Effects of Interleukin 12 on Immune Responses and Host Protection in Mice Infected with Intestinal Nematode Parasites

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Summary

The cytokine interleukin (IL) 12 stimulates T cell and natural killer cell production of interferon (IFN) γ and inhibits T cell production of IL-4. We investigated the effects of IL-12 on cytokine gene expression, immunoglobulin (Ig)E, mucosal mast cell, and eosinophil responses, and the course of infection in mice inoculated with the nematode parasite *Nippostrongylus brasiliensis*, as well as the IFN-γ dependence of these effects. IL-12 stimulated IFN-γ and IL-10 gene expression during primary and secondary N. brasiliensis infections and inhibited IL-3, IL-4, IL-5, and IL-9 gene expression during primary infections but had little inhibitory effect during secondary infections. IL-12 inhibited IgE, mucosal mast cell, and blood and tissue eosinophil responses during primary infections, but only eosinophil responses during secondary infections. IL-12 enhanced adult worm survival and egg production during primary, but not secondary infections. IL-12 needed to be administered by day 4 of a primary infection to inhibit IgE and mucosal mast cell responses, and by day 6 to strongly inhibit eosinophil responses and to enhance worm survival and fecundity. Anti-IFN-γ mAb inhibited the effects of IL-12 on IgE secretion, intestinal mucosal mastocytosis, and parasite survival and fecundity, but did not affect IL-12 inhibition of eosinophilia. These observations indicate that IL-12, if administered during the initiation of an immune response, can change the response from one that is characterized by the production of T helper (Th)2-associated cytokines to one characterized by the production of Th-1 associated cytokines. However, IL-12 treatment has less of an effect once the production of Th2-associated cytokines has become established. In addition, our results provide evidence that Th2-associated responses protect against, and/or Th1-associated responses exacerbate, nematode infections.

The nature of an immune response is defined not only by its specificity but also by the effector mechanisms that are induced. The selection of effector mechanisms is controlled, to a large extent, by the cytokines that are produced during the response. In the mouse, Th1-associated cytokines (IL-2, IFN-γ, and TNF-β) favor NK cell, CTL, and complement-fixing antibody (IgG3 and IgG2a) responses whereas Th2-associated cytokines (IL-4, IL-5, IL-9, and IL-10) favor noncomplement-fixing antibody (IgG1) and allergy-associated responses (IgE, mast cells, and eosinophils) (1, 2). T cell cytokine responses, in turn, are regulated by cytokines that can be produced early in an immune response by non-T cells (3–8). Recently the cytokine IL-12, which is produced by macrophages and B lymphocytes (6, 9, 10), has been found to induce Th1-associated responses by stimulating T cell and NK cell production of IFN-γ (4–6, 11) and by inhibiting T cell production of IL-4 (4–6). Stimulation of IFN-γ production and suppression of IL-4 production by IL-12 has been shown to enhance protective immunity against some intracellular parasites, such as *Leishmania major* (12, 13), to promote tumor immunity (14), and to suppress in vitro and in vivo IgE responses (15, 16). These observations suggest that

IL-12 may be clinically useful in the treatment of infectious diseases, cancer, and allergic disorders.

We wished to extend these observations by investigating: (a) whether IL-12 could inhibit the development of eosinophilia and mastocytosis as well as IgE production during a primary response; (b) whether IL-12 needed to be present at the initiation of the response to alter its course; (c) whether IL-12 could inhibit these phenomena during a secondary response; (d) whether IL-12 administration during a primary response would influence the characteristics of a subsequent secondary response; (e) whether IL-12 might inhibit protective immunity to infectious agents that typically induce a Th2-type cytokine response; and (f) whether IFN-γ was a critical mediator of the effects of IL-12 that we were studying. To investigate these issues we inoculated BALB/c mice with infective larvae of the nematode parasite, Nippostrongylus brasiliensis, which stimulates IL-3, IL-4, IL-5, and IL-9 cytokine production that induce IgE, eosinophil, and mast cell responses (17–20, and Madden, K., unpublished data). The results of our studies provide evidence that IL-12 is more effective at influencing a developing immune response than an established response, that eosinophil responses are more sensitive than mast cell or IgE responses to the inhibitory effects of IL-12, that IL-12 administered during a primary response can influence a subsequent secondary response, that IL-12 can exacerbate nematode infections, and that IFN-γ is a critical mediator of most of these effects of IL-12 in nematode-infected mice.

Materials and Methods

Animals. Female BALB/c mice were purchased from the Small Animals Division of the National Cancer Institute (Frederick, MD) and were used at age 6–12 wk. Experimental groups contained five mice each.

