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IFN-γ Regulates the Requirement for IL-17 in Proteoglycan-Induced Arthritis

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The contribution of the proinflammatory cytokines IFN-γ and IL-17 to the pathogenesis of experimental arthritis is controversial. In proteoglycan (PG)-induced arthritis (PGIA), severe arthritis is dependent on the production of IFN-γ, whereas IL-17 is dispensable. In collagen-induced arthritis and Ag-induced arthritis, although high levels of IFN-γ are secreted, disease is exacerbated in IFN-γ- or IFN-γ receptor-deficient mice due to the ability of IFN-γ to suppress IL-17 expression. In the current study, we investigated the effect of IFN-γ on the IL-17 response and its consequences in PGIA. In PG-immunized IFN-γ−/− mice, despite reduction in arthritis, the PG-specific CD4+ T cell IL-17 response was significantly increased. Elevated IL-17 contributed to development of arthritis, as disease in IFN-γ/IL-17−/− was significantly reduced in comparison with either IFN-γ−/− or IL-17−/− mice. A contribution of IFN-γ and IL-17 to the development of arthritis was also identified in T-bet−/− mice. PG-specific CD4+ T cells from T-bet−/− mice produced reduced IFN-γ and elevated concentrations of IL-17. Both IFN-γ and IL-17 contribute to arthritis, as T-bet−/− mice lacking IL-17 (T/bet/IL-17−/−) were resistant, whereas wild-type, T-bet−/−, and IL-17−/− mice were susceptible to PGIA. T cell proliferation and autoantibody production did not correlate with development of disease; however, expression of cytokines and chemokines in joint tissues demonstrate that IFN-γ and IL-17 cooperatively contribute to inflammation. These results demonstrate that both IFN-γ and IL-17 have the potential to induce PGIA, but it is the strength of the IFN-γ response that regulates the contribution of each of these Th effector cytokines to disease.


Rheumatoid arthritis (RA) is a chronic, progressive autoimmune disease primarily affecting the synovial joints and causing both significant morbidity and increased mortality (1). The etiology of the disease is unclear; however, RA is strongly linked to particular MHC alleles, implying that some aspect of the CD4+ T cell response to self-Ag is crucial to the autoimmune process. Proinflammatory CD4+ T cells have been divided into Th1, Th2, and Th17 subsets based on their production of IFN-γ, IL-4, and IL-17, respectively. Murine models of arthritis, proteoglycan-induced arthritis (PGIA), and collagen-induced arthritis (CIA), were originally classified as Th1-mediated diseases based on abundant IFN-γ production (2–4). IFN-γ has several proinflammatory properties that contribute to inflammation in arthritis. Activation of macrophages by IFN-γ results in induction of cytokines, NO, and superoxide production and expression of MHC class I and class II molecules (5–9). In PGIA, neutralization of IFN-γ inhibits arthritis and IFN-γ−/− mice developed arthritis with delayed onset and reduced severity in comparison with wild-type (WT) mice. However, IFN-γ−/− mice eventually succumb to arthritis, in some cases as severe as WT mice. These findings indicate that IFN-γ is an important proinflammatory cytokine promoting disease severity in PGIA (3, 4). In CIA, the role for IFN-γ is more complex. Complete elimination of IFN-γ or IFN-γ receptor signaling leads to exacerbation of disease (10–12). In contrast, neutralization of IFN-γ at an early stage of disease inhibits arthritis (13). It was originally thought that a failure to suppress T cell expansion and induce apoptosis and/or an increase in IL-1β was responsible for enhanced arthritis (14, 15). More recently, it was found that IFN-γ inhibits IL-17 production. IL-17 has emerged as an important proinflammatory T cell cytokine in several models of arthritis (16–18). Thus, the ability of IFN-γ to suppress Th17 cells appears to account for augmented disease in IFN-γ−/− or IFN-γ receptor-deficient mice in CIA and Ag-induced arthritis (AlA), as inhibition of IL-17 with neutralizing Abs suppressed arthritis (19, 20). Contrary to a dependence on IL-17 in CIA and AIA, in PGIA, IL-17–deficient mice develop arthritis similar to WT. To begin to resolve the controversy between these different models of arthritis, we investigated the IFN-γ-regulated IL-17 response in PGIA.

