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Immunology

Administration of interleukin 12 in combination with type II collagen induces severe arthritis in DBA/1 mice

(autoimmunity/T helper 1 cells/antibody response)

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ABSTRACT **The induction of arthritis in DBA/1 mice usually requires immunization with the antigen type II collagen emulsified with *Mycobacterium tuberculosis* in oil. Here we describe that interleukin 12 (IL-12) can replace mycobacteria and cause severe arthritis of DBA/1 mice when administered in combination with type II collagen. Immunization of DBA/1 mice with type II collagen emulsified in oil alone resulted in a weak immune response, and only a few animals (10–30%) developed arthritis. Administration of IL-12 for 5 days simultaneously with each immunization strongly enhanced the anti-type II collagen immune response. Collagen-specific interferon γ (IFN-γ) synthesis by *ex vivo* activated spleen cells was enhanced 3- to 10-fold. IFN-γ was almost completely produced by $CD4^+$ T cells. Furthermore, the production of collagen-specific IgG2a and IgG2b antibodies was upregulated 10- to 100-fold. As a consequence, the incidence of arthritis in the group of mice immunized with collagen plus IL-12 was very high (80–100%). The developing arthritis was severe, involving ≈50% of all limbs with strongly increased footpad thickness in most cases. Furthermore, histological examination revealed massive, mainly polymorphonuclear cell infiltration, synovial hyperplasia, cartilage and bone destruction, as well as new bone formation. In many cases, this resulted in the complete loss of joint structure. Neutralization of IFN-γ *in vivo* prevented the development of arthritis in collagen-immunized and IL-12-treated mice. In conclusion, our data show that *in vivo* administered IL-12 can profoundly upregulate a T helper 1-type autoimmune response, resulting in severe joint disease in DBA/1 mice.**

Intradermal injection of native type II collagen (CII), a major component of cartilage, in adjuvant induces an arthritogenic reaction in susceptible rats (1) and mice (2). In mice, immunization with CII emulsified in complete Freund's adjuvant (CFA) triggers arthritis more rapidly and with higher incidence than immunization with CII emulsified with incomplete Freund's adjuvant (IFA). In both species, the disease is characterized by synovial cell proliferation and infiltration of inflammatory cells into the synovium, the joint cavity, and the periarticular tissue. This is followed by cartilage and bone erosion with new bone formation resulting in destruction or ankylosis of joints (1–3). In many aspects, CII-induced arthritis in rats or mice resembles human rheumatoid arthritis (4). It is widely used as an experimental animal model of rheumatoid arthritis.

T and B lymphocytes are involved in the immune response to CII in mice (5). The susceptibility to arthritis is linked to the $H\text{-}2^q$ haplotype and is correlated with a high antibody response to the antigen (6, 7). However, some strains of mice elicit substantial antibody responses but fail to develop arthritis (6, 8). Studies by Watson and Townes (8) revealed that not the overall antibody production but rather the IgG2a (and to some degree IgG2b) response is associated with the development of arthritis. The importance of an antibody response in the pathogenesis of collagen-induced arthritis was recently documented by Durie *et al.* (9), who used an antibody against gp39 (the ligand for CD40) to prevent the humoral anti-collagen response and the development of arthritis in DBA/1 mice. However, the induction of severe chronic arthritis in naive mice requires the transfer of CII-specific antibodies in combination with specific $CD4^+$ T lymphocytes (10). In $H\text{-}2^q$ mice the CII-specific T cells appear to belong to the T helper 1 (Th1) subset of $CD4^+$ T lymphocytes. Upon antigen-specific *in vitro* stimulation with CII, spleen cells from immunized mice synthesize interferon γ (IFN-γ) (11). Because IFN-γ can induce immunoglobulin isotype switching to IgG2a (12), this cytokine might be involved in the regulation of collagen-specific IgG2a production. Interleukin 12 (IL-12) is a recently cloned (13) heterodimeric cytokine exerting many immunomodulatory effects reviewed in (refs. 14 and 15). These include the stimulation of IFN-γ synthesis and a strong enhancement of Th1 cell development (16, 17). This led us to test whether IL-12 administered to DBA/1 mice immunized with CII in IFA (without mycobacteria) would trigger an autoimmune response. Without IL-12 treatment, only a few animals developed arthritis, whereas nearly all IL-12-treated animals developed a severe destructive joint disease.

MATERIALS AND METHODS

Animals. DBA/1 mice were obtained from Charles River Breeding Laboratories. They were bred at the local animal facility. Female and male mice were used in the experiments at 8–12 weeks of age.