Immunological Reagents. Recombinant murine IL-12 was produced in serum-free medium by transfected CHO cells and purified by sequential chromatography on Q Sepharose Fast Flow (Pharmacia, Piscataway, NJ), Cellufine Sulfate (Amicon, Beverly, MA), and POROS 1 20 PE (PerSeptive Biosystems, Cambridge, MA) columns (21). IL-12 was diluted in PBS containing 1% BALB/c serum and was injected intraperitoneally in 0.1 ml. A rat IgG1 mAb that neutralizes IFN-γ (XMG-6) (22, 23) and a rat IgG2a mAb that binds mouse IgE (EM-95) (24) were produced in Pristane-primed nude mice and purified as previously described (22). A mouse IgE anti-TNP mAb (SPE-iv-7) (25), and a mouse IgG anti-FITC mAb (CG5, generated by Dr. Diana Goroff in the Department of Medicine, Uniformed Services University of the Health Sciences) were produced in Pristane-primed BALB/c mice and were purified by the same techniques. Rabbit anti–mouse IgE and goat anti–rabbit IgG antibodies were produced, absorbed, and affinity purified as described (26, 27). Sheep anti–mouse IgG1 antiserum was purchased from The Binding Site (San Diego, CA).

Eosinophil Counts. Eosinophils were counted from fresh blood samples with the Unopette test (Becton Dickinson & Co., Rutherford, NJ).

Evaluation of Mucosal Mast Cell Number. Tissue samples from 8–10-cm mid-jejunal segments from individual mice were prepared by the Swiss-roll technique, fixed in Carnoy's fixative, paraffin embedded, and processed for staining with Alcian blue and safranin (18). After a random selection of the first microscopic field (at a

magnification of 397), a total of 50 neighboring fields of a given section of rolled jejunum was scanned for mucosal mast cells (MMC)[1]. MMC were identified both in the villi and the crypts by their distinct intracellular granular staining with Alcian blue (18). All samples were evaluated blindly.

Histological Evaluation of Pulmonary Lesions. Lungs were fixed by intratracheal injection of Bouin-Hollande fixative and processed routinely. Paraffin sections were stained with Litt's modification of the Dominici stain (28). Sections were evaluated blindly and scored for the intensity of perivascular infiltrates and the approximate percentage of eosinophils in the infiltrates (29).

Parasites. N. brasiliensis was maintained and passed alternately in BALB/c and SW mice; infections were established by subcutaneous inoculation with 700 infective third-stage larvae. Infective third stage larvae were isolated with a Baermann apparatus after 7–10 d of culture, washed in saline, counted, and stored at 4°C until use. Mice were inoculated orally with 200 infective larvae, using a ball-tipped feeding tube. Parasite egg numbers were determined from group samples of feces collected for 1 h, while adult worm numbers of each sex were evaluated microscopically from individual mice after opening the small intestines with an enterotome. Aliquots of the contents of the small and large intestines and cecum were processed to determine the total number of eggs in each mouse. Worm fecundity was expressed by dividing the total number of eggs recovered from each mouse by the total number of female worms. In some experiments mice were cured of N. brasiliensis infection by treatment with a single oral dose of pyrantel pamoate (Strongid T; Pfizer Diagnostics, New York) administered through a feeding tube.

Antibody Assays. Serum IgG1 was quantitated by radial immunodiffusion, using a purified IgG1 mAb, CG5, as a standard. Serum IgE was quantitated by ELISA (30), using a purified IgE mAb, SPE-iv-7 (25), as a standard.

Isolation and Purification of RNA. RNase-free plastic and water were used throughout. Peyer's patch and mesenteric lymph node were homogenized in RNAzol (Tel-Test, Friendswood, TX) with a polytron (model PT3000; Brinkmann Instruments, Inc., Westbury, NY). Total RNA was isolated and quantitated as described (31). Purified RNA (10 µg) was electrophoresed on a 1% agarose gel containing ethidium bromide to check concentration and to verify that it was intact.

RT/PCR. A coupled RT/PCR was used to quantitate tissue RNA levels (31). Briefly, the RNA sample was reverse-transcribed with Superscript RT (GIBCO BRL, Gaithersburg, MD) and cytokine-specific primers and probes were used to amplify selected cytokines. For each cDNA product, the optimum number of cycles for PCR amplification was determined experimentally and was defined as the number of cycles that would achieve a detectable concentration well below saturating conditions (i.e., the quantity of cytokine PCR product was shown to vary linearly with the quantity of input cytokine mRNA). Relative concentrations of IFN-γ, IL-3, IL-4, IL-5, IL-9, and IL-10 mRNA were determined. Primers for the "housekeeping gene," hypoxanthine-guanine phosphoribosyltransferase (HPRT), were used in each experiment to verify that equal amounts of RNA were added in each PCR. All cytokine values were individually normalized to the corresponding HPRT values. Amplified PCR product was detected by Southern blot analysis (17), and the resultant signal was quantitated with a Phosphorimager (Molecular Dynamics, Sunnyvale, CA), which uses a phosphor screen instead of film to detect radioactive signals on the Southern blot.

