Dependence on Interferon-γ for the Spontaneous Occurrence of Arthritis in DBA/1 Mice

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Objective. Male DBA/1 mice are known to spontaneously develop arthritis in the hind legs. The present study was undertaken to investigate the role of endogenous interferon-γ (IFNγ) in the pathogenesis of this ankylosing enthesopathy.

Methods. The role of IFNγ was studied by examining the development of arthritis in IFNγ receptor-knockout (IFNγR-KO) DBA/1 mice as compared with wild-type mice, and by treatment of wild-type mice with monoclonal anti-IFNγ antibody. IFNγ-disrupted and wild-type mice were mixed and housed in the same cage, and clinical symptoms of arthritis were assessed weekly for at least 9 weeks. Histologic examination was performed at the end of the experiment.

Results. In DBA/1 wild-type mice, 70% of the animals developed clinical symptoms of spontaneous arthritis, such as redness and swelling of the proximal interphalangeal joints, toe stiffness, and ankylosis. As evident from microscopic evaluation, the arthritis was mainly characterized by formation of new cartilage and bone, originating at the entheses and leading to ankylosis. The incidence and severity of arthritis, both clinically and histologically, were significantly reduced in IFNγR-KO mice. In wild-type mice, neutralizing anti-IFNγ antibody inhibited the occurrence of the disease for the duration of treatment.

Conclusion. The results suggest that endogenous IFNγ plays an important role in the initial stages of spontaneous arthritis, and that the inflammatory components in its pathogenesis are more prominent than has been believed. In view of the similarity between this disease and spondylarthropathies in humans, the data suggest that endogenous IFNγ may also play a disease-promoting role in the human condition and thus may serve as a target for therapy.

The role of interferon-γ (IFNγ) in the pathogenesis of chronic arthritis has been a controversial issue. In particular, in current murine models, both disease-promoting and disease-limiting roles of endogenous IFNγ have been documented (1–8). We previously demonstrated that in collagen-induced arthritis (CIA) in DBA/1 mice, the apparent disease-limiting role of endogenous IFNγ is ascribed to the use of mycobacteria-containing Freund’s complete adjuvant in the induction of CIA (2). Strikingly, when the model was simplified by omitting the mycobacteria, i.e., using incomplete instead of complete adjuvant, the role of endogenous IFNγ was inverted into a disease-promoting effect.

In the present study, we further assessed the role of IFNγ in inflammatory joint diseases, taking advantage of the observation that after grouped caging and without any induction procedure, male DBA/1 mice develop a form of arthritis (9–11). In contrast to CIA, this spontaneously developing condition is predominantly characterized by formation of new cartilage and bone, which originates at the entheses and leads to ankylosis. With regard to the pathology in humans, ankylosing enthesitis is the hallmark feature of a group of clinical, pathologic, radiologic, and genetically related chronic arthropathies that are collectively designated spondylarthropathies (SpA) (12,13), comprising ankylosing spondylitis, psoriatic arthritis, arthritis associated with inflammatory
bowl disease, reactive arthritis, and an undifferentiated form of arthritis. Although rheumatoid arthritis (RA) and SpA are clinically and radiologically distinct, the same inflammatory mediators seem to be involved in their pathogeneses.

Of interest is a report (14) that describes higher expression levels of IFNγ and interleukin-1β in synovial tissues from RA patients as compared with those from SpA patients. Since these clinical observations are suggestive of at least quantitative differences in their pathogenesis, we considered it of interest to compare the role of endogenous cytokines in the corresponding murine models. In the present study, we addressed how ablation of IFNγ signaling affects spontaneous enthesitis in DBA/1 mice.

MATERIALS AND METHODS

Mice and experimental conditions. The generation and the basic characteristics of the mutant mouse strain (129/Sv/Ev) with a disruption in the gene coding for the α chain of the IFNγ receptor (IFNγ receptor knockout [IFNγR-KO]) have been described previously (15). These IFNγR-KO mice were backcrossed for 10 generations with DBA/1 wild-type mice, which were originally obtained from Jackson Laboratories (Bar Harbor, ME), to generate the DBA/1 IFNγR-KO mice used in the present study.