In this study, using several cytokine- and transcription factor-deficient mice, we show that a deficiency in IFN-γ converts PGIA from an IL-17–independent to an IL-17–dependent arthritis. In addition, we show that the transcription factor T-bet regulates IL-17 production. Moreover, T-bet−/− mice are susceptible to PGIA due to the combined effects of low IFN-γ and high IL-17 expression. Taken together, these results show that the strength of the IFN-γ response regulates whether IL-17 is expressed and its consequences in PGIA.

Materials and Methods

Mice

IFN-γ−/−, T-bet−/−, IL-17−/− mice were backcrossed to BALB/c for ≥10 generations. Double knockout mice were generated by intercrossing and selected for by PCR. WT, IFN-γ−/−, and T-bet−/− mice were from The Jackson Laboratory (Bar Harbor, ME) and maintained at the Rush
University Medical Center facility. Female WT and gene-deficient age-matched mice, 12–14 wk, were used in all experiments. All animal experiments were approved by the Institutional Animal Care and Use Committee at Rush University Medical Center (Chicago, IL).

**Induction and assessment of arthritis**

Human cartilage was obtained from The Orthopedic Tissue, Transplant, and Implant Repository of Rush University Medical Center, with the approval of the Institutional Review Board. Proteoglycan (PG) was isolated as previously described (21). Mice were immunized with 150 μg human PG i.p. as measured by protein in dimethylmethacrylate ammonium bromide (Sigma-Aldrich, St. Louis, MO) and boosted with 100 μg PG in dimethylmethacrylate ammonium bromide at weeks 3 and 6 (22). Mice were monitored for arthritis twice weekly and scored in a blinded manner. Arthritis severity was scored for each paw on a scale from 1–4 as described (22).

**Assessment of T cell activation by proliferation**

MACS purified CD4+ T cells (Miltenyi Biotech, Auburn, CA) (2.5 × 10^5 cells/ml) and irradiated (2500 rad) naïve spleen cells (2.5 × 10^5 cells/ml) were cultured in the presence or absence of PG (10 μg/ml) in RPMI 1640 complete media. Cells were incubated at 37°C in 5% CO2 for 5 d and were pulsed with ^[3]Hthymidine (0.5 μCi/well) for the last 18 h. Cell cultures were harvested and incorporated ^[3]Hthymidine was measured (MicroBeta Trilux, PerkinElmer, Waltham, MA).

**Detection of serum Ab titers by ELISA**

Sera from immunized mice were serially diluted and incubated with the immobilized human and mouse PG, and plate-bound human PG- or mouse PG-specific Ab was detected using peroxidase-conjugated rabbit IgG against mouse IgG1 and IgG2a (Zymed, San Francisco, CA). Data compared with a standard curve of unlabeled murine IgG1 and IgG2a (Southern Biotechnology Associates, Birmingham, AL).

**Assessment of intracellular cytokine production**

Spleen cells were incubated with ionomycin (500 ng/ml) and PMA (50 ng/ml) in the presence of GolgiStop (BD Biosciences, San Jose, CA) for 4 h. Cells were stained for extracellular CD4 then permeabilized with the Cytofix/Cytoperm Plus Kit (BD Biosciences) and stained for IFN-γ and IL-17 using specific Abs or isotype control (BD Biosciences). Data was acquired with FACSort (BD Biosciences) and analyzed using FlowJo software (TreeStar, Ashland, OR).

**Assessment of cytokines**

PureCD4+ T cells (Miltenyi Biotech) (2.5 × 10^5 cells/ml) were incubated in the presence or absence of PG (20 μg/ml) and naïve irradiated (2500 rad) spleen cells (2.5 × 10^5 cells/ml) in RPMI 1640 complete (4). Cytokines were measured from day 4 culture supernatants by both IFN-γ ELISA (BD Biosciences) and IL-17 ELISA kits (R&D Systems, Minneapolis, MN).

**Histology**

Hind joints were taken at week 12 and fixed in formalin, decalcified, embedded in paraffin, and stained with H&E. Cellular infiltration was assessed by a blinded observer using a scale of 0–4. Values represent mean ± SEM of n = 5–10 sections.