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[1] *Abbreviation used in this paper:* MMC, mucosal mast cell.

Results

IL-12 Inhibits IgE, Eosinophil, and Intestinal MMC Responses during a Primary N. brasiliensis Infection. To determine the effects of IL-12 on allergy-associated responses during a primary immunization, mice were inoculated with 700 *N. brasiliensis* third-stage larvae and injected intraperitoneally with vehicle or with 100 or 1,000 ng/d of IL-12. Based on preliminary toxicity studies, 5 d of IL-12 treatment was followed by 2 d without IL-12 in this and in most subsequent experiments. Mice were killed 13 d after parasite inoculation and blood eosinophil levels, intestinal mucosal mastocytosis, and serum IgE levels were evaluated (Fig. 1). IL-12 at both doses completely inhibited eosinophil responses. 1,000 ng/d of IL-12 also completely inhibited the IgE and MMC responses, whereas 100 ng/d of IL-12 inhibited the IgE response by ~90% and the intestinal MMC response by ~60%.

IL-12 Inhibition of N. brasiliensis–induced IgE and Intestinal MMC Responses is IFN-γ Dependent. To determine the IFN-γ dependence of IL-12 inhibition of *N. brasiliensis*–induced IgE, intestinal MMC, and eosinophil responses, mice inoculated with *N. brasiliensis* received no additional treatment or were injected daily with 1,000 ng of IL-12 ± 2 mg/wk of anti-IFN-γ mAb. 14 d after parasite inoculation, IL-12 treatment
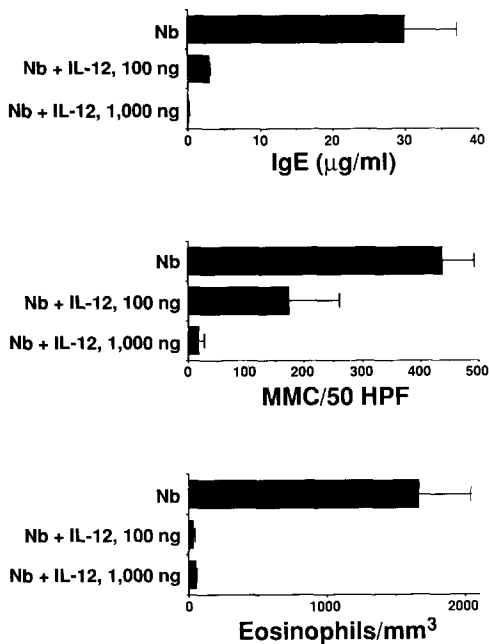

Figure 1. IL-12 suppresses IgE production, eosinophilia, and intestinal mucosal mastocytosis during a primary *N. brasiliensis* infection. BALB/c mice were inoculated subcutaneously with 700 *N. brasiliensis* third-stage larvae. Mice were injected intraperitoneally on days 0–4 and 7–11 with saline, 100 ng of IL-12, or 1,000 ng of IL-12/d. Mice were bled and killed 13 d after inoculation. Serum IgE levels, blood eosinophil counts, and numbers of intestinal mucosal mast cells/50 high-powered microscope fields were determined on samples from individual mice as described in Materials and Methods. Geometric means and standard errors are shown for IgE levels; arithmetic means and standard errors are shown for eosinophil and mast cell counts. Similar results were obtained in several additional experiments.
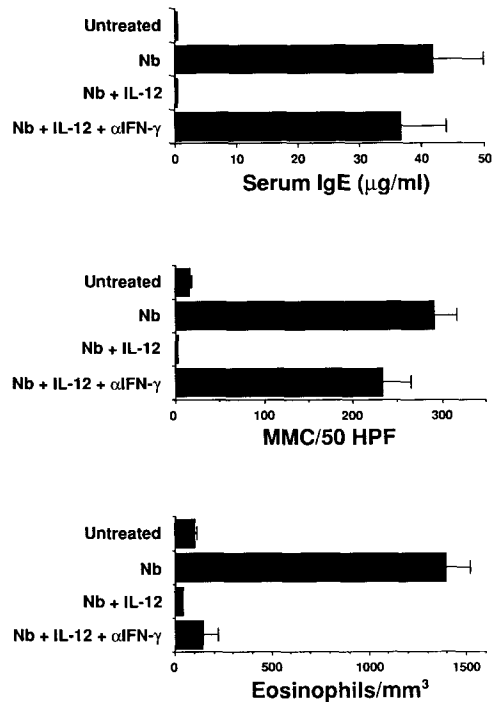