Collagen and Reagents. Chicken CII was purchased from Sigma. It was dissolved in 0.01 M acetic acid at a concentration of 4 mg/ml. IFA and CFA were from Difco. Purified murine recombinant IL-12 (>95% pure IL-12, as assessed by SDS/PAGE; <5 endotoxin units/mg, as determined by the *Limulus* amebocyte assay) was prepared from supernatant of transfected CHO cells (18). The monoclonal antibody (mAb) GK1.5 (19) against mouse CD4 and mAbs HO2.2 and 3.155 (20) against mouse CD8 were used to deplete the respective T-cell subset from whole spleen cell suspensions by complement-mediated lysis. The hybridoma XMG1.2 (21) secreting a neutralizing anti-IFN-γ mAb was a kind gift of T. Mosmann (University of Alberta, Edmonton, Canada). The antibody was purified from culture supernatants and used to neutralize IFN-γ *in vivo*. Rat IgG as a control was purchased from Dianova (Hamburg, Germany). A pair of mAbs recognizing different epitopes of IFN-γ (mAb R46A2 and biotinylated

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Abbreviations: CII, type II collagen; Th, T helper; IFN-γ, interferon γ; IL-12, interleukin 12; mAb, monoclonal antibody.
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mAb AN-18.17.24) were used to measure IFN-γ by ELISA (17). Streptavidin peroxidase was bought from Boehringer Mannheim. The peroxidase substrate 2,2'-azinobis(3-ethylbenzthiazoline 6-sulfonic acid) (ABTS) was obtained from Sigma.

Immunization. Chicken CII was emulsified with an equal volume of either IFA or CFA. The resulting emulsion was injected intradermally into the auriculars of both ears and one site at the back of each mouse. Each mouse received a total volume of 0.1 ml containing 100 μg of CII. Booster immunizations were performed with 100 μg of CII in PBS in a total volume of 0.1 ml injected i.p. Some mice received IL-12 (dissolved in PBS containing 1% syngeneic normal mouse serum) for 5 days simultaneously with each CII immunization by i.p. injection. IL-12 (50 ng/day in experiments 1–3; 1000 or 200 ng/day in experiments 4 and 5) was administered i.p. in a total volume of 0.2 ml starting 1 day before the CII immunization. Mice not treated with IL-12 received 0.2 ml of PBS with 1% normal mouse serum daily for 5 days. Some IL-12-treated animals received anti-IFN-γ mAb XMG1.2 (2 × 1 mg i.p. 1 day before and 2 days after the immunization with collagen) to neutralize endogenous IFN-γ or rat IgG as a control.

Determination of the Incidence of Arthritis and Measurement of Footpad Thickness. Arthritis was evaluated by examining daily all paws of individual mice for signs of arthritis. Data indicate the presence of definitive arthritis characterized by erythema and swelling of digits and/or paws without grading of severity. In addition, swelling was quantitated by measuring the thickness of the foot (dorsal/ventral) and the width of the ankle (malleolus lateral/medial) with a micrometer caliper (Mitutoyo, Japan).

Histology. Limbs were fixed in 4% buffered formalin and decalcified with EDTA. Paraffin-embedded material was cut into 5-μm-thick sections and stained with hematoxylin and eosin.

Assay for Detection of Anti-CII-Specific T-Cell Responses. Spleens of mice immunized with CII in IFA (without or with IL-12) were prepared at the end of the respective experiments (experiment 2, day 74; experiment 3, day 64). Tissue was minced through sterile wire mesh, resulting in single cell suspensions. Whole spleen cell suspensions and CD4- or CD8-depleted spleen cell suspensions were stimulated at a density of 10^6 cells per ml in 48-well culture plates with 100 μg of CII per ml. Activation supernatants were collected after 24, 48, and 72 hr and tested for IFN-γ by specific ELISA as described (17). CD4 or CD8 cell depletion was performed by incubating whole spleen cells with mAb GK1.5 or HO2.2 plus 3.155 and guinea pig complement. This procedure was repeated once. The remaining cells were then washed twice with PBS, counted again, and used at a density of 10^6 cells per ml in the experiments.