**Quantitative RT-PCR**

RNA was isolated from spleen and joint tissue using TRI Reagent (Molecular Research Center, Cincinnati, OH). RNA was then treated with DNase I (Invitrogen, Carlsbad, CA) digestion before reverse transcription. Reverse transcription was performed with SuperScript III (Invitrogen). Real-time PCR was performed with 1 μl reverse transcription product in an IQ5 real-time PCR detection system (Bio-Rad, Hercules, CA) by using QuantFast SYBR Green PCR MasterMix (Qiagen, Valencia, CA) 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 reaction, PCR amplification of the murine β-actin was performed for each sample. Relative fold induction was calculated using the equation 2^-DD^Ct where ΔΔCt is ΔCt(target gene) - ΔCt(β-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 joint spleen tissues.

**Statistical analysis**

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

**Results**

IFN-γ deficiency inhibits arthritis and enhances IL-17 production

We have demonstrated a requirement for IFN-γ in PGIA (4) and in the current study reconfirm these findings (Fig. 1A). WT and IFN-γ−/− mice were immunized with PG and the onset and severity of arthritis monitored over time. IFN-γ−/− mice developed arthritis with a delay in onset and reduced severity in comparison with WT mice; however, IFN-γ−/− mice often succumb to arthritis, in some cases as severely as WT mice. These findings indicate that IFN-γ is an important pathogenic factor governing disease severity in PGIA. Recent studies in murine models of arthritis CIA and AIA show that IFN-γ suppresses IL-17 production, leading to enhanced disease in IFN-γ-deficient mice (19, 20). Because arthritis is not exacerbated in IFN-γ−/− in PGIA, the regulation of IL-17 in vivo may be different. Therefore, we examined expression of cytokines from splenic CD4+ T cells. Purified CD4+ T cells from PG-immunized WT and IFN-γ−/− were restimulated in vitro with PG and irradiated naïve spleen cells. CD4+ T cells from WT mice secreted very little IL-17, whereas CD4+ T cells from IFN-γ−/− mice produced abundant IL-17 (Fig. 1B). Intracellular FACS analysis of ex vivo spleen cells gated on CD4+ T cells from PG-immunized mouse also revealed marked increase in the frequency of intracellular IL-17-positive CD4+ T cells in IFN-γ−/− mice (Fig. 1C, 1D). These data confirm reports by others that IFN-γ−/− suppresses IL-17 in vivo and demonstrate that IFN-γ regulates the balance between Th1 and Th17 cells in vivo. Thus, it was possible that the PG-induced arthritis that develops late in the IFN-γ−/− mice was dependent on IL-17.

Late-onset arthritis in IFN-γ−/− mice is IL-17 dependent

To determine whether elevated IL-17 in the IFN-γ−/− mice was responsible for late-onset arthritis observed in IFN-γ−/− mice, we bred IFN-γ−/− and IL-17−/− mice to generate double-deficient mice (IFN-γ−/−/IL-17−/−) and compared their development of arthritis to groups of age-matched WT, IFN-γ−/−, and IL-17−/− mice. As we have previously reported, WT and IL-17−/− mice are similarly susceptible to PGIA (22). However, the onset and severity of arthritis in IFN-γ−/−/IL-17−/− mice was significantly reduced in comparison with WT, IFN-γ−/−, and IL-17−/− mice (Fig. 2A). Histological examination of hind limb joints showed similar characteristics of acute arthritis in WT and IL-17−/− mice as previously reported (22). There was predominant polymorphonuclear and mononuclear cell infiltration in tissues and joint spaces with edema of the synovial and periarticular tissues accompanied by synovial hyperplasia. Cartilage destruction and disintegrating chondrocytes were also seen in the remaining articular surface in addition to extensive bone erosion as previously described (22, 23). Conversely, the cellular infiltration and histopathology were significantly reduced in IFN-γ−/− and further reduced in IFN-γ−/IL-17−/− mice (Fig. 2B, 2C).

Experimental autoimmune encephalomyelitis (EAE) and experimental autoimmune uveitis can be induced by either Th1 and Th17 cells; however, in EAE, the composition of the infiltrating cell populations are distinct, indicating that different populations of cells can trigger a clinically indistinguishable sign of disease (24, 25). In PGIA, despite a difference in the requirement for IFN-γ and IL-17, the composition of the cell populations infiltrating the synovial cavity in WT, IFN-γ−/−, and IL-17−/− strains was similar. Approximately 90% of the infiltrating cells were Gr-1/CD11b+ neutrophils ([22] and data not shown). There was insufficient synovial fluid to make this determination in the IFN-γ−/IL-17−/− mice.
These data demonstrate that IFN-γ and IL-17 can both contribute to the development of PGIA but that IL-17 comes into play only under conditions where IFN-γ levels are reduced.