Figure 2. IFN-γ dependence of the effects of IL-12 on allergy-associated responses to *N. brasiliensis* infection. Mice were left untreated or were inoculated with *N. brasiliensis* as in Fig. 1 and were injected with 1% BALB/c serum in saline or with 1,000 ng/d of IL-12 in the same vehicle on days 0–4 and 7–11. Some mice received 2 mg of rat anti–mouse IFN-γ mAb or an isotype-matched control mAb intravenously on days 0 and 7. Mice were bled and killed 14 d after the *N. brasiliensis* inoculation. Effects of IL-12 and anti-IFN-γ mAb on blood eosinophil counts were determined in a separate experiment that followed the same protocol, with the exception that the dose of IL-12 was reduced to 100 ng/day.

was found to strongly inhibit the *N. brasiliensis*–induced IgE, intestinal MMC, and eosinophil responses (Fig. 2). Although treatment with anti-IFN-γ mAb reversed IL-12 inhibition of the IgE and mast cell responses, the combination of IL-12 and anti-IFN-γ mAb still suppressed blood eosinophilia by >90%. Anti-IFN-γ mAb had similar effects on IgE and eosinophil responses in an experiment in which an IL-12 dose of 100 ng/d was used (data not shown). Examination of lungs demonstrated that *N. brasiliensis* induced eosinophil-rich perivascular infiltrates. No eosinophils were observed in the infiltrates of mice treated with 1,000 ng/d of IL-12, and the size of these pulmonary infiltrates was substantially reduced (Fig. 3). Anti-IFN-γ mAb reversed IL-12 inhibition of infiltrate size, but had little effect on IL-12 inhibition of infiltrate eosinophil content.

Effects of Delaying IL-12 Treatment on IgE, Intestinal MMC, and Eosinophil Responses to N. brasiliensis Inoculation. To determine how soon IL-12 needed to be administered after mice were inoculated with *N. brasiliensis* to inhibit IgE, eosinophil, and intestinal MMC responses, experiments were performed in which treatment with IL-12 (1,000 ng/d) was initiated at the time of, or 2, 4, 6, or 8 d after parasite inoculation and IgE, eosinophil, and intestinal MMC responses were evaluated 13 d after inoculation. IL-12 treatment initiated at 4 d
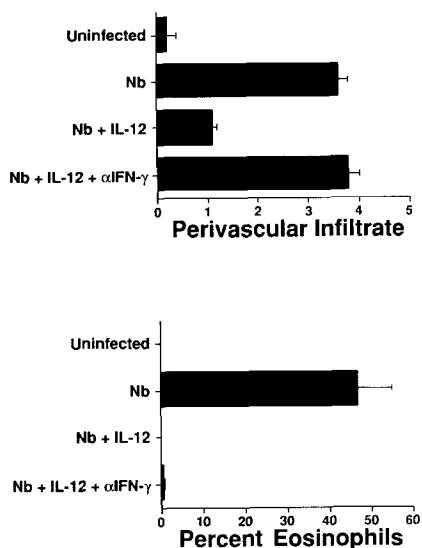
Figure 3. IL-12 inhibits tissue eosinophilia in response to *N. brasiliensis* infection. In the same experiment depicted in Fig. 2, in which 1,000 ng of IL-12 was injected per day, lungs were excised, fixed, and stained, and pulmonary perivascular infiltrate size and eosinophil content were evaluated. Arithmetic means and standard curves are shown.
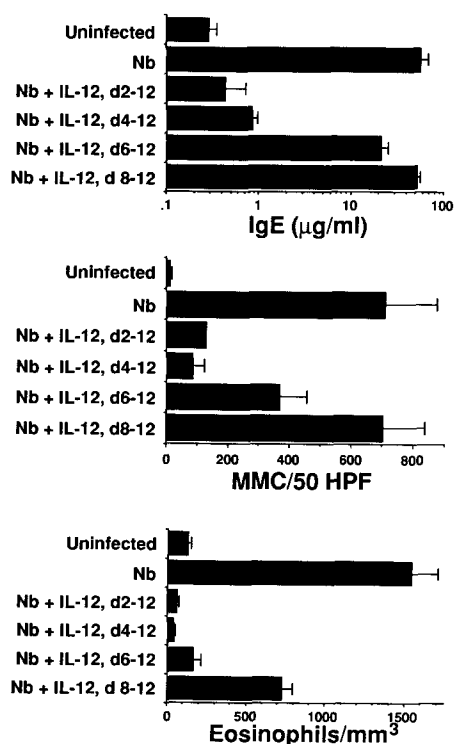
still inhibited these responses by >90% (Fig. 4). IL-12 treatment no longer inhibited the IgE response, and only partially inhibited the mast cell response if initiated at 6 d, but, in contrast, still inhibited the eosinophil response by >90%, and initiation of IL-12 treatment 2 d later still inhibited blood eosinophilia by ~50%.

