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Proteoglycan (Aggrecan)-Induced Arthritis in BALB/c Mice Is a Th1-Type Disease Regulated by Th2 Cytokines

Alison Finnegan, Katalin Mikecz, Ping Tao, and Tibor T. Glant

In animal models of arthritis induced with Ags or infectious agents, disease severity correlates with a dominant Th1-type response characterized by a higher ratio of IFN-γ to IL-4. Analysis of BALB/c mice revealed a genetic predisposition toward developing CD4+ Th2-type responses. The bias toward an IL-4-dominant response in BALB/c mice protects mice from severe Lyme-induced arthritis and spontaneous autoimmune disease. Since BALB/c mice immunized with proteoglycan develop severe arthritis, we were interested in testing whether arthritis is associated with a Th2-type response and thus is different from other arthritic models. BALB/c mice immunized with proteoglycan generated a higher ratio of IFN-γ to IL-4 that peaks at the onset of arthritis. We investigated whether when Th1 cells were dominant, disease outcome could be modified with pharmacological amounts of Th2 cytokines. Treatment with IL-4 prevented disease and induced a switch from a Th1-type to a Th2-type response. Proinflammatory cytokine mRNA transcripts were reduced in joints of cytokine-treated mice. Th2 cytokine therapy at the time of maximum joint inflammation also suppressed symptoms of disease. Despite the predisposition of BALB/c mice to a Th2-type response, proteoglycan-induced arthritis is a Th1-type disease. The effectiveness of IL-4 treatment was particularly striking because in other models of arthritis, treatment in a similar manner with IL-4 was not sufficient to inhibit arthritis. The effective control of arthritis and the switch from a Th1 to Th2 response suggest that levels of endogenous IL-4 in BALB/c mice may increase their responsiveness to Th2 cytokine therapy. The Journal of Immunology, 1999, 163: 5383–5390.

The differentiation of naïve CD4+ T cells into mature effector cells is conducted by secretion of an array of cytokines that dictate the nature of the immune response against infectious organisms and self-Ags. Activation of the CD4+ Th1 subset that produces IL-2, IFN-γ, and TNF-β is necessary for elimination of intracellular pathogens; however, these cells elicit the inflammation and tissue destruction observed in autoimmune diseases. The Th2 subset, which produces IL-4, IL-5, IL-6, IL-10, and IL-13, controls humoral immunity to extracellular organisms, but inhibits cell-mediated inflammatory responses (1–6). The development of Th1 or Th2 subsets from naïve cells to effector cells is regulated by the presence of specific cytokines in the microenvironment at the time of T cell priming. For the Th1 subset, IL-12 is a necessary cytokine for differentiation, whereas for Th2 cells, IL-4 is critical (7–12).

Several human autoimmune diseases and their corresponding animal models are characterized by the dominance of Th1-type cells (13–15). Analysis of T cell subsets in patients with rheumatoid arthritis and in mice with collagen-induced arthritis suggests that Th1-type cells dominate over Th2-type cells (6, 16–19). For example, in type II-collagen-induced arthritis (DBA/1, H-2b), a higher ratio of IFN-γ to IL-4 is produced from lymph node cultures of collagen-immunized mice, which peaks at the onset of disease symptoms (5).

Strain-specific differences in cytokine production have been exploited to help us understand the mechanisms responsible for cytokine control of susceptibility and resistance to infectious agents and autoimmune disease. BALB/c mice infected with Leishmania major fail to eliminate the organism, which correlates with the development of a predominant Th2-type response (2, 20, 21). The bias toward a Th2 response in BALB/c mice is potentially regulated at two distinct stages of the immune response (22–24). Early on there is an increase in the frequency of Th2 cells producing IL-4, which is influenced by a locus on chromosome 16 (21). Later in the immune response a premature loss of IL-12 responsiveness occurs because of reduced IL-12R expression, which is caused by a locus on chromosome 11 (20). Although a Th2-type response in BALB/c mice is detrimental with regard to parasites, it may be protective in the case of autoimmunity (25, 26). In a model of spontaneous autoimmune disease, Scott et al. showed that BALB/c mice are resistant to disease, whereas B10.D2 mice are susceptible (25). In addition, BALB/c mice infected with the spirochete Borrelia burgdorferi showed only mild Lyme-induced arthritis (26). Resistance to autoimmune disease in both these models correlates with naïve cell differentiation toward a Th2-type effector cell (25, 26).