Reduction cytokine and chemokine transcripts in joints of IFN-γ−/− and IFN-γ/IL-17−/− mice

PG-specific T cells and autoantibodies are required for the development of PGIA; neither serum Abs nor T cells alone are able to transfer arthritis (26, 27). PG-specific T cell proliferation was not reduced in IL-17−/−, IFN-γ−/−, and IFN-γ/IL-17−/− mice in comparison with WT (data not shown) and did not correlate with the development of arthritis. There was no difference in PG-specific IgG1 response between WT, IFN-γ−/−, and IL-17−/−; however, a significantly lower IgG1 response was observed in IFN-γ/IL-17−/− mice (data not shown), and the PG-specific IgG2a response was reduced in the IFN-γ−/− and IFN-γ/IL-17−/− mice as expected, as IFN-γ is responsible for IgG2a isotype switch (data not shown).

To directly assess how a deficiency in IFN-γ and IL-17 affected the microenvironment favorable for cellular infiltration into the synovial tissue, we evaluated the expression of several inflammatory mediators in the hind paws of PG-immunized mice. We selected several cytokines (IL-1β, IL-6, TNF-α, and GM-CSF), chemokines (CXCL1, CXCL2, CXCL9, CXCL10, CXCL11, CXCL12, CCL2, CCL3, CCL8, and CCL20), and NO synthase (NOS) based on their known activity in RA, their induction by IFN-γ or IL-17, and our previous identification in prearthritic and arthritic joints in PGIA (28–32). We obtained RNA from ankle joints of PG-immunized WT, IL-17−/−, and IFN-γ−/− mice at a time point when joint swelling was on the rise, with the exception of joints from IFN-γ/IL-17−/− mice, which were not swollen. Although measuring protein levels for the cytokines and chemokines would have been ideal, synovial fluid could not be obtained from IFN-γ/IL-17−/− mice. Transcripts of several cytokines and chemokines were not consistently increased in immunized WT mice; these include TNF-α, GM-CSF, CXCL9, CXCL10, CXCL11, and CXCL12 (data not shown).

In contrast, joint tissues from WT and IL-17−/− mice expressed reproducibly elevated transcripts for IL-1β, IL-6, NOS, CXCL1, CXCL2, CCL2, CCL3, CCL8, and CCL20 (Fig. 3C). In IFN-γ−/− and IFN-γ/IL-17−/− joint tissues, two patterns of cytokine and chemokine expression emerged. In IFN-γ−/− mice, IL-1β, IL-6, NOS, CXCL1, CXCL2, CCL2, CCL3, CCL8, and CCL20 transcripts were significantly decreased in comparison with either WT or IL-17−/− mice, whereas in IFN-γ/IL-17−/− mice, IL-6, CCL2, CCL8, and CCL20 were further reduced in comparison with IFN-γ−/− mice. These data demonstrate that IFN-γ either directly or indirectly plays a major role in regulating expression of cytokines and chemokines that are important for inflammation and cellular infiltration in the joint.

In addition, these data show that in the absence IFN-γ, IL-17 was proinflammatory and further contributed to the expression of cytokines/chemokines in joint tissue.

T-bet−/− mice are susceptible to PGIA

T-bet is a major transcription factor regulating the production of IFN-γ Th1 cells (33). Given the importance of IFN-γ in PGIA, we anticipated that T-bet would be essential for induction of arthritis. However, the kinetics and severity of arthritis development in T-bet−/− mice was similar to WT mice (Fig. 4A, 4B). We speculated that sufficient proinflammatory cytokines might be produced that permit the development of arthritis in the absence of T-bet as IFN-γ can be expressed through TCR signaling or IL-12 activation of T cells (34). Also, T-bet is important for regulation of IL-17 as T-bet−/− T cells increase production of IL-17 (35). Thus, the combination of low IFN-γ and high IL-17 might be sufficient to induce arthritis.

