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IL-27 Induces a Th1 Immune Response and Susceptibility to Experimental Arthritis

Yanxia Cao,† Paul D. Doodes,* Tibor T. Glant,‡ and Alison Finnegan2g†

IL-27 is the newest member of the cytokine family comprised of IL-12 and IL-23. IL-27 was originally described as a cytokine that along with IL-12 induces the differentiation of naive precursor T cells into Th1 effector cells. This activity has been called into question based on evidence in infectious disease and autoimmune models in which IL-27 is not absolutely required for the generation of IFN-γ, and IL-27 plays a regulatory role in controlling inflammation. We have previously reported in proteoglycan-induced arthritis (PGIA), a model of rheumatoid arthritis, that severe arthritis is dependent on the production of IFN-γ. In this study, we report that IL-27 was expressed in spleen and joint tissues of arthritic mice. We determined the involvement of IL-27 in PGIA by assessing the progression of arthritis in IL-27R−/− mice. Development of arthritis in IL-27R−/− mice was delayed and severity reduced in comparison with IL-27R+/+ littermate controls. Histology confirmed a reduction in joint cellularity, cartilage destruction, and bone erosion. Diminished arthritis was associated with fewer T cells producing IFN-γ and decreased IFN-γ secretion overtime. Moreover, the frequency of IL-4- and IL-17-expressing T cells and the production of IL-4 and IL-17 were similar in IL-27R−/− mice and controls. Our results indicate that IL-27 is critically involved in the induction of inflammation in PGIA. IL-27 functions by inducing the differentiation of IFN-γ-producing T cells in vivo that are essential for the development of arthritis. The Journal of Immunology, 2008, 180: 922–930.

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2g Address correspondence and reprint requests to Dr. Alison Finnegan, Department of Medicine, Section of Rheumatology, Rush University Medical Center, 1735 West Harrison Avenue, Chicago, IL 60612. E-mail address: Alison_Finnegan@rush.edu
3 Abbreviations used in this paper: EBI3, EBV-induced gene 3; CIA, collagen-induced arthritis; Ct, cycle threshold; EAE, experimental autoimmune encephalomyelitis; PGIA, proteoglycan-induced arthritis; Tg, transgenic; PG, proteoglycan; TCCR, T cell cytokine receptor.

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**Materials and Methods**

**Mice**

T cell cytokine receptor-deficient mice (designated in this study as IL-27R−/−) were provided by F. de Sauvage (Genentech, South San Francisco, CA) (2). IL-27R−/− mice were backcrossed to BALB/c (Charles River Laboratories) for seven generations and then intercrossed to obtained IL-27R−/− and IL-27R+/− littermates. Female BALB/c age-matched IL-27R−/− and IL-27R+/− littermates 12–14 wk of age were used in all experiments. TCR transgenic (Tg) mice expressing a TCR specific for a dominant epitope of human PG were used to test primary responses to IL-27 (28, 29). 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 from patients undergoing joint replacement surgery, and was provided through the Orthopedic Tissue, Transplant, and Implant Repository of Rush University Medical Center, with the approval of the Institutional Review Board. PG (aggrecan) was extracted from pooled cartilage samples, as previously described (23). Female mice were immunized i.p. with 150 μg of human PG measured as protein, emulsified in dimethyldioctadecylammonium bromide (DDA) (Sigma-Aldrich), as previously described (30, 31), and boosted with 100 μg of PG/DDA at 3 and 6 wk.

Mice were monitored for arthritis and scored in a blinded manner. Paws were scored for arthritis on a scale from 1 to 4, as follows: 0, normal; 1, mild swelling affecting several digits or mild swelling of the paw; 2, moderate erythema and swelling of the paw; 3, severe swelling affecting the entire paw and with hardening of the articular tissue. Each animal received a cumulative score ranging from 0 to 16, based on incidence of joint inflammation and damage. Hind limbs were dissected, decalcified, embedded in paraffin, and sectioned at 6 μm. Sagittal sections were stained with H&E, and cellular infiltration was measured on a scale from 0 to 4, with 0 being no infiltrating cells and 4 being massive infiltration.