Several years ago we demonstrated that immunization of BALB/c mice (H-2b) with human cartilage proteoglycan (PG) induced progressive polyarthritis and spondylitis (27–29). Clinical features and histological studies of the diarthrodial joints and spines of those mice revealed many similarities with rheumatoid arthritis (27). CD4+ T cells have been implicated in the development of PG-induced arthritis by observations that anti-CD4 MAb treatment prevents arthritis and that the transfer of disease requires T cells from arthritic animals (30–32). Based on the predisposition of BALB/c mice to develop Th2 effector cells, we were interested in determining whether the induction of arthritis in BALB/c mice...
is associated with a Th2-type response and thus is different from other models of arthritis. Alternatively, if PG-induced arthritis is a Th1-type disease, then PG immunization and subsequent induction of arthritis override the genetic predisposition of BALB/c mice to a Th2-type response. In this study we monitored the development of Th1/Th2 dominance in PG-immunized mice to gain insight into the regulation of PG-induced arthritis by Th1 and Th2 subsets.

Materials and Methods
Preparation of PG monomer (aggrecan)
Human cartilage tissue was obtained at the time of joint replacement surgery. PG of adult articular cartilage were prepared as previously described (27), (33). Briefly, cartilage pieces were frozen, and 20-µm cryostat sections were extracted with 4 M guanidinium chloride in 50 mM sodium acetate, pH 5.8, containing protease inhibitors at 4°C. High buoyant density PG monomers (aggrecan) were prepared by dissociative cesium chloride gradient centrifugation. PG were digested with protease-free chondroitinase ABC (Seikagaku America, Rockville, MD) overnight at 37°C and then further purified on a Sephacryl S-200 column (Pharmacia Biotech, Uppsala, Sweden).

Induction of arthritis and treatment with cytokines
Female BALB/c mice (Charles River Breeding Laboratories, Wilmington, MA; Kingston K51 colony) were injected i.p. with 100 µg of cartilage PG measured as protein on days 0, 7, 28, and 49. The first and fourth injections of PG were given in CFA (Difco, Detroit, MI) and the second and third injection of PG in IFA (Difco) as previously described (27, 28, 33). Cytokines IL-4 and IL-10 were a gift from Schering-Plough Research Institute (Kenilworth, NJ). Cytokines were administered i.p. at a concentration of 5 µg/mouse for 21 consecutive days. In some experiments, cytokines were administered at the prearthritic stage of the disease (i.e., 3 days before or 3 days after the fourth PG injection), and in others they were given after the appearance of joint swelling (~2–3 wk after the fourth PG injection). Control animals received PBS i.p. at the time of cytokine treatment.

Assessment of arthritis
Joint swelling was recorded every third day as the cumulative forepaw and hindpaw thickness in millimeters of wrist and ankle joints of each mouse, measured in both frontal and sagittal directions with a micrometer. In some experiments (Fig. 6) the cumulative change in joint thickness in millimeters was recorded. Animals were considered arthritic if the cumulative joint thickness increased by 1 mm. Histological studies were performed to determine the extent of joint damage. Hind paws were dissected, fixed, and decalcified before being embedded in paraffin and sectioned at 6-µm thickness in millimeters with a cryostat section (Fisher Scientific). Sections were stained with hematoxylin and eosin. Sections were examined by a blinded histologist, and arthritic changes were scored as normal (none), mild, moderate, or severe.