Effect of IL-12 on IgE, Eosinophil, and Mast Cell Responses to a Second N. brasiliensis Infection. The inability of IL-12 to block IgE and intestinal MMC responses to a primary *N. brasiliensis* infection when IL-12 administration was initiated 6 d after parasite inoculation suggested that IL-12 might also fail to inhibit these responses if administered for the first time during a second infection. To examine this possibility, mice were reinoculated with *N. brasiliensis* 33 d after an initial inoculation. Treatment of these mice with 1,000 ng/d of IL-12, starting on the day of reinoculation with *N. brasiliensis*, failed to inhibit IgE or intestinal MMC responses in these mice, but inhibited the blood eosinophil response to the second infection by >90% (Fig. 5). The presence of eosinophils in perivascular pulmonary infiltrates was similarly inhibited by IL-12 (data not shown). However, whereas anti-IFN-γ mAb had little effect on IL-12 inhibition of eosinophilia during a primary *N. brasiliensis* infection, it con-
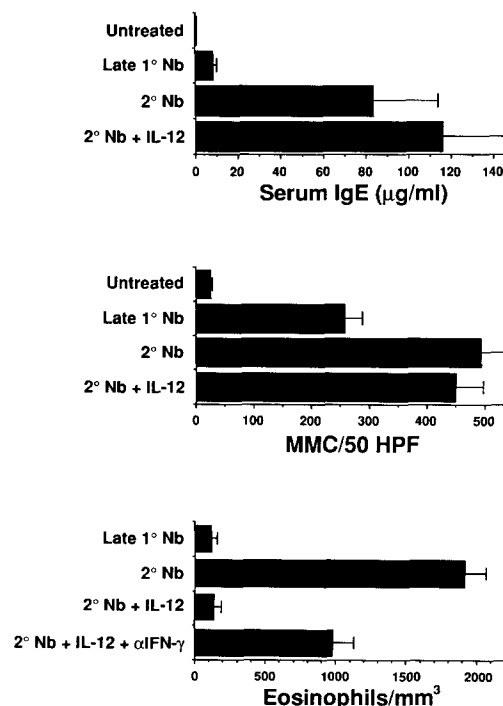

Figure 4. Effects of delayed initiation of IL-12 treatment on IgE, MMC, and eosinophil responses in *N. brasiliensis*-infected mice. BALB/c mice that were inoculated with *N. brasiliensis* larvae received either no further treatment or daily intraperitoneal injections of 1,000 ng of IL-12 that started 2, 4, 6, or 8 d after inoculation. Mice were bled and killed 13 d after worm inoculation and serum IgE levels, numbers of intestinal mucosal mast cells per 50 microscope high-power fields, and blood eosinophil counts were determined. Means and standard errors are shown.

Figure 5. Effects of IL-12 on the IgE, MMC, and blood eosinophil responses to a secondary *N. brasiliensis* infection. BALB/c mice were left untreated or were infected with *N. brasiliensis* larvae. Some mice (*late 1° Nb*) received no further treatment; others (*2° Nb*) were reinoculated with *N. brasiliensis* larvae 33 d after the initial inoculation. Some reinoculated mice were treated with 1,000 ng/d i.p. of IL-12, starting on the day of the second inoculation with *N. brasiliensis*, and some of these mice were also injected intravenously with 2 mg/wk of rat anti–mouse IFN-γ mAb. Mice were bled and killed 13 d after the second inoculation. Means and standard errors are shown.
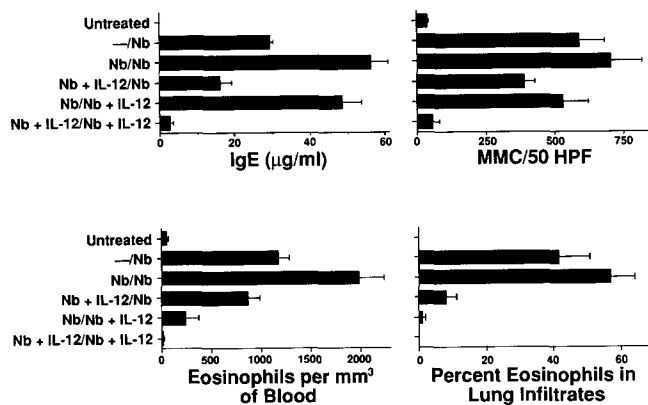
Figure 6. Treatment with IL-12 during a primary *N. brasiliensis* infection inhibits IgE, MMC, and eosinophil responses during the second infection. BALB/c mice were left untreated or were inoculated with *N. brasiliensis* larvae. Two groups of infected mice were treated were 1,000 ng/d i.p. of IL-12 for 9 d. Infected mice were treated with pyrantel pamoate to cure residual infection 9 d after the initial inoculation and were reinoculated with *N. brasiliensis* 26 d later. At the time of the second worm inoculation, IL-12 treatment (1,000 ng/d) was restarted for one group of mice that had received IL-12 during the primary infection and was initiated for one group of mice that had not initially received IL-12. One group of mice that was originally left untreated was given a primary inoculum of *N. brasiliensis* at this time. Mice were bled and killed 11 d later. Serum IgE levels, blood eosinophil counts, percentages of eosinophils in pulmonary perivascular infiltrates, and intestinal MMC numbers were determined. A diagonal line (/) is used in this figure to separate treatment given during primary and secondary infections.