**Detection of anti-proteoglycan Ab by ELISA**

Mice immunized with human PG were bled from the orbital plexus, and serum was obtained and examined for Abs against mouse proteoglycan and human proteoglycan by ELISA.

Enzyme immunoassay tissue culture 96 half-area plates (Costar) were coated overnight at 4°C with 0.5 μg of chondroitinase ABC-digested human PG, or 0.75 μg of native mouse proteoglycan in carbonate buffer. Serum was serially diluted in buffer (PBS/0.5% Tween 20). Serially diluted serum samples and internal standards samples (pooled sera from arthritic mice) were incubated with immobilized PG. Abs were detected with HRP-labeled rabbit anti-mouse IgG1 and IgG2a (Zymed Laboratories), which were then detected with the substrate o-phenylenediamine. The relative concentration of Ab was determined from a standard curve of known concentrations of unlabelled murine IgG1 and IgG2a Ab (Southern Biotechnology Associates). Data represent the mean ± SD of quadruplicate cultures. *p < 0.05. Data are representative of two separate experiments.

**Assessment of T cell activation by proliferation**

CD4+ (2.5 × 105 cells/well) purified by AutoMACS selection (Miltenyi Biotech) were stimulated with PG (10 μg/ml) and irradiated (2500 rad) spleen cells (2.5 × 105 cells/well) from naive IL-27R−/− mice. Cells were cultured in quadruplicate in 96-well Falcon plates (Fisher Scientific) in RPMI 1640 medium containing 10% FCS, 100 μg/ml penicillin, 100 μg/ml streptomycin, and 2 mM l-glutamine (complete medium). Cultures were pulsed on day 4 with [3H]thymidine overnight and examined for proliferation. In other experiments, as indicated in figure legends, CD4+ T cells from naive PG-specific Tg mice were stimulated with irradiated naive BALB/c mice spleens under suboptimal concentrations of PG (5 μg/ml) in the presence or absence of IL-27 (1 ng/ml; eBiosciences). Cells were cultured in quadruplicate in 96-well Falcon plates (Fisher Scientific) in serum-free HL-1 medium containing 100 μg/ml penicillin, 100 μg/ml streptomycin, and 2 mM l-glutamine. Cultures were pulsed on day 2 with [3H]thymidine overnight and examined for proliferation.

**Assessment of cytokines**

For cytokine production, nylon wool-purified T cells (2.5 × 105 cells/well) from peritoneal exudates (pooled from four to six mice) or CD4+ T cells (2.5 × 105 cells/well) selected by AutoMACS from spleen of PG-immunized mice were stimulated with PG (10 μg/ml) and irradiated (2500 rad) spleen cells (2.5 × 105 cells/well) from naive IL-27R−/− mice. Cells were cultured in quadruplicate in 96-well Falcon plates (Fisher Scientific) in complete medium. Supernatants were harvested at day 4 for the detection of IFN-γ, IL-4, and IL-17 cytokines, and 24 h for the detection of IL-2 by conventional sandwich ELISA (BD Biosciences). For spleen cytokine production, spleen cells from PG-immunized mice were isolated and cultured (2 × 107/ml) in complete medium in 24-well Falcon plates (Fisher Scientific) in medium alone or PG (20 μg/ml). Supernatants were harvested and assayed by ELISA for TNF-α, IL-6 (BD Biosciences), and IL-2 (R&D Systems). The number of CD4+ T cells secreting cytokines was assessed by ELISPOT. Capture Ab was incubated overnight at 4°C on sterile Multiscreen plates (Millipore), and then blocked for 1 h before addition of CD4+ T cells (1.5 × 105 cells/well) selected by AutoMACS from spleen of PG-immunized mice and stimulated with PG (2 μg/ml) and irradiated (2500 rad) spleen cells (2.0 × 105 cells/well) from naive IL-27R−/− mice. Cells were incubated for 48 h (IFN-γ) and 72 h (IL-17 and IL-4), and cytokines were detected with cytokine-specific Abs. Plates were dried and read using ELISPOT reader and software (ImmunoSpot Analyzer and ImmunoSpot software; CTL Analyzers).