To begin to examine this possibility, we assessed CD4+ T cell cytokine production from PG-immunized WT and T-bet−/− mice. CD4+ T cells from T-bet−/− mice secreted significantly less IFN-γ than CD4+ T cells from WT mice (Fig. 4B). In contrast, CD4+ T cells from WT mice secreted very little IL-17 in comparison with abundant production by T-bet−/− T cells (Fig. 4B). Intracellular FACS analysis of ex vivo spleen cells gated on CD4+ T cells from PG-immunized mice revealed a similar pattern; the frequency of intracellular IL-17–positive CD4+ T cells in T-bet−/− was significantly higher than in WT. (Fig. 4C, 4D). These data demonstrate that T-bet is important in the production of IFN-γ and in the inhibition of IL-17 by CD4+ T cells in PGIA. In addition, because IFN-γ was not completely suppressed, the residual IFN-γ and the increase in IL-17 might account for the susceptibility to PGIA in T-bet−/− mice.

Development of arthritis in T-bet−/− mice is dependent on IL-17

To determine whether the elevated levels of IL-17 in the T-bet−/− mice promoted arthritis, we bred mice deficient in T-bet and IL-17 to generate T-bet/IL-17−/− mice and compared the development of disease in age-matched WT, T-bet−/−, and IL-17−/− mice.

FIGURE 1. IFN-γ deficiency inhibits PGIA and enhances IL-17 production. Groups of age-matched WT (closed circles) (n = 15) and IFN-γ−/− (open circles) (n = 15) mice were immunized with PG in adjuvant three times at 3-wk intervals and monitored. Arthritis score (A, left panel) is the sum of paw inflammation scores in individual mice divided by the total number of arthritis mice. Incidence (right panel) denotes the percentage of mice that developed PGIA. B, Purified splenic CD4+ T cells from PG immunized mice were cultured in the presence of PG and supernatants harvested at day 4 and examined by ELISA for IFN-γ (black bar) or IL-17 (gray bar). C, FACS analysis of intracellular staining for IFN-γ (left panel) and IL-17 (right panel) in PG-immunized WT and IFN-γ−/− mice. D, Spleen cells from immunized WT (left panel) and IFN-γ−/− (right panel) mice were examined by FACS for intracellular IFN-γ and IL-17 (gated on CD4+ T cells). Asterisks denote significant differences (p < 0.05) compared with WT. Data are representative of two to four experiments.

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between IFN-γ and IL-17 incidence (g2). IL-17 production did not correlate with the development of arthritis (data not shown). We showed that loss of IFN-γ substantially reduced expression of inflammatory mediators in the joint. However, in T-bet−/− mice, IFN-γ expression was reduced but not ablated. We assessed the expression of inflammatory mediators in joints of PG-immunized mice. Transcripts from WT and IL-17−/− mice were compared with transcripts from T-bet−/− and T-bet/IL-17−/− mice (Fig. 6C). In T-bet−/− mice, only NOS, CXCL1, CCL2, and CCL20 were inhibited in comparison with WT and IL-17−/− mice, suggesting that the residual IFN-γ produced in T-bet−/− mice was inflammatory and responsible for elevated expression of IL-1β, IL-6, CXCL2 CCL3, and CCL8 transcripts. Similar to the IFN-γ/IL-17−/− mice, in the T-bet/IL-17−/− mice, IL-6, CCL2, CCL8, and CCL20 were reduced in comparison with IL-17−/− and in some cases T-bet−/− mice, indicating that T-bet is protective.

Discussion

This study was designed to determine how the pathogenic and protective properties of IFN-γ control chronic autoimmune disease. We have previously shown that in mice deficient in IFN-γ, PGIA is suppressed but not eliminated, demonstrating that IFN-γ is pathogenic in this disease model (3, 4). In the current study, we found that there was an increase in expression of IL-17 by CD4+ T cells in T-bet−/− mice compared with either WT, T-bet−/−, or IL-17−/− mice (Fig. 5A). The histological picture in T-bet−/− mice was similar to WT and IL-17−/− mice with polymorphonuclear and mononuclear infiltration accompanied by edema of the synovium and periarticular tissue. Cellular infiltration coincided with cartilage destruction and bone erosion. This histological picture was substantially reduced in T-bet/IL-17−/− mice compared with the WT, T-bet−/−, and IL-17−/− controls (Fig. 5B, 5C). Thus, in T-bet−/− mice, the increase in IL-17 together with the T-bet-independent IFN-γ response may account for PGIA susceptibility.