siderably decreased the ability of IL-12 to block eosinophilia during a second *N. brasiliensis* infection. The differential effects of IL-12 on IgE and eosinophil responses during primary and secondary nematode infections were also seen in mice inoculated with another intestinal nematode, *Heligmosomoides polygyrus* (data not shown).

Treatment of Mice with IL-12 during a Primary N. brasiliensis Infection Decreases IgE, MMC, and Eosinophil Responses to a Second Infection. The failure of IL-12 to block IgE and MMC responses to a second *N. brasiliensis* infection might reflect either different susceptibilities of virgin or memory T cells to the effects of IL-12 or irreversible programming of T cell cytokine responses that occurred during the initial infection. To distinguish between these possibilities we examined whether allergy-associated responses during a second *N. brasiliensis* infection could be inhibited by treating mice with IL-12 during a primary infection or during both primary and secondary worm infections. Because IL-12 inhibits worm expulsion during a primary *N. brasiliensis* infection (see below), primary infections were terminated by treating mice with pyrantel pamoate. IL-12 treatment during a primary *N. brasiliensis* infection inhibited the IgE response to a challenge infection by ~70%, the intestinal MMC response to a challenge infection by ~45%, the blood eosinophil response to a challenge infection by ~55%, and the pulmonary eosinophil response to a challenge infection by ~85%. IL-12 treatment during both primary and secondary *N. brasiliensis* responses blocked the eosinophil, intestinal MMC, and IgE responses to the second *N. brasiliensis* infection by >95% (Fig. 6). In the same experiment, IL-12 treatment that was initiated at the time of the challenge infection inhibited eosinophil responses, but did not significantly inhibit IgE or MMC responses.

Effects of IL-12 on Cytokine Responses to Primary and Secondary N. brasiliensis Infections. Inasmuch as IL-4 is required for the generation of IgE responses (32, 33), IL-5 for the generation of eosinophil responses (34, 35), and IL-3, IL-4, and IL-9 all contribute to the generation of intestinal MMC responses in nematode-infected mice (18, and Madden, K., unpublished observations), our observations suggested that
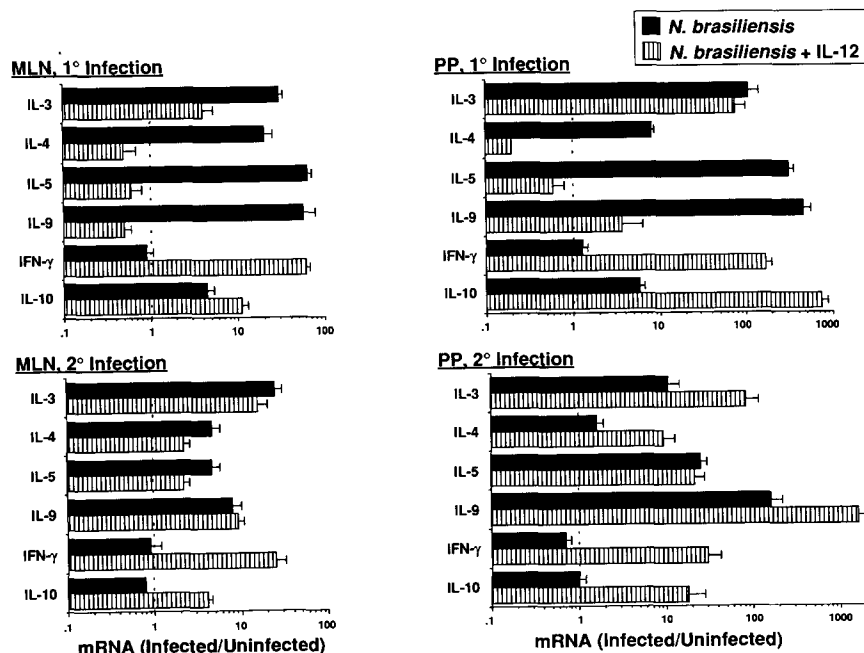