IFNγR-KO and wild-type mice were bred and maintained under non–specific pathogen–free conditions in our Experimental Animal Center (University of Leuven). At age 12 weeks (± 2 days), male IFNγR-KO and wild-type mice, all coming from different litters, were mixed and housed together in the same cage. Each cage contained 2–3 IFNγR-KO mice and 2–3 wild-type mice. For experiments in which the wild-type mice were treated with monoclonal antibodies, the anti–IFNγ–treated mice and control–treated mice were housed together in the same cage.

Representative mice were routinely screened for viral pathogens at the ICLAS Reference Centre for Rodent Viruses (University Medical Center St-Radboud, Nijmegen, The Netherlands) and for bacterial and parasitic pathogens at La Mayenne (Laboratoire Vétérinaire Départemental, Laval, France). These mice were found to be negative for all murine pathogens commonly tested in these centers, i.e., common viral agents (K virus, polyomavirus, mouse adenovirus, mouse hepatitis virus, Sendai virus, vaccinia virus, reovirus type 3, Theiler’s murine encephalomyelitis virus, minute virus of mice, mouse parvovirus, mouse cytomegalovirus, and mouse pneumonia virus), common bacterial pathogens (Bordetella bronchiseptica, Citrobacter freundii, Corynebacterium kutcheri, Mycoplasma species, Salmonella, Streptococcus β-haemolyticus, Streptococcus pneumoniae, Streptobacillus moniliformis, and Escherichia coli), and parasites (gastrointestinal helminths, Giardia species, Spironucleus species, Trichomonas, Klossiella species, Eimeria species, and Entamoeba muris).

Anti–IFNγ and control antibodies. Neutralizing monoclonal antibody (rat IgG2a) against murine IFNγ and monoclonal irrelevant rat IgG2a (indicated as control IgG) were obtained as described previously (3). The neutralizing titer of anti–IFNγ antibody (endpoint dilution corresponding to 50% neutralization of the antiviral effect of 30 units/ml of mouse IFNγ on mouse L929 cells infected with mengovirus) was 1/400,000 units/ml. Mice were treated weekly, from age 12 weeks to age 19 weeks, with anti–IFNγ antibody or control IgG at a concentration of 0.5 mg in 0.2 ml saline (intraperitoneally). Batches of anti–IFNγ and control IgG were tested for endotoxin content by a chromogenic Limulus ameboocyte lysate assay (KabiVitrum, Stockholm, Sweden) and were found to contain <2 ng/ml endotoxin.

Macroscopic scoring of arthritis. Mice were examined once weekly and scored macroscopically for signs of arthritis. Scores were as follows: 0 (no symptoms), 1 (redness and swelling in one toe), 2 (redness and swelling in more than one toe), 3 (toe stiffness), and 4 (deformity and/or ankylosis).

Histology. For histologic examination, mice were killed by cervical dislocation, and the hind paws were dissected and split into forefeet and ankles. All tissues were fixed for 16 hours in formaldehyde, followed by 72 hours of decalcification using Decal (Serva, Heidelberg, Germany), and further processed for paraffin embedding. Forefeet were cut in a transversal plane, and ankles in a sagittal plane. Consecutive sections were made of the distal interphalangeal, proximal interphalangeal (PIP), and metatarsophalangeal (MTP) joints, as well as the tibiocalcaneal and talocalcaneal joints. The sections were stained with hematoxylin and eosin.

Microscopic scoring of arthritis. Four stages were recognized in the disease, as previously described (9). These stages were cell proliferation (score 1), cartilage formation (score 2), bone formation (score 3), and joint space bridging or ankylosis (score 4). In each mouse, a cumulative score for all of the PIP, MTP, and ankle joints of the 2 hind limbs was calculated. The sections were scored by an investigator who was unaware of the genotype of the mice.