Assay for Detection of Serum Anti-CII Antibodies. Mice were bled during the course of and at the end of the experiments to determine (by ELISA) antibodies specific for native CII in the sera. Briefly, ELISA plates (Maxisorb; Nunc, Wiesbaden, F.R.G.) were coated overnight at 4°C with antigen (10 μg of native CII per ml; 50 μl per well) in phosphate buffer (0.1 M NaH_2PO_4, pH 9.2) followed by overnight incubation with a 2% skimmed milk powder solution to saturate nonspecific binding sites. Serial 1:5 dilutions of the immune sera in PBS with 0.1% Tween 20 were added (45 min per step) followed by incubation with isotype-specific (IgG1, IgG2a, IgG2b) biotinylated anti-mouse antisera (The Binding Site, Birmingham, U.K.) or biotinylated sheep anti-mouse immunoglobulin antiserum (total immunoglobulin), streptavidin peroxidase (1:5000) in PBS with 0.1% Tween 20, and the substrate ABTS in reaction buffer (40 mM citric acid, pH 4.5, 0.0075% H_2O_2). ELISA plates were read at 414 nm after 20–60 min in a microplate reader (Immuno Reader NJ-2000; Nunc, Wiesbaden, Germany). Plates were washed three times between each step with PBS containing 0.1% Tween 20. A standard curve was generated from serial 1:2 dilutions of a standard serum to calculate the antibody content (in arbitrary units/ml; 1 unit = dilution with A_{414} of 0.5) in different sera.

RESULTS

High Incidence of Arthritis in DBA/1 Mice Immunized with CII in IFA Plus IL-12. DBA/1 mice of the Mainz colony develop arthritis with an incidence of 50–70% or 10–30% when immunized with CII in CFA or IFA, respectively. When CFA was used as adjuvant the joint disease observed was usually severe, involving several limbs with extensive paw swelling and bone deformation. In the case of IFA as adjuvant, only a few animals develop arthritis, which is usually less severe and sometimes restricted to one or two digits. Because mycobacteria can induce the synthesis of IL-12 by macrophages, we tested whether IL-12 could replace mycobacteria and trigger/enhance an (auto)immune response resulting in arthritis in DBA/1 mice immunized with chicken CII in IFA. Mice were treated with IL-12 for 5 days (day −1 to day +3) simultaneously with the primary and booster immunizations. Data of five independent experiments are presented in Table 1. They clearly document that administration of IL-12 stimulated an autoimmune response. Joint disease developed with a very high incidence (80–100%). This was evident in male (experiments 1, 3, and 4) and female (experiments 2 and 5) DBA/1 mice. In experiment 3, the effect of IL-12 treatment was compared directly between groups of mice immunized with CII in CFA or IFA. Two of 10 mice in the IFA group and 5 of 10 mice in the CFA group had developed arthritis at day 64 after primary immunization. Administration of IL-12 strongly promoted the development of joint disease in the IFA group (10 of 10 mice) and also in the CFA group (9 of 10 mice). These data indicate that IL-12 is a potent adjuvant promoting autoimmune arthritis in DBA/1 mice when administered in combination with CII even in the absence of mycobacterial adjuvant.

Table 1. Time course of arthritis in CII-immunized DBA/1 mice with or without IL-12

		No. of arthritic mice/total no. of mice			
Exp.	Day	CII in IFA	CII in IFA + IL-12	CII in CFA	CII in CFA + IL-12
1	35	0/4	2/4		
	47	0/4	4/4		
2	36	0/10	2/9		
	52	0/10	8/9		
	74	2/9	8/9		
3	37	1/10	5/10	4/10	4/10
	51	2/10	7/10	5/10	7/10
	64	2/10	10/10	5/10	9/10
4	37	2/5	3/9		
	53	2/5	5/9		
	74	1/5	8/9		
5	36	1/10	3/15		
	42	1/10	8/15		
	104	2/10	13/15		

Female mice were used in Exps. 2 and 5; male mice were used in Exps. 1, 3, and 4. All mice received a primary intradermal injection of 100 μg of CII in IFA or CFA and were booster injected i.p. (100 μg of CII in PBS) after 3 weeks. Mice were booster injected again in Exp. 1 at day 31, in Exp. 2 at days 31 and 42, in Exp. 3 at day 49, in Exp. 4 at day 42, and in Exp. 5 at day 74. Some groups of mice received i.p. injections of IL-12 parallel to each CII immunization (5 days, 50 ng daily in Exps. 1–3; 1000 or 200 ng in Exps. 4 and 5). Each mouse was examined visually for signs of arthritis (erythema, swelling of digits or paws) two to four times a week.