Figure 7. Effect of IL-12 on cytokine gene expression in *N. brasiliensis*–inoculated mice. BALB/c mice were left untreated or were inoculated with *N. brasiliensis* larvae. Some mice were killed 8 d after inoculation; some of these received 1,000 ng/d i.p. of IL-12 (5 d/wk), starting at the time of parasite inoculation. Some mice were reinoculated 29 d after the initial inoculation and killed 5 d after the second inoculation. Some of these mice received 1,000 ng/d i.p. of IL-12 (5 d/wk), starting at the time of the second inoculation. RNA was prepared from mesenteric lymph nodes (*MLN*) and Peyer's patch (*PP*) of killed mice and reverse transcribed. cDNA was amplified by PCR, using HPRT, IL-3, IL-4, IL-5, IL-9, IL-10, and IFN-γ–specific primer pairs. PCR products were electrophoresed and quantitated by Phosphorimager analysis of Southern blots hybridized with labeled, cytokine-specific probes. All mRNA levels are expressed relative to the mean levels found in the same organs of uninfected mice, which are arbitrarily given a value of 1, and are normalized for differences in HPRT mRNA levels. Means and standard errors are shown.
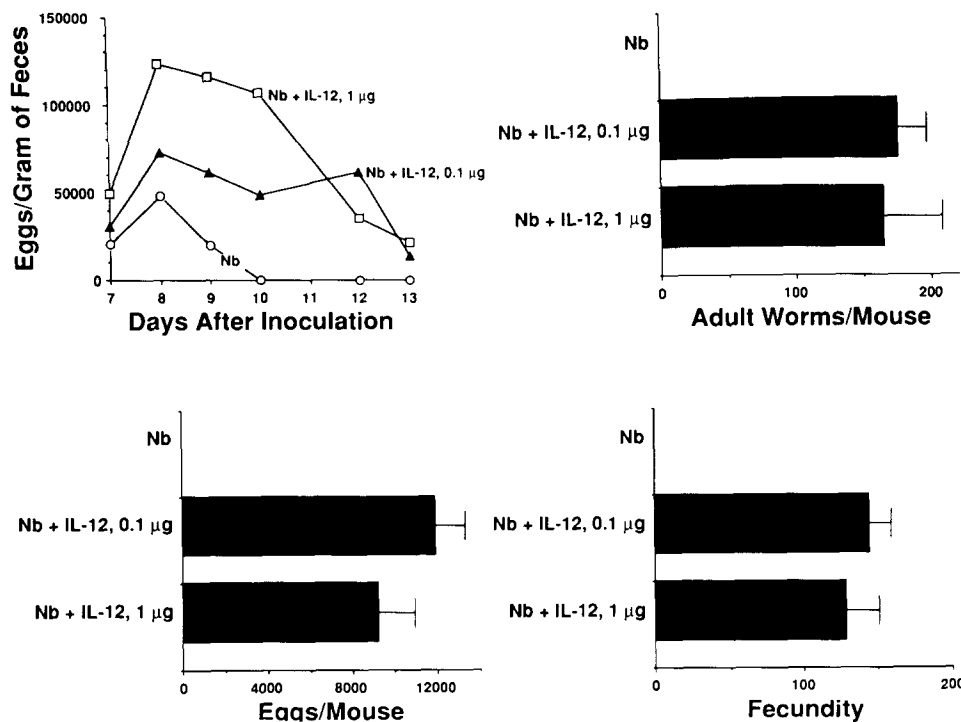
Figure 8. IL-12 suppress protective immunity to N. *brasiliensis*. The effects of treating N. *brasiliensis*-inoculated mice with 100 or 1,000 ng/d i.p. of IL-12 on egg production (determined by daily quantitation of eggs per gram of feces), and on adult worm numbers, parasite eggs per mouse, and worm fecundity 13 d after inoculation were determined in the same experiment shown in Fig. 1. Determination of eggs per gram of feces were made on pools of feces from five mice. Other determinations were made with individual mice and are depicted as arithmetic means ± standard errors.