RESULTS

Spontaneous arthritis in wild-type and IFNγR-KO DBA/1 mice. We found that the wild-type male DBA/1 mice spontaneously developed macroscopically detectable arthritis, with an incidence and characteristics similar to those described in published reports (9–11). Representative images of the most frequently occurring symptoms are shown in Figure 1. Only the hind limbs were affected, usually with initial manifestations of redness and swelling in 1 or 2 of the PIP joints (Figures 1A and B). Although in some of the animals, these inflammatory signs persisted until the end of the experiment, in other animals, the signs of inflammation disappeared but the joint remained deformed. As a result, toe stiffness, which was assessable by placing mice
on a wire grid (Figure 1C), was often observed. Ankylosis of the ankles (Figure 1D) was another common feature.

As evident from microscopic evaluation (Figures 1E and H), the disease process typically affected the entheses of the PIP and ankle joints. Different stages could be recognized, as follows: stage 1 = enthesial cell proliferation, stage 2 = cartilage formation, stage 3 = bone enthesophyte formation, and stage 4 = joint space bridging and ankylosis. This cascade is suggestive of the occurrence of heterotopic enchondral bone formation. In contrast to the microscopic characteristics of CIA, synovitis was seen infrequently and the articular cartilage remained unaffected in these animals with spontaneously occurring arthritis.

To evaluate the possible role of endogenous IFNγ in the pathogenesis of this condition, IFNγR-KO and wild-type mice, beginning at the age of 12 weeks, were scored once a week for the development of joint inflammation and deformity. In view of reports that have suggested that spontaneously developing arthritis is influenced by behavioral factors (11), and to avoid cage-specific effects, mixed groups of IFNγR-KO and age-matched wild-type mice were housed together in the same cages. Four independent experiments of this type were conducted.

The cumulative incidence of arthritis and the average disease scores obtained over a 9-week observation period in 1 representative experiment are shown in Figures 2A and B, respectively. In the wild-type animals, symptoms of arthritis appeared 2 weeks after grouping of the mice. For the following 3 weeks, the prevalence of arthritis in wild-type mice increased to reach a maximum of 70% at age 16 weeks. In sharp contrast, among the IFNγR-KO mice, only 1 of 12 (8.3%) developed signs of arthritis during the entire time course of the experiment (Figure 2A). Accordingly, the mean clinical group score of arthritis was significantly lower in the IFNγR-KO mice than in their wild-type counterparts (Figure 2B). Combined results of the 4 experiments are shown in Figures 2C and D. The observations in mice were recorded for at least 9 weeks postgrouping. In total, 23 of 30 wild-type mice developed spontaneous arthritis as compared with only 3 of 27 IFNγR-KO mice (Figure 2C). Moreover, in the affected IFNγR-KO mice, the symptoms of arthritis stayed at or below a score of 2, i.e., redness and swelling in more than one toe. In contrast, toe stiffness and ankylosis or deformity, corresponding to arthritis scores of 3 and 4, respectively, occurred exclusively in the wild-type mice (Figure 2D).

To ascertain whether the differences in the macroscopic scores between the IFNγR-KO and wild-type mice correlated with differences in pathology, the hind limbs of all mice from 1 representative experiment (illustrated in Figures 2A and B) were examined histologically. The sections were scored as described in Materials and Methods, by an investigator who was
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blind to the genetic status of the animals. Whereas 8 of 10 wild-type mice showed microscopic signs of arthritis, only 1 of 12 mutant mice developed microscopic symptoms of arthritis (i.e., score 1). The difference in group histopathologic score between the wild-type mice (mean \( \pm \text{SEM} 5.40 \pm 1.42 \)) and IFN-\( \gamma \)-R KO mice (0.08 \( \pm \) 0.08) was statistically significant (\( P < 0.0005 \), by Mann-Whitney U test).