Severe Destructive Joint Disease Develops in IL-12-Treated DBA/1 Mice Immunized with CII. To characterize the severity of arthritis, measurements of footpad and ankle thickness as well as histological studies were undertaken. In general, only a few DBA/1 mice immunized with CII in IFA develop arthritis. This is shown in Fig. 1, where results of measuring footpad thickness [comparing day 14 (before onset of arthritis) with day 65] of the hindpaws are presented. In this particular experiment, 2 of 10 mice of the group not treated with IL-12 developed arthritis and showed significant swelling of one paw per mouse. In other experiments, mice of similarly treated groups had a less severe arthritis, involving only some digits without swelling of the paws. In contrast, all (10/10) mice of the IL-12-treated group suffered from severe arthritis with erythema and extensive footpad swelling (see Fig. 1) of at least one limb (one mouse showed no swelling of the hindlimbs but had a swollen forelimb instead). Measurements of the ankle width from the medial to the lateral malleolus gave similar results (not shown). The overall limb involvement in the collagen-IFA-immunized group was 2/36 in experiment 2 and 5/40 in experiment 3, whereas it was 22/36 (experiment 2) and 26/40 (experiment 3) in the group of mice also treated with IL-12. The animals were sacrificed on day 65, the swollen paws were amputated, and longitudinal sections were examined histologically. The joints (hindlimb) of nonarthritic mice of the IL-12-untreated group appeared histologically normal (Fig. 2*A*). In contrast, the arthritic joints (hindlimb) of the IL-12-treated mouse (Fig. 2*B*) were characterized by an extensive inflammatory reaction of the synovia showing fibrin exudation and intensive infiltration of polymorphonuclear cells and macrophages. The synovial lining cells exhibited hypertrophic and hyperplastic changes as a result of activation and proliferation. Clearly, severe damage of the articular surface appeared, consisting of destruction of the cartilage and marginal subchondral erosion of bones followed by intense osteosclerosis and bone remodeling. Taken together, the results obtained by measuring footpad thickness and histopathologic examination of arthritic paws clearly demonstrate that immunization of DBA/1 mice with chicken CII combined with administration of IL-12 induces a severe destructive arthritis.

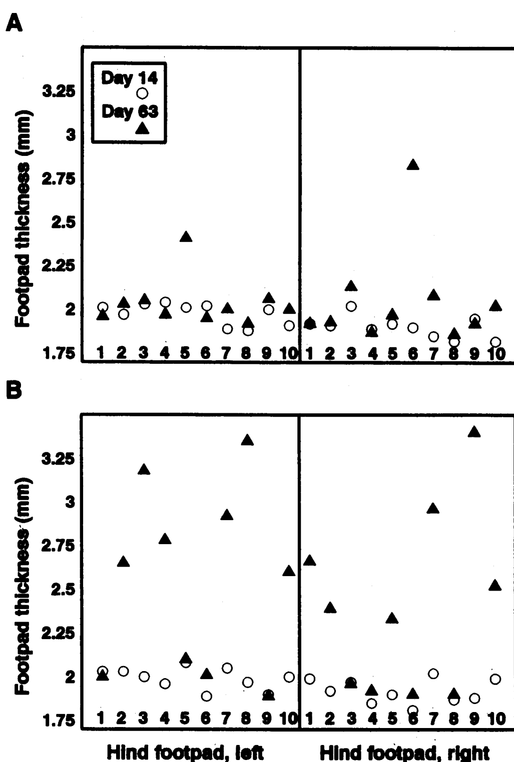

FIG. 1. Comparison of footpad thickness of CII-immunized DBA/1 mice with or without IL-12 treatment. DBA/1 mice were immunized with CII in IFA without (*A*) or with (*B*) administration of IL-12 (see Table 1, experiment 3). The thickness of the hind footpads was measured before the onset of arthritis at day 14 and at day 63 with a micrometer caliper. Values represent footpad thickness in mm (day 14 vs. day 63) for each individual hind footpad of all mice of the two groups.