**Quantitative RT-PCR**

RNA was isolated from spleen and joint tissue using TRI-Reagent (Molecular Research Center). RNA was further subjected to DNase I (Invitrogen Life Technologies) digestion before reverse transcription. Reverse transcription was performed with SuperScript III (Invitrogen Life Sciences).
Technologies). Real-time PCR was performed with 1 μl of reverse-transcription product in an IQ5 real-time PCR detection system (Bio-Rad) by using IQ SYBR Green PCR Supermix (Bio-Rad), according to the manufacturer’s guidelines. The PCR cycling conditions were as follows: 50 cycles of 15 s at 95°C, 20 s at 60°C, and 30 s at 72°C. All samples were run in triplicate. To verify that equivalent amounts of RNA were added to each PCR, PCR amplification of the murine β-actin was performed for each sample. Relative fold induction was calculated using the formula $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct = Ct_{\text{target gene}} - Ct_{\text{actin}}$ and $Ct$ is the cycle at which the threshold is crossed. PCR product quality was monitored using post-PCR melting curve analysis. Controls were from naive nonimmunized and target genes from PG-immunized IL-27R+/+ and IL-27R−/− joint and spleen tissues.

**Statistical analysis**

The nonparametric Mann-Whitney U test was used to compare data for statistical significance. Significance is based on a $p$ value < 0.05.

**FIGURE 2.** IL-27 expression in PGIA. IL-27R+/+ were immunized i.p. with human PG in adjuvant three times at 3-wk intervals and monitored for the incidence and severity of PGIA. A, IL-27 and IFN-γ production from arthritic mice spleen cells stimulated with PG. B, Ebi3 and IL-27 mRNA transcripts from arthritic joints and spleen cells stimulated with PG. Joint tissue and spleen from nonimmune mice were used to calculate the relative fold increase in cytokine RNA. Suppression of PGIA onset and severity in IL-27R−/− mice. IL-27R+/+ and IL-27R−/− mice were immunized and monitored for the incidence, arthritic score, and severity of PGIA at given time points. Incidence (C) denotes the percentage of mice that develop PGIA. Arthritic score (D) is the sum of all paws inflamed and noninflamed in individual mice divided by the total number of mice. Severity (E) (cumulative arthritis score) is the sum of paw inflammation scores in individual mice divided by the total number of arthritic mice. Values are the mean ± SEM. * $p < 0.05$ for IL-27R+/+ ($n = 10$) vs IL-27R−/− ($n = 11$). Data are representative of four separate experiments.

**FIGURE 3.** Suppression of histopathology in the joint of IL-27R−/− mice. Hind limbs of mice were dissected, fixed in formalin, decalcified, and embedded in paraffin. The tissue sections were stained with H&E. Sections are from representative ankle joints from IL-27R+/+ (A and C) and IL-27R−/− mice (B and D). Magnification ×4 (A and B) and ×10 (C and D). Thick arrow indicates an area of cartilage destruction, and thin arrow indicates an area of bone erosion. Cellular infiltration (E) was measured on a scale of 0–4. Values are the mean ± SEM. * $p < 0.05$ for IL-27R+/+ ($n = 12$) vs IL-27R−/− ($n = 17$) for two separate experiments.
IL-27 activates primary proteoglycan-specific T cells to proliferate and produce IFN-γ

Several reports indicate that IL-27 augments T cell proliferation and IFN-γ production (1, 2). We were therefore interested in determining whether IL-27 contributes to the Th1 phenotype in PGIA. We initially examined whether IL-27 augmented CD4+ T cell proliferation and IFN-γ production in PG-specific TCR Tg mice. IL-27 enhanced PG-specific T cell proliferation under a suboptimal concentration of PG (Fig. 1A). Similarly, IL-27 stimulated IFN-γ production and IFN-γ mRNA expression significantly higher than either PG or IL-27 alone (Fig. 1, B and C). These data confirm previous reports on the ability of IL-27 to induce proliferation and IFN-γ production in naive T cells (1, 8).

