly through T cell secretion of RANKL (60). There are several overlapping effects of IFN-γ, IL-1β, IL-6, and TNF with IL-17, suggesting that in PGIA the lack of one of these cytokines might be easily compensated for by the presence of the others.

It is now apparent that IFN-γ regulates the development of Th17 cells (26, 28) and disease severity in a number of autoimmune models (21, 42, 61–63). Likewise, there is some evidence that IL-17 may regulate IFN-γ production. Komiya et al. (21) report that myelin oligodendrocyte glycoprotein-specific, IFN-γ-producing T cells are increased in IL-17−/− mice. IL-4 also inhibits Th17 production (26, 28); however at present there is no evidence that IL-17 regulates IL-4 production. Our results do not support a role for IL-17 in regulation of either IFN-γ or IL4 production in PGIA, as PG-specific CD4+ T cells from WT and IL-17−/− mice produce equivalent amounts of IFN-γ and IL-4. As a measure of the efficacy of T cell responses in IL-17−/− mice, we examined the proliferation of CD4+ T cells in response to PG. We found that the IL-17−/− CD4+ cells proliferate significantly more than WT cells. These data are in accord with the documented anti-proliferative effects of IL-17 seen in intestinal epithelial cells (51) and could contribute to the robust inflammation seen in the IL-17−/− mice.

Several models of induced and spontaneous arthritides and other autoimmune diseases are dependent on IL-17 (21, 42–46), whereas several are dependent on IFN-γ (5–11, 41, 64). There are a number of possible mechanisms to explain the lack of reliance on IL-17. First, the dominant IFN-γ response in PGIA appears sufficient to drive the production of proinflammatory cytokines such as TNF, IL-1β, and IL-6 in the absence of IL-17. Second, the consequence of a strong IFN-γ response is the inhibition of IL-17, making it less important in inflammation. Thus, we would predict that if the IFN-γ response was reduced, a role of IL-17 might be uncovered. It is presently unclear what drives the IFN-γ response in PGIA, but because Th1 and Th17 cells differentiate under the influence of different cytokines it is likely that the cytokine milieu in which disease is initiated determines the T cell phenotype. It is also possible that IFN-γ and IL-17 act at different phases of disease. For example, in CIA early blockade of IFN-γ inhibits disease while late blockade exasperates disease (65). Similarly, in a model of uveoretinitis IL-17 was important for disease severity only late in the maintenance phase (45).

Despite strong evidence for IL-17 in some autoimmune disease models, data supporting a role for IL-17 in RA are inconclusive. Recent reports show increased IL-17 in RA synovial fluids and in the T cell areas of RA synovial tissue (66, 67). IL-17 was also overexpressed in serum and activated PBMC cultures of patients with RA (68). However, another study found that the frequency of Th17 cells in the joints of RA patients was significantly decreased compared with that in the peripheral blood of the same RA patients (69). IFN-γ and its receptor IFN-γR are significantly up-regulated in the joints of RA patients compared with patients with osteoarthritis (70). Also, a genetic linkage of RA to IFN-γ is detected in affected sibling-paired families (71). The role of IFN-γ and IL-17 in RA remains the subject of intense research. Moreover, as the differences between mouse and human Th subset biology become more evident (72–74), we should be cautious assuming physiological relevance to any one model, particularly in light of the heterogeneity of human disease (75–77).

In this study, using an established murine model of RA, we have shown that IL-17 is unnecessary for the onset or severity of inflammation in PGIA. We further show that neutrophil recruitment to inflamed joints and bone erosion are unaffected by a deficiency in IL-17.

Disclosures
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References

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