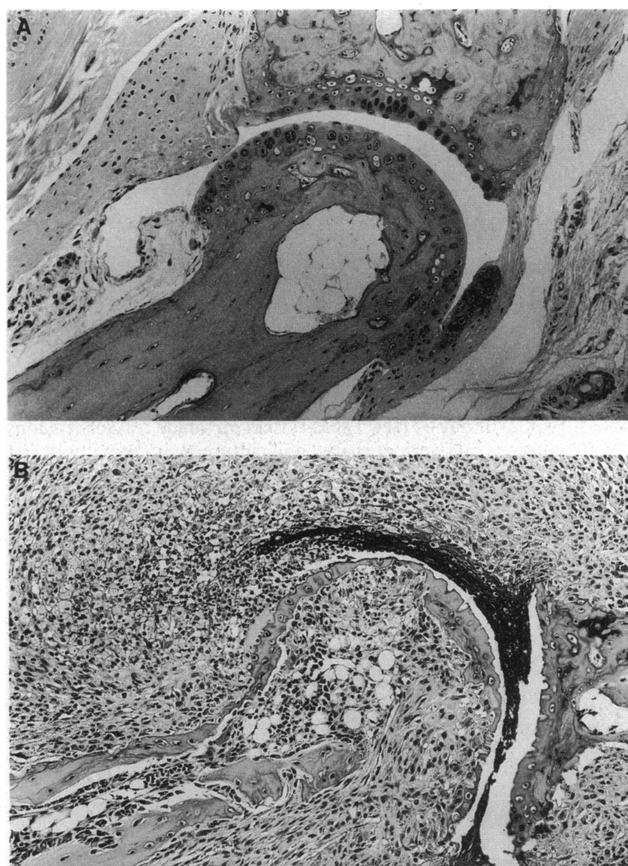

FIG. 2. Histological comparison of sections of a normal hindpaw from a nonarthritic mouse with the hindpaw from an arthritic mouse. (*A*) Normal metatarsophalangeal joint of a nonarthritic mouse. (Hematoxylin and eosin; ×98.) (*B*) Metatarsophalangeal joint of an arthritic mouse with totally destroyed articular surfaces and severe inflammatory reaction. (Hematoxylin and eosin; ×98.) The nonarthritic mouse (*A*) was immunized intradermally with 100 μg of chicken CII in IFA and received booster immunizations with 100 μg of CII in PBS i.p. at days 21 and 49. The arthritic mouse (*B*) was treated with IL-12 (5 days; 50 ng/day) simultaneously with each immunization. Mice were sacrificed at day 65.

Treatment with IL-12 Promotes the Development of Collagen-Specific $CD4^+$ T Cells Producing IFN-γ. The cellular immune response to CII in DBA/1 mice was investigated by stimulating spleen cells *ex vivo* with collagen and measuring the synthesis of IFN-γ after 24, 48, and 72 hr. Maximal levels were reached after 48 hr. These data are presented in Table 2 (experiment 2 for female mice, experiment 3 for male mice). In mice of both sexes, administration of IL-12 promoted the development of T cells producing IFN-γ. Spleen cells from mice immunized with CII in IFA produced rather low amounts (10–20 units/ml) of IFN-γ upon antigen-specific activation *in vitro*. There was no significant difference between arthritic and nonarthritic mice regarding the synthesis of IFN-γ (unpublished data). Treating mice simultaneously with the immunizations with IL-12 increased the production of IFN-γ by spleen cells ≈4-fold (male) or ≈12-fold (female). To determine the nature of the IFN-γ-producing cells, $CD4^+$ T cells were

Table 2. Treatment with IL-12 *in vivo* results in augmented IFN-γ production by $CD4^+$ T cells *ex vivo*

		IFN-γ, units/ml	
Exp.	Treatment	Spleen cells	Spleen cells + CII
2	CII in IFA	1.2	13.8
	CII in IFA + IL-12	4.6	176.3
3	CII in IFA	1.5	10.4
	CII in IFA + IL-12	2.2	36.7
	$CD4^+$ depleted	0.6	0.8
	$CD8^+$ depleted	4.1	30.0

Spleen cells from arthritic mice (two or three per group) immunized with CII in IFA or CII in IFA + IL-12 were prepared at day 74 (see Table 1, Exp. 2) or day 64 (see Table 1, Exp. 3). Single cell suspensions (3×10^6 cells per ml) were prepared and cultured with or without CII (100 μg/ml). In addition, in Exp. 3, spleen cells from the CII in IFA + IL-12 group were depleted of either $CD4^+$ or $CD8^+$ T cells. Forty-eight hours later, the activation supernatants were collected and the amount of IFN-γ was determined by ELISA.

depleted prior to antigen-specific activation. This treatment eliminated IFN-γ synthesis. Depletion of $CD8^+$ T cells had no effect. Thus, administration of IL-12 to DBA/1 mice immunized with CII led to increased IFN-γ synthesis by $CD4^+$ Th1-like cells.

The Humoral Immune Response to CII Is Strongly Upregulated in IL-12-Treated Mice. Antibodies specific for CII, especially of the IgG2a subclass, play a major role in the pathogenesis of arthritis in the model described here (6–9). Therefore, we investigated the collagen-specific overall antibody production as well as the IgG subclass pattern in the sera of female and male DBA/1 mice. Data derived from sera taken at two time points—one during the course of the experiment and a second at the end—are presented in Fig. 3. The mean of all mice of different groups (IL-12-treated vs. nontreated) is presented regardless of whether or not the mice had developed arthritis. In general, arthritic mice of the CII in IFA group had relatively high antibody titers, reaching those of IL-12-treated mice. Mice of both sexes immunized with CII in IFA produced substantial amounts of IgG1, IgG2a, and IgG2b. Administration of IL-12 enhanced the synthesis of CII-specific total immunoglobulin by a factor of 3–6. However, the IgG subclasses were affected differentially. IgG1 antibodies were upregulated only slightly (2-fold). In contrast, CII-specific antibodies of the IgG2b and IgG2a subclasses were upregulated 3- to 18-fold and 10- to 37-fold, respectively. The increase in antibody synthesis triggered by IL-12 was generally somewhat more pronounced in female than in male mice due to a lower background in female mice. Thus, IL-12 treatment strongly augmented the production of those IgG subclasses (IgG2a and IgG2b) that are known to be of critical importance in the pathogenesis of collagen-induced arthritis.