IL-27 is required for the development of PGIA

We next assessed whether IL-27 was produced in PGIA. Spleen cells from arthritic mice were restimulated in vitro with PG, and supernatants were examined for IL-27 and IFN-γ production by ELISA. IFN-γ and IL-27 were produced in spleen cell cultures activated with PG (Fig. 2A). We used real-time PCR to quantify the relative transcript abundance of IL-27 in spleen and arthritic joint tissues of PG-immunized mice. Relative to nonimmune mice, a 5- to 6-fold increase in Ebi3 (which encodes EBI3) and Il27 (which encodes for p28) in joint tissues of arthritic mice was observed. Similarly, Ebi3 and Il27 mRNA transcripts were increased in spleen cells of arthritic mice (Fig. 2B). Macrophages and dendritic cells are the primary source of IL-27, suggesting that the increase in Ebi3 and Il27 transcripts in the joint tissues of arthritic mice could be due to infiltration of migrating cells into the joint or to the activation of resident synoviocytes.

Although studies have indicated that IL-27 is important for the differentiation of naive CD4+ T cells into Th1 effector cells, in several infectious and noninfectious disease models, IL-27R-deficient mice generate robust Th1 responses characterized by enhanced IFN-γ and IL-2 production. Given the importance of IFN-γ in susceptibility to arthritis in PGIA, we assessed the requirement for IL-27 signaling in PGIA. Wild-type (IL-27R+/+) and IL-27R-deficient (IL-27R-/-) mice were immunized with proteoglycan and evaluated for arthritis incidence...
and severity (Fig. 2, C–E). IL-27R \(^{+/+}\) mice developed arthritis with 100% incidence, whereas the incidence of arthritis in IL-27R \(^{-/-}\) mice was significantly inhibited over many weeks. In the small percentage of IL-27R \(^{-/-}\) mice that developed arthritis, in general disease was less severe than IL-27R \(^{+/+}\) mice (Fig. 2, C–E). Similar results were observed in four separate experiments. These data demonstrate that IL-27 signaling was critical for the development of PGIA.

To determine the extent of inflammation, we examined ankle joint histology in IL-27R \(^{+/+}\) and IL-27R \(^{-/-}\) mice, as depicted in Fig. 3, A–D. The histologic picture in IL-27R \(^{+/+}\) was characteristic of acute arthritis. Mononuclear and polymorphonuclear cell infiltration was abundant in the tissues and joint spaces. There was edema of the synovial and periarticular tissues accompanied by synovial hyperplasia. Cartilage erosion and disintegrating chondrocytes were also seen in the remaining layer of the articular surface. Conversely, cellular infiltrate and histopathological signs of arthritis were significantly reduced in IL-27R \(^{-/-}\) (Fig. 3, B, D, and E). In the IL-27R \(^{-/-}\) mice, the lack of paw erythema and swelling correlated with the absence of cellular infiltrate and cartilage destruction.

Proinflammatory cytokines play a major role in driving inflammation in arthritis. We therefore measured mRNA transcripts for TNF-\(\alpha\), IL-6, and IL-1\(\beta\) using RNA isolated from joint tissues of IL-27R \(^{+/+}\) and IL-27R \(^{-/-}\) mice immunized with PG. There was a significant decrease in TNF-\(\alpha\), IL-6, and IL-1\(\beta\) transcripts in IL-27R \(^{-/-}\) mice in comparison with IL-27R \(^{+/+}\) mice (Fig. 4A). In addition, T cell-derived cytokine transcripts for IFN-\(\gamma\) and IL-17