**Counteracting effects of anti-IFN\( \gamma \) on the development of spontaneous arthritis in wild-type mice.** To evaluate IFN\( \gamma \) as a therapeutic target and to verify that the lower susceptibility of IFN-\( \gamma \)-R-KO mice was indeed due to deficiency in IFN\( \gamma \) functioning, and not to any unidentified collateral effect of the genetic difference between the animals, we tested whether treatment of wild-type mice with neutralizing anti-IFN\( \gamma \) monoclonal antibodies would reduce the occurrence of the joint lesions. Two groups of wild-type mice (age 12 weeks) (\( n = 8 \) each) were treated for 7 weeks with either anti-IFN\( \gamma \) antibodies or irrelevant control IgG in accordance with a dose regimen as previously described for CIA (3). Figure 3 shows the mean clinical scores and cumulative incidences of spontaneous arthritis in the mice up to age 27 weeks. Symptoms in control IgG–treated animals started to appear in 2 of 8 mice at age 14 weeks, and over the complete course of the observation period, 6 of the 8 mice developed arthritis. In contrast, only a single mouse of the anti-IFN\( \gamma \) antibody–treated group developed joint symptoms, with a score much lower than the mean score in control IgG–treated mice.

After termination of the antibody treatment (at age 19 weeks), the incidence of arthritis in the anti-IFN\( \gamma \)-treated mice increased, but the average clinical score for the following 2 weeks remained significantly lower than that in the control IgG–treated animals. Thus, at the age of 21 weeks, the average score was 0.50 \( \pm \) 0.27 (mean \( \pm \) SEM) in the anti-IFN\( \gamma \)-treated mice versus 2.25 \( \pm \) 0.73 in the control IgG–treated mice (\( P < 0.05 \), by Mann-Whitney U test). Eight weeks after termination of the treatment, the difference in score between the 2 groups of mice was still evident, but did not reach statistical significance (0.88 \( \pm \) 0.35 in the anti-IFN\( \gamma \) group versus 2.75 \( \pm \) 0.80 in the control group; \( P = 0.06 \)).
DISCUSSION

In the present study, we have confirmed the findings of earlier studies (9–11) by demonstrating that wild-type male DBA/1 mice spontaneously develop arthritis of the hind paws. In addition, we have shown that these lesions fail to develop in congenic IFNαRKO mice or in wild-type mice treated with anti-IFNα antibody. A first point of discussion is that although DBA/1 mice are widely used in many laboratories, spontaneous occurrence of arthritis in this mouse strain has so far been reported by only one other research consortium (9–11). However, the signs of this condition are relatively modest and require a long observation period to be noticed. In addition, grouped caging of mice from different litters is essential for the lesions to develop. Therefore, the disease can easily go unnoticed when mice are kept under usual circumstances.

The possibility that our colony is genetically more sensitive than are colonies at large is unlikely, since we found that DBA/1 mice newly purchased from 2 different commercial sources (Harlan Laboratories, Horst, The Netherlands and Jackson Laboratories, Bar Harbor, ME) developed the condition as frequently as did mice from our local colony (Matthys P, et al: unpublished data). Alternatively, bacterial infection or the presence of a peculiar microbial intestinal flora may render the mice more sensitive. The role of bacterial infection in reactive arthritis is, of course, well established, but its possible role in other SpA remains uncertain (16). Consequently, although we could not detect any infection from a number of common mouse pathogens (see Materials and Methods), we cannot exclude the possibility that an unidentified microorganism is involved in the pathogenesis of the spontaneously developing arthritis.