Assessment of cytokine production by spleen cells in vitro
Spleens were obtained 1 wk after each injection with PG. Single-cell suspensions were prepared as previously described (34). Splenocytes (2.0 × 10^7 cells/ml) were incubated in 24-well Falcon plates (Fisher Scientific, Pittsburgh, PA) in RPMI 1640 medium containing 7% FBS (Life Technologies, Grand Island, NY), 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM l-glutamine, 50 mM 2-ME, 1 mM sodium pyruvate, 0.01 mM non-essential amino acids, and 10 mM HEPES. Cells were stimulated in the presence of 10 µg/ml recombinant PG. Supernatants were harvested on day 3 for IL-4 and IL-10 and on day 5 for IFN-γ and then assayed by ELISA. The zero time point indicates the baseline values for unimmunized mice. Values represent the mean and SEM of cytokine production for spleen cells in response to PG of five mice. *, Data that are statistically significant (p < 0.05) between IFN-γ production and IL-4 or IL-10 production.

Cytokine mRNA in joint tissue
Hind paws were homogenized with a Polytron homogenizer (KRI Works, Cincinnati, OH) on ice. Homogenate was centrifuged to remove large debris, and RNA was extracted with TRI Reagent (Molecular Research, Cincinnati, OH). RNase protection assays were performed on 10 µg of RNA using the Riboquant MultiProbe RNase Protection Assay System (Phar-Mingen) according to the directions of the manufacturer. The mCh3b template was used to detect a set of cytokines (TNF-α, IFN-γ, IL-2, IL-4, IL-6, IFN-γ, IFN-β, TGF-β1, TGF-β2, TGF-β3, and MIF) as well as the housekeeping gene GAPDH and L3. Labeled [α-32P]UTP antisense RNA was synthesized by in vitro transcription from a cDNA template provided in the kit. Antisense RNA probe was purified by phenol/chloroform extraction and ethanol precipitation, and then hybridized with mRNA samples at 56°C overnight. RNA was used to digested ssRNA. Protected dsRNA was purified by phenol/chloroform extraction and ethanol precipitation. The samples were electrophoresed on a 5% denaturing polyacrylamide gel. The gel was dried and exposed to a phosphorimager screen. Radioactivity of the samples was measured and analyzed by scanning densitometry on a STORM PhosphorImager (Molecular Dynamics, Sunnyvale, CA). The level of mRNA for each cytokine was expressed as the ratio (in units of density) of each cytokine to GAPDH.

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

Results
Kinetics of cytokine secretion after immunization with PG
To determine whether BALB/c mice generated a Th1 or a Th2 response after immunization with PG, spleens were harvested from PG-immunized mice 1 wk after each injection with Ag. As shown in Fig. 1, a PG-specific cytokine response from spleen cells of room temperature and then washed twice in PBS-Tween 20. Biotinylated anti-IL-4 or anti-IL-10 was added for 1 h at room temperature, polyclonal rabbit anti-IFN-γ was added for 2 h at 37°C, and plates were washed extensively. For detection of rabbit anti-IFN-γ, biotinylated anti-rabbit IgG was incubated for 30 min at 37°C, and plates were washed extensively. The substrate 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) and 0.012% H2O2 were added to all wells. The color reaction was read on an ELISA reader (Couter, Hialeah, FL) at 405 nm.