![FIGURE 6. Substantial reduction in the production and frequency of T cells secreting IFN-\(\gamma\) in IL-27R \(^{-/-}\) mice. IL-27R \(^{+/+}\) and IL-27R \(^{-/-}\) mice were immunized, as described in Fig. 2. Peritoneal exudate cells and spleens from three to four mice were obtained at 4, 8, and 11 wk after immunization. T cells from peritoneal exudates and CD4\(^+\) T cells from spleen were stimulated with proteoglycan in the presence of irradiated naive spleen cells and assayed for IFN-\(\gamma\), IL-4, and IL-17 production by ELISA and the frequency of T cells producing IFN-\(\gamma\), IL-4, and IL-17 by ELISPOT. Peritoneal exudate T cells (A, D, and G), CD4\(^+\) spleen T cells (B, E, and H), and CD4\(^+\) spleen T cells ELISPOT (C, F, and I). Values are the mean \(\pm\) SEM of quadruplicate cultures. * \(p<0.05\).]
were also significantly reduced in IL-27R−/− mice (Fig. 4B). The reduction in cytokines reflects the lack of inflammation in the IL-27R−/− mice. These results demonstrate a necessity for IL-27 in the development of inflammation in PGIA.

PG-specific Ab production and T cell activation in IL-27R-deficient mice

Proteoglycan-specific CD4+ T cells and autoantibodies are necessary for the development of PGIA; neither T cells nor serum Abs are capable of inducing arthritis on their own (30, 32). It has recently been shown that IL-27 inhibits IgG1 and induces IgG2a class switch in B cells (33). Therefore, we next tested whether in vivo IL-27 signaling alters PG-specific IgG1 and IgG2a isotype expression. PG-immunized mice were bled periodically over the course of disease development. The concentration of PG-specific Abs increased over time, but the differences between IL-27R+/+ and IL-27R−/− mice remained constant. The data in Fig. 5, A and B, represent Ab concentrations at 11 wk after immunization with PG. There was no significant difference in the IgG1 Abs specific for human or mouse PG (Fig. 5, A and B). For the PG-specific IgG2a isotype, Abs specific for human were always significantly reduced in IL-27R−/− in comparison with IL-27R+/+ mice, whereas Abs specific for mouse PG were reduced, but the data did not reach significance (Fig. 5, A and B).

In Fig. 1, we showed that primary PG-specific T cell proliferation was augmented in the presence of IL-27. We assessed PG-specific CD4+ T cell proliferation at 4, 6, 8, and 11 wk after immunization with PG. CD4+ T cells were purified from spleen and stimulated in vitro with irradiated naive spleen cells plus Ag. Data in Fig. 5C are representative of T cell proliferation at 4 wk after immunization. There was no difference in proteoglycan-specific T cell proliferation between IL-27R+/+ and IL-27R−/− mice. T cell proliferation at later time points, weeks 6, 8, and 11, also demonstrated no difference between mice sufficient and deficient in IL-27R expression (data not shown). These data suggest that although IL-27 may be important for primary T cell proliferation, by 4 wk after T cell sensitization to PG, IL-27R-deficient T cells were able to overcome the early deficit in IL-27.

Recent data indicate IL-27 limits the production of IL-2 (12, 20, 21). Therefore, we examined the production of IL-2 in spleen T cells from IL-27+/+ and IL-27−/− mice immunized with proteoglycan. There was no difference between proteoglycan-specific IL-2 production between IL-27R+/+ and IL-27R−/− mice (Fig. 5D).

IL-27 is required for the production of IFN-γ

IFN-γ is a critical cytokine that controls the onset and severity of PGIA. We next examined whether the production of IFN-γ correlated with inhibition of arthritis in IL-27R−/− mice. T cell IFN-γ production was examined in several different ways. Because PGIA is induced by injection of PG into the peritoneal cavity, we isolated the peritoneal exudate. We surmised that there may be a higher frequency of PG-specific T cells that migrate to the site of Ag. At 4 and 8 wk, T cells were purified and restimulated in culture with PG in the presence of irradiated naive spleen cells. At the same time points, spleen CD4+ T cells were restimulated with PG and naive APCs. Supernatants were assayed for the production of IFN-γ by ELISA. At 4, 8, and 11 wk, CD4+ T cells were assessed for the frequency of IFN-γ-producing T cells by ELISPOT. From the same cultures, IL-4 and IL-17 were measured. T cells isolated from the peritoneal cavity produced substantially more IFN-γ than spleen T cells, suggesting that PG-specific T cells migrate to the peritoneal cavity. Furthermore, T cells from IL-27R−/− mice produced significantly less IFN-γ whether from peritoneal exudates or spleen. The frequency of IFN-γ-positive CD4+ T cells from IL-27R−/− mice was similarly reduced in comparison with IL-27R+/+ mice (Fig. 6, A–C).