Reduced cytokine and chemokine transcribes in joints of T-bet−/− and T-bet/IL-17−/− mice

As described above, PG-specific T cell activation and autoantibody production did not correlate with the development of arthritis (data not shown). We showed that loss of IFN-γ substantially reduced expression of inflammatory mediators in the joint. However, in T-bet−/− mice, IFN-γ expression was reduced but not ablated. We assessed the expression of inflammatory mediators in joints of PG-immunized mice. Transcripts from WT and IL-17−/− mice were compared with transcripts from T-bet−/− and T-bet/IL-17−/− mice (Fig. 6C). In T-bet−/− mice, only NOS, CXCL1, CCL2, and CCL20 were inhibited in comparison with WT and IL-17−/− mice, suggesting that the residual IFN-γ produced in T-bet−/− mice was inflammatory and responsible for elevated expression of IL-1β, IL-6, CXCL2 CCL3, and CCL8 transcripts. Similar to the IFN-γ/IL-17−/− mice, in the T-bet/IL-17−/− mice, IL-6, CCL2, CCL8, and CCL20 were reduced in comparison with IL-17−/− and in some cases T-bet−/− mice, indicating that T-bet is protective.
expression by IFN-γ is ablated or reduced, indicating that IL-17 can function as a proinflammatory cytokine in PGIA. Although production of either IFN-γ or IL-17 completely inhibited PGIA. These data suggest that the increase in IL-17 in T-bet−/− mice together with the remaining IFN-γ contribute to inflammation in PGIA. Although our data correlate CD4+ T cell IFN-γ and IL-17 with the development of PGIA, recent data in EAE suggest that T-bet−/− development of disease is independent of IFN-γ and IL-17 (38). In EAE, therapeutic administration of small interfering RNA specific for T-bet (39) or a deficiency in T-bet inhibits disease (40). In addition, T-bet is essential for the development of CIA induced with mAbs against type II collagen (41). Several studies have highlighted the role of T-bet in B cell, NK cells, NK T cells, dendritic cells, and CD8+ T cells, and it is an important transcription factor for functions other than IFN-γ production, thus the difference in the requirement for T-bet may be model specific (42–46). This is specifically illustrated in models of colitis, where in oxazolone-induced colitis, T-bet is protective, whereas in colitis induced by transfer of CD4+ CD62L+ T cells into an immunodeficient host, T-bet is pathogenic (47).

What is the mechanism by which IFN-γ and IL-17 contribute to arthritis in PGIA? Because there was no clear correlate in T and B cell responses with the development of PGIA, we assessed the effect of IFN-γ and IL-17 on inflammatory microenvironment of the joint by examining the expression of joint tissue cytokine and chemokine RNA transcripts. The RNA transcripts for CXCL1, CXCL2, CCL2, CCL3, CCL8, and CCL20 are upregulated in arthritic joints of WT and IL-17−/− mice. These chemokines are chemotactic for macrophages, neutrophils, lymphocytes, and dendritic cells. The role of CXCL1 and CXCL2 in neutrophil/macrophage recruitment correlates with the dominance of these cell populations in the synovial tissue and fluid in PGIA. In IFN-γ−/− mice, there was a significant reduction in CXCL1, CXCL2, CCL2, CCL3, CCL8, and CCL20 with a further reduction in CCL2, CCL8, and CCL20 in the IFN-γ−/−IL-17−/− mice. The reduction in chemokine RNA expression correlates with a reduction in cellular infiltration and histopathology in IFN-γ and IFN-γ/IL-17−/− mice.

IFN-γ is documented to both induce and suppress chemokine expression and neutrophil recruitment, which may be important in the progression of a normal inflammatory response. For example, in a model of peritonitis induced with Staphylococcus epidermidis, a deficiency in IFN-γ reduces the influx of neutrophils into the peritoneal cavity and the expression of neutrophil chemokine CXC chemokines (CXCL1 and CXCL2) through a mechanism that involves IL-1 and IL-6. As infection proceeds, IFN-γ regulates
neutrophil removal initiated by suppression of CXC chemokines (48–50). Thus, IFN-γ can act sequentially as pro- and then as anti-inflammatory in regulating leukocyte influx. The proinflammatory properties of IFN-γ are also evident in mice infected with pneumonia virus, where IFN-γ is important for CCL3 expression (51). In addition, IFN-γ activates CCL2 expression in IL-1β–activated RA synovial fibroblast (52). In CIA, IFN-γ is shown to inhibit neutrophil recruitment by suppressing CXCL5 expression (53). In addition, in RA synovial fibroblast, IFN-γ inhibits IL-1β–induced CXCL8 expression (52).