FIGURE 1. Kinetics of IFN-γ, IL-4, and IL-10 production in vitro after immunization with PG. Mice were immunized with PG on days 0, 7, 28, and 49, and spleens were harvested from five mice 1 wk after each injection with Ag. Spleen cells were cultured in medium alone or PG at 100 µg/ml. Supernatants were harvested on day 3 for IL-4 and IL-10 and on day 5 for IFN-γ and then assayed by ELISA. The zero time point indicates the baseline values for unimmunized mice. Values represent the mean and SEM of cytokine production by spleen cells in response to PG of five mice. *, Data that are statistically significant (p < 0.05) between IFN-γ production and IL-4 or IL-10 production.
The PG schedule is the same as in either IL-4 or IL-10. The higher ratio of IFN-γ from BALB/c mice secreted a significantly higher ratio of IFN-γ. At this time point and at subsequent time points spleen cells from BALB/c mice first became apparent after the third injection with Th2 cytokine treatment before the onset of arthritis inhibiting development of inflammation. A, IL-10 (n = 8) and PBS (n = 8) treatment. Animals were injected with PG on days 0, 7, 28, and 49 and were treated with cytokines (5 μg, i.p.) daily from days 46–67. B, IL-4 (n = 9), IL-10 (n = 9), IL-4 plus IL-10 (n = 9), and PBS (n = 9) treatment. The PG schedule is the same as in A, but with cytokines (5 μg, i.p.) administered on days 52–73. C, IL-4 (n = 9), IL-4 plus IL-10 (n = 10), and PBS (n = 6) treatment. Cytokine treatment was the same as in A. The arrow indicates the last day of PG immunization. “Days” indicate days after the initiation of cytokine treatment. Data represent the mean of paw swelling in millimeters. *, Data that are significantly different (p < 0.05) between controls (PBS) and cytokine-treated animals. Error bars are omitted for clarity.

BALB/c mice first became apparent after the third injection with Ag. At this time point and at subsequent time points spleen cells from BALB/c mice secreted a significantly higher ratio of IFN-γ to either IL-4 or IL-10. The higher ratio of IFN-γ to IL-4 in BALB/c mice immunized with PG is characteristic of a Th1-type response and suggests that, similar to the collagen-induced arthritis model, PG-induced arthritis is a Th1-type disease.

The Th2 cytokine IL-4 prevented arthritis in PG-immunized animals

To determine whether joint inflammation can be manipulated by administration of recombinant Th2 type cytokines, IL-4 and IL-10 were injected individually and in combination (5 μg of each cytokine) i.p. daily for 21 days at the prearthritic stage of the disease (i.e., 3 days before or after the last PG injection). In the first experiment the daily injection of IL-10 moderately reduced joint swelling in PG-immunized mice (Fig. 2A). Control mice (seven of eight) that received PBS developed progressive arthritis within 10 days (Fig. 2A and Table I), whereas five of eight mice that received IL-10 developed arthritis by day 21 of treatment. The IL-10 effect was transient: after cessation of IL-10 treatment mice began to develop inflammation. The moderate effect of IL-10 on suppression of joint swelling may result from the short half-life of this anti-inflammatory cytokine in vivo (35).

In the second experiment, cytokine therapy with IL-4, IL-10, and a combination of both IL-4 and IL-10 began 3 days after the fourth injection with Ag. IL-4 completely suppressed the development of arthritis (Fig. 2B and Table I). Moreover, IL-10 alone in this experiment significantly suppressed the development of arthritis (Fig. 2B and Table I). The difference in the severity of arthritis between the first (Fig. 2A) and the second (Fig. 2B) experiment was due to variation between groups of animals. It is possible that less severe arthritis, as depicted in Fig. 2A, may be more easily suppressed by IL-10. In a third experiment IL-4 was injected alone and in combination with IL-10 beginning at the same prearthritic stage of the disease as described for the first experiment. Complete suppression of arthritis was observed in both cytokine-treated groups (Fig. 2C). After cytokine treatment was terminated on day 21, joint swelling remained suppressed for 15–20 days before significant inflammation was observed. When examined on day 55, there was no difference in the severity of joint inflammation between the PBS-treated and cytokine-treated groups (Fig. 2C and Table I).

Table I. Incidence of arthritis in IL-4- and IL-10-treated mice

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<th>Treatment</th>
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<sup>a</sup> Animals were treated with IL-4 and IL-10 as described in Materials and Methods. Animals were considered arthritic when the accumulative joint thickness increased 1 mm.

<sup>b</sup> Incidence of arthritis in Expt. 3 was determined on day 94.