We have previously reported that IL-4 functions to reduce the severity of PGIA (23). Because it has been suggested that IL-27 inhibits Th2 responses in infectious and noninfectious responses, we next tested whether an increase in IL-4 production in IL-27R−/− mice might be the explanation for reduction of arthritis in the absence of IL-27 signaling. Our results indicate that IL-27R−/− and IL-27R+/+ cells were generally equivalent with respect to IL-4 production when evaluated either by the number of cells expressing IL-4 or by the secretion of IL-4 over time. IL-4 was only transiently inhibited at 4 wk in the T cell peritoneal exudate cultures (Fig. 6, D–F). Thus, suppression of PGIA in IL-27R−/− mice was not due to an increase in IL-4 production.

It has recently been found that IL-27 plays an essential role in the inhibition of IL-17 production (14, 15). Both groups show that neuroinflammation is exacerbated in the absence of IL-27 due to an increase in IL-17 expression. Because we found arthritis was
inhibited in the absence of IL-27R. IL-17 is unlikely to play a major role in PGIA. Similar to reported findings, we confirmed in vitro that IL-27 inhibited IL-17 production in anti-CD3-activated CD4⁺ T cells (data not shown). Assessment of IL-17 levels from PG-activated T cells obtained from peritoneal exudates or spleen indicated that very little IL-17 was produced. However, there was a transient rise in IL-17 production in IL-27R⁻/⁻ in comparison with IL-27R⁺/⁺ mice at 4 wk in spleen CD4⁺ T cells that was not sustained at later time points (Fig. 6, G–I). These data indicate in vivo levels of IL-17 were not affected by a deficiency in IL-27R and that there was no correlation between IL-17 expression and suppression of PGIA in the IL-27R⁻/⁻ mice. Overall, the results described in this study lead to the conclusion that IL-27R⁻/⁻ cells produce less IFN-γ and that the reduction in IFN-γ contributes to resistance in the development of PGIA.

Reduced production of proinflammatory cytokines in IL-27R⁻/⁻ mice in PGIA

Because of the critical importance of IFN-γ in the pathogenesis of PGIA, we determined whether the decrease in the propensity to produce IFN-γ in the IL-27R⁻/⁻ mice led to a decrease systemic expression of inflammatory cytokines. We therefore measured mRNA transcripts for TNF-α, IL-1β, IL-6, Ebi3, and IL-27 and TNF-α, IL-1β, IL-6, and IL-27 production from spleen cultures stimulated with proteoglycan from IL-27R⁺/⁺ and IL-27R⁻/⁻ mice. There was a significant decrease in TNF-α, IL-1β, Ebi3, and IL-27 transcripts and PG-stimulated TNF-α, IL-6, and IL-27 secretion in IL-27R⁻/⁻ mice in comparison with IL-27R⁺/⁺ mice (Fig. 7). These proinflammatory cytokines are most likely produced by activated macrophages and dendritic cells in the spleen. The reduced production of IFN-γ by CD4⁺ T cells may result in decrease in macrophage and dendritic cell activation and in turn cytokine production. Thus, the reduction in arthritis in the IL-27R⁻/⁻ mice could be a consequence of inhibition of several proinflammatory pathways.