The IL-12-Induced Increase in Antibody Production and the Development of Arthritis Are IFN-γ-Dependent. One of the major effects of IL-12 is the upregulation of IFN-γ production. To investigate whether the effects of IL-12 in CII-induced arthritis are IFN-γ dependent, a group of CII-immunized mice received IL-12 in combination with the anti-IFN-γ mAb XMG1.2. None of 10 mice of this group developed arthritis until day 64 after the primary immunization, whereas 10 of 10 mice of the IL-12-treated group and 2 of 10 mice of the group not treated with IL-12 showed signs of joint disease. Similarly, the 19- and 7.5-fold increases in the serum levels of CII-specific IgG2a and IgG2b antibodies of CII-immunized and IL-12-treated mice, compared to the antibody levels in the sera of mice immunized with CII in IFA without administration of IL-12 (IgG2a, 38,000 ± 30,000 units/ml; IgG2b, 71,000 ± 108,000 units/ml), were completely prevented when CII-immunized animals received IL-12 in combination with an anti-IFN-γ mAb. Thus, the IL-12-triggered enhancement of the CII-specific humoral immune response and the induction of arthritis depend on synthesis of endogenous IFN-γ.

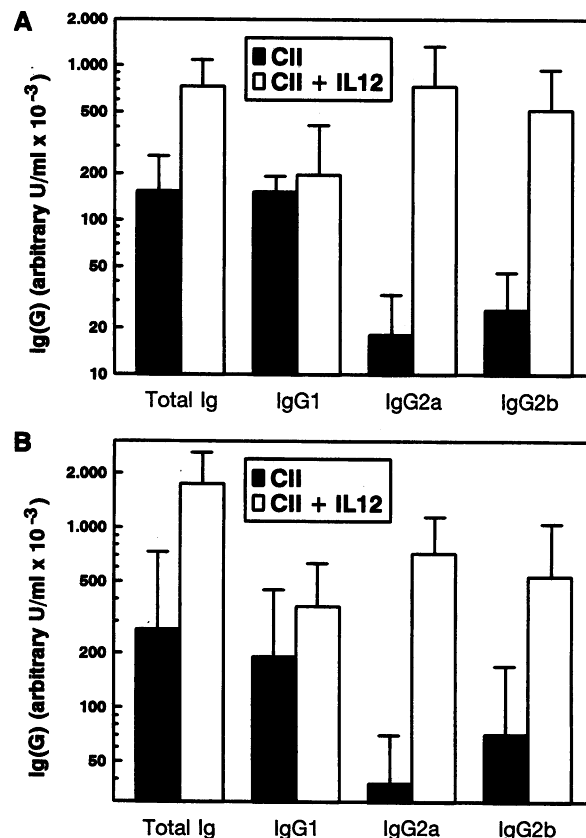

FIG. 3. Comparison of serum anti-CII antibody titers of DBA/1 mice immunized with CII in IFA with or without IL-12. Mean values ± SD for total immunoglobulin, IgG1, IgG2a, and IgG2b in arbitrary units/ml are shown for groups of mice immunized with CII in IFA or with CII in IFA plus IL-12 treatment. (*A*) Results for female DBA/1 mice bled at day 74 (from experiment 2). (*B*) Results for male DBA/1 mice bled at day 64 (from experiment 3; see Table 1 legend).

DISCUSSION

A major advance in understanding how the type of a developing immune reaction is regulated was the discovery made by Hsieh *et al.* (16) that certain bacteria can directly induce the synthesis of IL-12 by antigen presenting macrophages and that IL-12 promotes the differentiation of naive Th lymphocytes into Th1 cells. This effect of IL-12 is also evident *in vivo* where it stimulates a protective Th1 response in *Leishmania major*-infected BALB/c mice, which, without IL-12 treatment, would develop a disseminated infection (22, 23).