**IL-4 completely prevented the histopathologic characteristics associated with arthritis**

It has been shown previously that the paw swelling and redness observed in response to immunization with PG accompany histopathologic changes that lead to extensive cartilage and bone destruction over time. We therefore assessed the effect of cytokine therapy on joint histology from the groups of animals shown in Fig. 2B. Animals were sacrificed on day 21, and ankle and knee sections were examined. As shown in Fig. 3A, in the control group there was severe arthritis in the ankle joints, with edema of the
synovial and periarticular tissues accompanied by synovial hyperplasia. Mononuclear and polymorphonuclear cell infiltration was abundant in the tissue and in the joint space, and early signs of cartilage damage and hyperplastic chondrocytes were observed. These early cellular changes were found in three of eight mice in the control group on day 21. In the IL-4-, IL-10-, and IL-4- plus IL-10-treated animals, no or very mild histopathologic signs of inflammation were noted in the ankle joint (Fig. 3, B, C, and D, respectively). These results demonstrate that treatment with Th2 cytokines prevented both the external symptoms of inflammation

FIGURE 3. IL-4 prevented the histopathology associated with the development of PG-induced arthritis. Sections of hind paws were examined by a blinded histologist and scored as normal, mild, moderate, or severe. Sections of representative ankle joints from control (A), IL-4-treated (B), IL-10-treated (C), and IL-4- plus IL-10-treated (D) animals. Sections were stained with hematoxylin and eosin. Bar = 400 μm.

FIGURE 4. Th2 cytokine treatment reduced IFN-γ and increased IL-4 and IL-10 production by spleen cells. PBS- and cytokine-treated animals (groups depicted in Fig. 2B) were sacrificed immediately after 21 days of cytokine treatment. Spleen cells were cultured in the absence PG (spontaneous) or the presence of Ag (PG-induced). Supernatants were harvested and assayed by ELISA for IFN-γ, IL-4, and IL-10 as described in Materials and Methods. Values represent the mean and SEM of cytokine levels from cells of eight mice per treatment group. *, Data that are statistically significant (p < 0.05) between control (PBS) and cytokine-treated animals.
(swelling and redness) and the internal consequences of joint inflammation (cellular infiltration and synovial hyperplasia).

Treatment with Th2 cytokines inhibits the PG-specific IFN-γ response
To determine whether treatment with Th2 cytokines affects the levels of Th1 and Th2 cytokines, spleen cells were harvested immediately after 21 days of cytokine treatment from PG-immunized and cytokine- or PBS-treated groups (n = 8/group) and assayed in vitro with and without stimulation with PG. Culture supernatants were harvested on day 3 for IL-4 and IL-10 and on day 5 for IFN-γ. As shown in Fig. 4, spleen cells from immunized animals, cultured in the absence of PG, spontaneously produced elevated levels of cytokines. The IFN-γ levels in the animals treated with IL-4 and IL-4 plus IL-10 were significantly lower than those in PBS-treated controls in both unstimulated (spontaneous) and PG-stimulated cultures (Fig. 4A). Conversely, IL-4 levels were moderately increased and reached significantly elevated levels in spleen cell cultures from animals treated with IL-10 alone (spontaneous release) or with IL-4 plus IL-10 (PG-specific release; Fig. 4B). The IL-10 level was significantly increased in the IL-10-treated groups and was detected as either a spontaneous or a PG-specific response (Fig. 4C). The reduction in the Th1 cytokine, IFN-γ, and the increase in the Th2 cytokines, IL-4 and IL-10, indicated a shift from a Th1-type to a Th2-type immune response in the cytokine-treated animals compared with PBS-treated controls. This shift to a Th2-type response most likely contributed to the prevention of arthritis. Interestingly, treatment with IL-10 appeared to enhance IL-10 secretion from spleen cells, suggesting that IL-10 might function as a differentiation factor for Th2 cells.