Discussion

The present study was designed to define the role of IL-27R expression in autoimmune arthritis and to determine whether the expression of IL-27R is necessary for development of arthritis and Th1 cell activation. Previous studies indicate that IL-27R signaling enhances primary T cell activation and IFN-γ production. We showed in the PGIA model that primary PG-specific T cell proliferation and the production of IFN-γ are augmented in the presence of IL-27, confirming that IL-27 is an important differentiation factor for Th1 cells. We have previously reported that in the absence of IFN-γ or STAT4, the onset and severity of PGIA are substantially reduced, indicating that PGIA is mediated by Th1 cells (24). The presence of IL-27 in the spleen and IL-27 mRNA transcripts in joint tissues of arthritic mice suggested that IL-27 was involved in driving the Th1-type inflammatory response in PGIA. To directly demonstrate the contribution of IL-27 signaling to the development of PGIA, we used mice deficient in IL-27R expression. The consequence of IL-27R deficiency was a significant delay in the onset and severity of arthritis. The reduction in erythema and swelling of paws was reflected in a decrease in cellular infiltration in the joint, and reduced synovial hyperplasia and cartilage destruction. Moreover, there was a marked decrease in inflammatory cytokine mRNA transcript expression in joint tissues of IL-27R⁻/⁻ in comparison with IL-27R⁺/⁺ mice.

IL-27R signaling supports Th1 responses through the induction of several essential components necessary for Th1 cell differentiation. IL-27 induces the transcription factors STAT1 and T-bet, which in turn activate IL-12Rβ2 and IFN-γ genes (6, 8, 9). Our data show that in IL-27R⁻/⁻ mice there was a reduction in the frequency of IFN-γ secretion CD4⁺ T cell and in the production of IFN-γ. Similar to IL-12, IL-27 can also activate STAT4, a signaling pathway necessary for polarization of Th1 cells into effector cells (9). In PGIA, STAT4 is required because STAT4⁻/⁻ mice are resistant to arthritis induction (24). In addition, we observed that IL-12-deficient mice (p40⁻/⁻ and p35⁻/⁻) are less susceptible to arthritis than wild-type mice (data not shown). Thus, IL-12 and IL-27 may both contribute to the activation of STAT4 in PGIA. In addition to the role of STAT4 in IFN-γ transcription, STAT4 regulates the expression of a number of genes involved in inflammation and Th1 responses that probably contribute to induction of arthritis (34–37).

PG-specific Abs are necessary for the development of arthritis. Thus, the reduction in PG-specific IgG2a isotype could contribute to the suppression of arthritis in IL-27R-deficient mice. There are two possible pathways by which IL-27 regulates IgG2a class switch. IL-27 induces IgG2a expression directly through the activation of T-bet (33) and indirectly through activation of IFN-γ (38). Interruption of the IL-27 signaling through either of these pathways would result in reduction of IgG2a isotype switch observed in PG-sensitized IL-27R-deficient mice.

In agreement with our results, IL-27R-deficient mice infected with either L. monocytogenes or Leishmania major have an impaired IFN-γ response and are less resistant to infection (2, 3). However, in L. major infection, a reduction in IFN-γ correlates with a rise in IL-4 production, and disease resistance could be restored by neutralization of IL-4. IL-27 is known to inhibit GATA-3, a critical transcription factor for Th2 differentiation (9). Thus, the ability of IL-27 to inhibit GATA-3 expression, rather than its requirement for Th1 differentiation, may be crucial to controlling L. major infection. Similarly, IL-27 controls T. cruzi parasitemia by suppressing Th2 responses (13). We have previously reported that PGIA is regulated in IL-4- and STAT6-deficient mice, indicating that IL-4 regulates the severity of arthritis (24). However, despite the regulatory role of IL-4 in PGIA, we found that the lack of IL-27 signaling has no effect on the number of IL-4-secreting cells or on the amount of IL-4 produced. Hence, an increase in IL-4 in IL-27R⁻/⁻ is not involved in suppression of PGIA.