The chemokine CCL20 is recognized by CCR6, which is expressed by Th17 cells and has been reported to preferentially recruit Th17 cells to the inflamed joint (31). Also, it has been found that IL-17 synergizes with TNF-α to upregulate the expression of CXCL1 and CCL20 (51, 54). Our data show that several chemokines that are reported to be involved in the recruitment of IL-17 and in IL-17 synergistic activity are activated by IFN-γ. Importantly, these chemokines are decreased despite the increase in IL-17 expression that occurs in the IFN-γ−/− mice. It is clear that for some of these chemokines, CCL2, CCL8, and CCL20, IL-17 is also important for their expression. Thus, these data suggest that under circumstances where IFN-γ is unable to regulate IL-17 production, IFN-γ and IL-17 can cooperate in the activation of chemokine expression and the recruitment of cell to the joint.

We observed a similar regulation of chemokine expression in T-bet−/− mice in which IFN-γ was reduced but not eliminated. Expression of chemokine RNA transcripts in the joint tissue reflects the presence of residual IFN-γ as some chemokines; CCL2, CCL3, and CCL8 were no longer reduced in T-bet−/− mice. However, elimination of IL-17 resulted in suppression of several chemokine. These data further support the cooperation of IFN-γ and IL-17 in chemokine expression in joint tissue, although whether this effect is directly or indirect is not known.

Cytokine transcripts for IL-1β, IL-6, and NOS were consistently detected in WT and IL-17−/− mice. Our data show that similar to chemokine expression, these cytokines were suppressed in IFN-γ−deficient mice and further inhibited in IFN-γ/IL-17−/− mice. In T-bet−/− mice, only NOS was reduced and IL-6 and NOS further reduced in T-bet/IL-17−/− mice. IL-1β and IL-6 activate chemokine expression so that the regulation of chemokine expression by IFN-γ and IL-17 may be indirectly through cytokine expression. Taken together, both the pathogenic and protective properties of IFN-γ are evident in cytokine and chemokine expression in the joint tissue, although the mechanism by which this occurs is unclear.

The respective role of Th1 and Th17 cells in RA remains unclear. Elevated levels of IFN-γ protein and RNA transcripts have been identified in synovial tissues and fluid in patients with RA (55–57), and a clinical trial involving IFN-γ blockade showed significant improvement (58). IL-17 is also expressed in some but not all patients with RA in sera, synovial fluid, and synovial biopsies (31, 59, 60). The overlapping inflammatory roles of Th phenotypes demonstrated in...
this study suggest a mechanism by which patients may be refractory to therapeutic interventions focused on unique components of one inflammatory pathway (i.e., anti–IFN-γ Ab), whereas blockade of common downstream effectors (i.e., anti-TNF Ab) showed greater success. The highly heterogeneous nature of human disease suggests that animal models may represent different subtypes of RA, each of which may respond differently to a given therapeutic intervention. It is possible that the subtypes of RA, and therefore perhaps the nature of Th involvement, are a consequence of different etiologies that result in a similar disease phenotype. If this is the case, the efficacy of biological therapies needs to be tailored for the specific nature of inflammation of an individual or to focus on elements common to both inflammatory pathways. It is important to clarify how the proinflammatory and anti-inflammatory properties control the outcome of autoimmune disease, as therapeutic interventions may disrupt a balance that could inadvertently lead to increased disease severity.

Recent data demonstrate that inflammation in experimental autoimmune uveitis and EAE models are not solely dependent upon the Th17 subset, as had been suggested, but rather either Th1 or Th17 subsets can mediate pathology (24, 25). We confirmed and extended these findings in a Th1-mediated model of RA, demonstrating that a robust IFN-γ response in WT mice readily suppresses the IL-17 response, making the contribution of IL-17 to the development of arthritis in IFN-γ-deficient mice as a result of unrestricted Th17 response. J. Immunol. 179: 6228–6236.