Levels of IFN-γ, TNF-α, TNF-β, and IL-6 transcripts were reduced in the joints of Th2 cytokine-treated animals
To determine whether reduced mRNA expression for IFN-γ and other cytokines also occurred in the joints of Th2 cytokine-treated animals, RNase protection assays were performed. We found that the relative levels of mRNA for IFN-γ, TNF-α, and IL-6 in peripheral joints were significantly inhibited in mice treated with IL-4, IL-10, and IL-4 plus IL-10 compared with those PBS-treated controls (Fig. 5). LTβ expression was also significantly suppressed in the IL-10-treated mice. The observation that TNF-β showed no difference between control and cytokine-treated mice indicated that suppression of mRNA synthesis was selective. Furthermore, there were no differences between IFN-β, TGF-β1, TGF-β2, TGF-β3, and MIF mRNA levels (data not shown). These findings suggest that Th2 cytokine treatment dramatically suppressed the expression of proinflammatory cytokines, particularly those associated with arthritis.

Th2 cytokines suppress acute joint swelling
The effect of Th2 cytokine treatment on acute symptoms of disease was tested by monitoring joint swelling in PG-immunized BALB/c mice. At the peak of inflammation (i.e., at the time of maximum joint swelling 2–4 wk after the fourth injection of PG), the animals were injected i.p. with PBS, IL-4, IL-10, or IL-4 plus IL-10 daily for 21 days, and joint swelling was monitored by measurement with a caliper. Upon administration of IL-4 or IL-4 plus IL-10, joint thickness immediately began to decrease (Fig. 6A). Shortly after the cytokine treatment was stopped, swelling increased, but never reached its original level (Fig. 6B). These data suggest that IL-4 alone or in combination with IL-10 suppresses joint swelling.
Because the effect is immediate, it seems likely that the mechanism involves direct suppression of inflammation, possibly through inhibition of inflammatory cytokine expression and cell migration.

When sections of ankle joints were assessed for histopathological changes in the animals depicted in Fig. 6a, immediately after cessation of cytokine treatment essentially peripheral joints in all groups showed similar histopathology. Despite the reduction in the clinical symptoms of joint inflammation (redness and swelling), there was no difference in the histopathologic changes between the control and cytokine-treated mice if the treatment began at the peak of arthritis (data not shown). Therefore, it is possible that much of the joint damage occurred before cytokine therapy. Further studies of the effects of Th2 cytokine treatment, initiated earlier in the course of the disease, are needed to determine when damage occurs and can be prevented.

**Cytokine expression after therapeutic application of Th2 cytokines in arthritic mice**

To determine whether there was any change in Th1 and Th2 cytokine levels in acutely arthritic animals treated with Th2 cytokines, the production of IFN-γ, IL-4, and IL-10 was assayed in animals treated with PBS, IL-4, IL-10, and IL-4 plus IL-10 on the day after cessation of cytokine treatment. There was no statistically significant difference between PBS-treated and cytokine-treated groups in the quantity of IL-4, IL-10, or IFN-γ secreted by spleen cells either spontaneously or in response to PG in vitro (data not shown).

Because treatment with Th2 cytokines significantly reduced joint thickness (Fig. 6, A and B), we sought to determine whether changes in the expression levels of proinflammatory cytokines contributed to the reduction in joint swelling. In the IL-4-treated group, the levels of TNF-α and LTβ mRNA in joint tissues were significantly reduced (Fig. 7). Surprisingly, in the IL-10-treated group mRNA levels of TNF-α and LTβ were significantly increased (Fig. 7). The combination of IL-4 and IL-10 treatment resulted in TNF-α and LTβ mRNA levels similar to those in PBS-treated control animals (Fig. 7). There was no change in the mRNA levels for IL-6, TNF-β, and IFN-γ (Fig. 7) and for IFN-β, TGF-β1, TGF-β2, TGF-β3, and MIF (data not shown). These data indicated that IL-4 selectively decreased, and IL-10 increased, the levels of TNF-α and LTβ, and combination therapy was not beneficial.