The experiments presented here demonstrate that IL-12 can promote autoimmune joint disease in susceptible mice *in vivo.* Immunization with CII in IFA induced arthritis in a few animals only. Coadministration of IL-12 amplified the anti-CII immune response. CII-specific production of the Th1-type cytokine IFN-γ by *ex vivo* activated $CD4^+$ splenic T cells was increased, and synthesis of the "Th1-dependent" complement-fixing IgG2a and IgG2b antibodies specific for CII was strongly upregulated. In parallel, a severe destructive joint disease developed in such mice. The histological changes observed in the joints of these mice were very similar to those seen in human rheumatoid arthritis. The development of arthritis in IL-12-treated mice was completely prevented by coadministration of a monoclonal antibody against IFN-γ. In parallel, the

IgG2a and IgG2b antibody response was reduced to the level observed in the sera of mice immunized with CII in IFA only. Thus, administration of IL-12 (in an IFN-γ-dependent way) can trigger a Th1-mediated autoimmune joint disease in CII-primed DBA/1 mice *in vivo*. This extends *in vitro* findings describing the generation of pathogen-specific Th1 clones from patients with reactive arthritis (24–26) or patients with rheumatoid arthritis (27, 28), implicating Th1 cells to be involved in the pathogenesis of arthritis. The results described here are also in agreement with a recent report by Simon *et al.* (29), who demonstrated the expression of a Th1-type cytokine pattern in synovial tissues from rheumatoid arthritis patients. The ability of lipopolysaccharide (LPS) from Gram-negative bacteria as well as so far uncharacterized products from Gram-positive bacteria, mycobacteria, and other microorganisms (16, 30) to directly induce the synthesis of IL-12 by macrophages might play a crucial role in the pathogenesis of reactive arthritis or even in rheumatoid arthritis by promoting Th cell differentiation into a Th1-like direction and by up-regulating the synthesis of complement-fixing IgG antibodies. Secretion of IL-12 by macrophages stimulated with LPS would also explain the T-cell dependency of the adjuvant effect of LPS in the model of experimental autoimmune thyroiditis (31). Similar to the results obtained with IL-12 in experimental arthritis, injection of LPS (or CFA) in combination with thyroglobulin induced a destructive thyroiditis, characterized by the production of autoantibodies and infiltration of mononuclear cells into the thyroid gland.

At later stages of autoimmune arthritis, local synthesis of cytokines such as tumor necrosis factor (TNF) is probably responsible for progression of inflammation to a destructive arthritis, because anti-TNF reagents ameliorated arthritis in DBA/1 mice (32, 33). Furthermore, transgenic mice with a deregulated expression of TNF develop chronic destructive polyarthritis (34). Proinflammatory cytokines such as TNF are probably released by macrophages primarily. Panayi *et al.* (35) suggested that in the pathogenesis of rheumatoid arthritis "the T cell is the conductor of an orchestra playing a tune written by the antigen-presenting cell." Regarding this, a Th1 cell might be the best conductor.

Taken together, the experiments described here demonstrate that the development of a Th1-like immune reaction associated with high levels of IgG2a (IgG2b) antibodies is of critical importance for development of severe arthritis in the animal model of CII-induced joint disease. By virtue of its ability to preferentially enhance this response, IL-12 may play a crucial role in the development of autoimmune arthritis and other Th1-type autoimmune diseases such as diabetes in NOD mice, experimental allergic encephalomyelitis, or experimental thyroiditis.