The high susceptibility of DBA/1 mice to both CIA and spontaneous arthritis has raised the possibility of a common pathologic mechanism (10). In fact, on macroscopic examination, the lesions superficially resembled those seen in CIA and caused similar defects in function, i.e., redness, swelling, and ankylosis (10,11). However, pathologically, the lesions were distinct from those in classic CIA in mice, most importantly because of the presence of ankylosing enthesitis which was characterized by enthesial cell proliferation and subsequent endochondral bone formation, leading to joint space bridging and ankylosis. In contrast, only minimal proliferative synovitis, without large immune cell infiltrates, was seen. Articular cartilage and the underlying bone were rarely eroded. The enthesis seems to be the primary target organ in this murine experimental model, as was also suggested to be the case in human SpA (12).

Not only the histologic appearance, but also the pathogenesis of this condition differs, at least in part, from that of CIA. In particular, development of spontaneous arthritis has been reported to show no requirement for T cells (9), whereas such a requirement does apply in CIA. Although T cells were not completely absent in either the T cell receptor α/β knockout mice or the T cell receptor γ/δ knockout mice used in these studies, the role of T cells in spontaneous arthritis seems limited, particularly since no T cell infiltrates were recognized on pathologic examination (11). Since T cells are a rich source of IFNγ, and IFNγ has a demonstrated impact on CIA, we explored the issue of whether this cytokine plays a role in spontaneous arthritis. Using both IFNγR-KO mice and neutralizing monoclonal antibodies, we found evidence that endogenous IFNγ does significantly contribute to the development of this condition, although it is not strictly required.

Our finding that IFNγ is a disease-promoting factor in spontaneously developing arthritis, together with the reported noninvolvement of T cells, suggests that spontaneous arthritis, in contrast to CIA, is predominantly of an innate immune pathologic nature, involving other IFNγ-producing cells such as natural killer cells or myeloid cells. One more argument for the predominance of innate immune pathology is the observation that the affected joints do not contain major histocompatibility complex (MHC) class II–expressing cells (11). Interestingly, neutrophils have recently been found to contain intracellular stores of IFNγ during primary Salmonella infection (17). Activation of natural killer cells and neutrophils does not require interaction in the MHC class II context.

The stimulus for activation of these or other cells in spontaneous arthritis needs to be identified, but may be behavioral in nature. Indeed, important features of spontaneous arthritis in DBA/1 mice include the restriction of its development to males and the requirement that mice be housed in groups of at least 3 per cage and that they be derived from different litters. Intriguingly, the disease occurrence also remains limited to the hind paws (9–11). Conceivably, chronic physical straining of the hind legs, due to continued aggressive posture or action, might be the local triggering factor. Alternatively, systemic production of IFNγ may act as an enhancing factor for inflammatory events in the strained connective tissues of the joints. Of interest, the human SpA also have a nonsymmetric sex distribution, since these conditions primarily affect men. Likewise, psychological
components such as stress play a role in exacerbations. Even so, local injury or overuse may trigger an attack of SpA enthesitis or arthritis in susceptible patients (18).

In previous studies by us and from other laboratories, both disease-promoting and disease-limiting roles of IFNγ in murine CIA have been documented (1–6). Most significant was the observation that the disease-limiting role depends on the use of mycobacterial adjuvant. With partial or complete omission of this adjuvant, endogenous IFNγ acts as a disease-promoting agent (2). The finding that this is also true for spontaneous arthritis in DBA/1 mice reinforces the antiinflammatory role of IFNγ in classic Freund’s complete adjuvant–assisted CIA, since it acts as an artefact deriving from the presence of the mycobacteria in the adjuvant. Therefore, the cellular target and mechanism of action of IFNγ in spontaneous arthritis and in CIA induced without bacterial adjuvant (using Freund’s incomplete adjuvant) may be similar.

The primary reason for studying murine models of arthritis is to acquire insight into the pathogenetic factors and mechanisms at work in human arthritic diseases. Our observation, that in DBA/1 mice endogenous IFNγ strongly enhances spontaneous development of periarticular lesions, suggests that in human patients, the overall inflammatory status may, likewise, constitute a contributory factor to the progression of the lesions. Similarly, the role of IFNγ, natural killer cells, and neutrophils in human SpA should be investigated more closely.

REFERENCES