Recently, several anti-inflammatory properties of IL-27 have been uncovered that may provide an explanation for some of the conflicting outcomes in IL-27R signaling. This is illustrated in IL-27R-deficient mice, in which exposure to T. cruzi, T. gondii, or M. tuberculosis results in an excess production of proinflammatory cytokines, including IFN-γ (11–13). These findings indicate IL-27 is not absolutely required for the generation of IFN-γ and reveal a regulatory role of IL-27 in controlling inflammation. The robust Th1 response in IL-27R-deficient mice infected with T. gondii controls parasite replication, but is unable to down-regulate the protective inflammatory responses, which results in the lethality of disease (12, 13). Similarly, IL-27R-deficient mice infected with T. cruzi develop liver necrosis as well as enhanced sensitivity to Con A-induced hepatitis (13, 16). Because liver disease improves with neutralization of IFN-γ, IL-27 appears to function in these models of infectious disease as a regulator of Th1-mediated inflammation (13). However, this is clearly not the case in PGIA, in which IFN-γ and several proinflammatory cytokines are down-regulated in the absence of IL-27R signaling. Why might IL-27 function to promote Th1 responses in PGIA, but suppress Th1 responses in T. cruzi or T. gondii infection? One possibility is that the threshold of Th1 activation determines whether IL-27 promotes or suppresses Th1 responses (39). Infection with T. gondii elicits high levels of IL-12 production (12). This strong Th1-polarizing condition could
result in IL-27 delivering a negative signal to suppress IFN-γ production. In PGIA, the susceptible strain BALB/c is bias toward a Th2 response due to a premature loss of IL-12 responsiveness because of reduced IL-12R expression (40–42). Under these suboptimal polarizing conditions, IL-27 primary activity is to suppress differentiation of Th1.

Two recent reports indicate IL-27 is involved in the regulation of Th17 cell activity (14, 15). EAE is dependent on IL-17-producing T cells, and in IL-27R−/− mice exacerbation of EAE is associated with an increase in IL-17 expression in CD4 T cells (14, 43). Similarly, chronic infection with T. gondii in mice lacking IL-27R leads to lethal CD4-mediated neuroinflammation associated with an aggravated IL-17 response despite control of parasite replication (14). Because IFN-γ regulates IL-17 production (44, 45), augmented IL-17 expression could be due to a failure to produce IFN-γ in the IL-27R-deficient mice. In support of this, absence of IFN-γ in EAE leads to an increase in IL-17 production and exacerbation in disease symptoms (43, 45), and in M. tuberculosis-infected mice lacking IFN-γ there is an increase in the number of IL-17-producing T cells (46). In PGIA, we clearly show that IL-27 does not play a major role in suppressing IL-17 production. In IL-27R−/−, there was a transient rise in IL-17 secretion at 4 wk after immunization with PG, but this was not sustained at 8 wk and there was no difference in the number of CD4+ T cells secreting IL-17 at 4, 8, or 11 wk.

How does PGIA compare with another well-studied model of arthritis, CIA? In PGIA, a deficiency in IFN-γ results in resistance to development of arthritis, indicating that PGIA model of arthritis is Th1 mediated. However, in CIA, a deficiency in IFN-γ-R results in exacerbation of disease (26, 27). The recent findings that IFN-γ is an important inhibitor of IL-17 and that a deficiency in IL-17 or inhibition of IL-17 suppresses CIA indicate that CIA is an IL-17-mediated disease (44, 45, 47, 48). Because Th1 and Th17 cells are differentiated by different sets of cytokines, it is likely that the cytokine milieu in which PGIA and CIA are initiated determines the T cell phenotype of the disease (49).

In addition to the MHC and non-MHC gene differences between the two models, there are other differences. The development of PGIA is recessive, whereas CIA is a dominant disease. Another difference is the higher incidence of arthritis in female mice, whereas the reciprocal is true in CIA (22, 50). How these dissimilarities contribute to the T cell cytokine dependence is presently unknown. It is important to point out that whereas no animal model is identical with rheumatoid arthritis, they remain highly relevant to human disease because they provide important information on the mechanisms underlying human disease and they provide insight for the development of novel therapeutic agents.

In summary, IL-27 regulation of T cell immune responses is complex. The circumstances under which IL-27 is required for Th1 cell differentiation are not obvious. However, for PGIA, in which IFN-γ unambiguously regulates the severity of arthritis, IL-27 is an important cytokine for driving the Th1 response.

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Disclosures

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