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1. Trentham, D. E., Townes, A. S. & Kang, A. H. (1977) *J. Exp. Med.* **146,** 857–875.
2. Courtenay, J. S., Dallman, M. J., Dayan, A. D., Martin, A. & Mosedale, B. (1980) *Nature (London)* **283,** 666–668.
3. Stuart, J. M., Townes, A. S. & Kang, A. H. (1984) *Annu. Rev. Immunol.* **2,** 199–218.
4. Trentham, D. E. (1982) *Arthritis Rheum.* **25,** 911–916.
5. Nowack, H., Hahn, E. & Timpl, R. (1976) *Immunology* **30,** 29–32.
6. Wooley, P. H., Luthra, H. S., Stuart, J. M. & David, C. S. (1981) *J. Exp. Med.* **154,** 688–700.
7. Holmdahl, R., Rubin, K., Klareskog, L., Larsson, E. & Wigzell, H. (1986) *Arthritis Rheum.* **29,** 400–410.
8. Watson, W. C. & Townes, A. S. (1985) *J. Exp. Med.* **162,** 1878–1891.
9. Durie, F. H., Fava, R. A., Foy, T. M., Aruffo, A., Ledbetter, J. A. & Noelle, R. J. (1993) *Science* **261,** 1328–1330.
10. Seki, N., Sudo, Y., Yoshioka, T., Sugihara, S., Fujitsu, T., Sakuma, S., Ogawa, T., Hamaoka, T., Senoh, H. & Fujiwara, H. (1988) *J. Immunol.* **140,** 1477–1484.
11. Myers, L. K., Rosloniec, E. F., Seyer, J. M., Stuart, J. M. & Kang, A. H. (1993) *J. Immunol.* **150,** 4652–4658.
12. Snapper, C. M. & Paul, W. E. (1987) *Science* **236,** 944–947.
13. Schoenhaut, D. S., Chua, A. O., Wolitzky, A. G., Quinn, P. M., Dwyer, C. M., McComas, W., Familletti, P. C., Gately, M. K. & Gubler, U. (1992) *J. Immunol.* **148,** 3433–3440.
14. Brunda, M. J. (1994) *J. Leukocyte Biol.* **55,** 280–288.
15. Wolf, S. F., Sieburth, D. & Sypek, J. (1994) *Stem Cells* **12,** 154–168.
16. Hsieh, C. S., Macatonia, S. E., Tripp, C. S., Wolf, S. F., O'Garra, A. & Murphy, K. M. (1993) *Science* **260,** 547–549.
17. Schmitt, E., Hoehn, P., Germann, T. & Rüde, E. (1994) *Eur. J. Immunol.* **24,** 343–347.
18. Gately, M. K., Warrier, R. R., Honasoge, S., Carvajal, D. M., Faherty, D. A., Connaughton, S. E., Anderson, T. D., Sarmiento, U., Hubbard, B. R. & Murphy, M. (1994) *Int. Immunol.* **6,** 157–167.
19. Wilde, D. B., Marrack, P., Kappler, J., Dialynas, D. P. & Fitch, F. W. (1983) *J. Immunol.* **131,** 2178–2183.
20. Sarmiento, M., Dialynas, D. P., Laincki, D. W., Wall, K. A., Lorber, M. I., Loken, M. R. & Fitch, F. W. (1982) *Immunol. Rev.* **68,** 139–169.
21. Cherwinski, H. M., Schumacher, J. H., Brown, K. D. & Mosmann, T. R. (1987) *J. Exp. Med.* **166,** 1229–1244.
22. Heinzel, F. P., Schoenhaut, D. S., Rerko, R. M., Rosser, L. E. & Gately, M. K. (1993) *J. Exp. Med.* **177,** 1505–1509.
23. Sypek, J. P., Chung, C. L., Mayor, S. E., Subramanyam, J. M., Goldman, S. J., Sieburth, D. S., Wolf, S. F. & Schaub, R. G. (1993) *J. Exp. Med.* **177,** 1797–1802.
24. Yssel, H., Shanafelt, M. C., Soderberg, C., Schneider, P. V., Anzola, J. & Peltz, G. (1991) *J. Exp. Med.* **174,** 593–601.
25. Schlaak, J., Hermann, E., Ringhoffer, M., Probst, P., Gallati, H., Meyer zum Büschenfelde, K. H. & Fleischer, B. (1992) *Eur. J. Immunol.* **22,** 2771–2776.
26. Lahesmaa, R., Yssel, H., Batsford, S., Luukkainen, R., Mottonen, T., Steinman, L. & Peltz, G. (1992) *J. Immunol.* **148,** 3079–3085.
27. Miltenburg, A. M., van Laar, J. M., de Kuiper, R., Daha, M. R. & Breedveld, F. C. (1992) *Scand. J. Immunol.* **35,** 603–610.
28. Quayle, A. J., Chomarat, P., Miossec, P., Kjeldsen Kragh, J., Forre, O. & Natvig, J. B. (1993) *Scand. J. Immunol.* **38,** 75–82.
29. Simon, A. K., Seipelt, E. & Sieper, J. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 8562–8566.
30. D'Andrea, A., Rengaraju, M., Valiante, N. M., Chehimi, J., Kubin, M., Aste, M., Chan, S. H., Kobayashi, M., Young, D., Nickbarg, E., Chizzonite, R., Wolf, S. F. & Trinchieri, G. (1992) *J. Exp. Med.* **176,** 1387–1398.
31. Esquivel, P. S., Rose, N. R. & Kong, Y-C. M. (1977) *J. Exp. Med.* **145,** 1250–1263.
32. Thorbecke, G. J., Shah, R., Leu, C. H., Kuruvilla, A. P., Hardison, A. M. & Palladino, M. A. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 7375–7379.
33. Williams, R. O., Feldmann, M. & Maini, R. N. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 9784–9788.
34. Keffer, J., Probert, L., Cazlaris, H., Georgopoulos, S., Kaslaris, E., Kioussis, D. & Kollias, G. (1991) *EMBO J.* **10,** 4025–4031.
35. Panayi, G. S., Lanchbury, J. S. & Kingsley, G. H. (1992) *Arthritis Rheum.* **35,** 729–735.