**Discussion**

Because BALB/c mice are genetically predisposed to a Th2-type immune response, we sought to determine whether the induction of PG arthritis in BALB/c mice is associated with a Th1- or a Th2-type response and whether it differs from other arthritic diseases. We showed here that immunization of BALB/c mice with PG induces a higher ratio of IFN-γ to IL-4, indicating that PG-induced arthritis is a Th1-type response. A PG-specific cytokine response was first observed after the third injection with Ag and may have developed sometime between the second and third injections (i.e., between 2 and 5 wk postimmunization). Throughout the immune response to PG, IFN-γ was always dominant over IL-4 and IL-10. Similar to PG-induced arthritis, other autoimmune models induced by immunization with heterologous Ags required Ag priming with mycobacteria (e.g., in CFA), which is known to promote Th1 activation (36). These results indicate that immunization with PG in CFA is a sufficient Th1 stimulus to overcome the genetic inclination toward development of a Th2-type response in BALB/c mice.

Our next question was whether higher levels of endogenous IL-4 in BALB/c mice make them more responsive to treatment with Th2 cytokines. We found that treatment of PG-immunized BALB/c mice with IL-4 alone before the onset of arthritis completely prevented the development of joint inflammation, whereas IL-10 alone only moderately suppressed disease. In collagen-induced arthritis in DBA/1 mice, IL-4 alone had no effect when administered at the time of arthritis onset (37). However, if IL-4 was continuously administered from the onset of collagen immunization, arthritis was suppressed (38). In group A streptococcal cell wall-induced arthritis, IL-4 had only a minimal effect on the acute onset of disease (39). Although we did not administer cytokines continuously, IL-4 was still able to suppress joint inflammation completely. A possible explanation for the difference between these studies might be related to the concentration of IL-4. If BALB/c mice endogenously produce higher levels of IL-4 in response to Ag, the amount of exogenous IL-4 needed to suppress inflammation might be reduced.

In the present study, because IL-4 completely inhibited the development of arthritis, no additional inhibitory effects could be attributed to IL-10. However, in collagen-induced arthritis, combined treatment with both IL-4 and IL-10 was needed to suppress disease significantly (37). Combined therapy with IL-4 and IL-10 was also shown to be the most effective therapy in murine streptococcal cell wall arthritis (40). These results further support the idea that the higher levels of IL-4 in BALB/c mice might make them more responsive to Th2 cytokine therapy.

We were next interested in determining whether the treatment of BALB/c mice with Th2 cytokines before the onset of arthritis resulted in a shift from a Th1-type to a Th2-type profile. Indeed, the PG-specific cytokine response measured in vitro revealed a significant reduction in the level of IFN-γ in the IL-4- and IL-10-treated groups (Fig. 3A). Conversely, there was an increase in IL-4 and IL-10 production by spleen cells of mice treated with IL-10 or IL-4 plus IL-10 (Fig. 3, B and C). The requirement for IL-4, and to a lesser extent for IL-10, in the differentiation of Th2 effector cells suggests that the presence of exogenous IL-4 and IL-10 could convert an ongoing or committed PG Th1-type to a Th2-type response (10, 41–43). In the mice treated with IL-4 or IL-4 plus IL-10, arthritis was suppressed between 15 and 20 days after cessation of treatment, and the severity of arthritis during the relapse never reached that in the control animals (Fig. 2C). If there are insufficient numbers of Th2 cytokine-producing T cells present, suppression of disease might be incomplete and transient. Initiation of cytokine treatment...
earlier in the course of immunization or treatment for longer per-
iods of time could suppress arthritis completely.

To determine whether the proinflammatory cytokine levels were
reduced in the joints of Th2 cytokine-treated animals cytokine
mRNA transcripts were examined. We found that the failure to
develop joint swelling correlated with reduced levels of proinflam-
matory cytokine transcripts; IL-4 and IL-10 alone and in com-
pbination were potent inhibitors of TNF-α, IL-6, TNF-β, and IFN-γ
mRNA in joints. Because both IL-4 and IL-10 inhibit expression of
proinflammatory cytokines by activated macrophages and syno-
vocyttes (44–47), the reduction in proinflammatory cytokine
TNF-α contributes to the suppression of mediators that cause the
cartilage and bone destruction in arthritis. IL-10 also exerts its
effects by down-regulating macrophage expression of class II MHC,
costimulatory molecules ICAM-1, B7-1, and B7-2 and the arthritic response in collagen-


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