IL-12 suppresses the production of these cytokines during primary, but not secondary, N. *brasiliensis* infections. To test this hypothesis, we quantitated IL-3, IL-4, IL-5, IL-9, IL-10, and IFN-γ mRNA levels in mesenteric lymph node and Peyer's patch 8 d after primary or 5 d after secondary N. *brasiliensis* infections. These time points and organs were chosen because they were the times and sites of peak cytokine gene expression (17, and Madden, K., unpublished data). IL-12 treatment during a primary N. *brasiliensis* infection constrained IL-4, IL-5, and IL-9 gene expression to below or near baseline levels in both mesenteric lymph node and Peyer's patch and significantly decreased the level of IL-3 mRNA in mesenteric lymph node, but not Peyer's patch (Fig. 7). In contrast, during a secondary N. *brasiliensis* infection IL-12 treatment had little effect on mesenteric lymph node expression of these cytokine genes and enhanced IL-3, IL-4, and IL-9 gene expression in Peyer's patch. As has been observed in other systems (16), IL-12 enhanced IFN-γ and IL-10 gene expression during both primary and secondary N. *brasiliensis* infections.

Effect of IL-12 on the Course of a Nematode Infection. In the same experiments shown above we examined the effect of IL-12 on adult worm survival and egg production (fecundity). IL-12 (either 100 or 1,000 ng/d) increased egg production and suppressed adult worm expulsion in N. *brasiliensis*-infected mice (Fig. 8). These effects were still substantial if IL-12 administration was delayed until 6 d after N. *brasiliensis* inoculation, but were limited or neglible if IL-12 treatment was started 8 d after inoculation (Fig. 9 and data not shown). Anti-IFN-γ mAb blocked most of the IL-12–induced increases in fecundity and adult worm survival (Fig. 10). During a second N. *brasiliensis* infection, no nematode eggs were detectable in mouse feces 8 d after inoculation and no adult
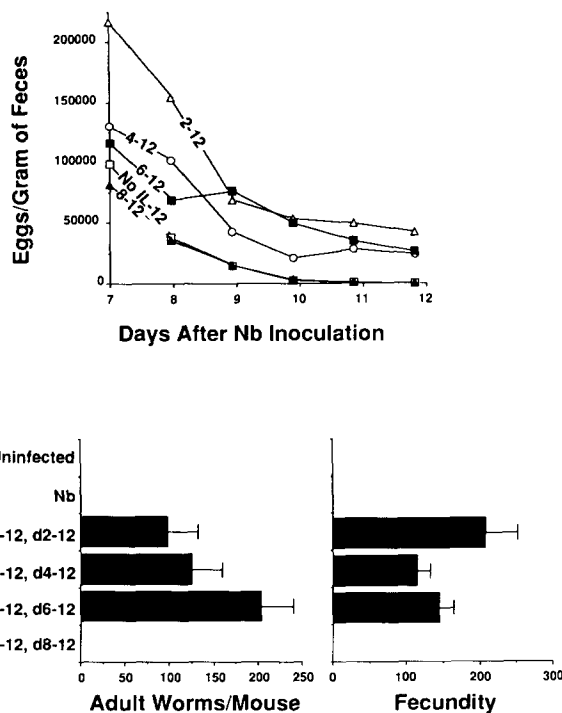

Days After Nb Inoculation

Figure 9. Effects of delayed initiation of IL-12 treatment on egg production and adult worm survival in N. *brasiliensis*-inoculated mice. The ability of IL-12 (1,000 ng/day) to enhance parasite egg production and adult worm survival when initiated 2–8 d after worm inoculation was studied in the same experiment shown in Fig. 4. Numbers of eggs per gram of feces and adult worms and worm fecundity were determined as described in Fig. 8.
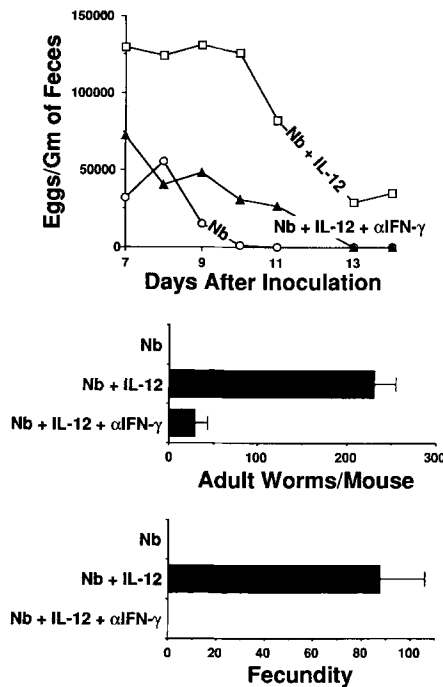
Figure 10. IFN-γ dependence of IL-12 effects on adult worm survival and fecundity in *N. brasiliensis*-inoculated mice. Anti-IFN-γ mAb inhibition of the effects of IL-12 on adult worm survival and egg production during a primary *N. brasiliensis* infection was studied in an experiment similar to that shown in Fig. 2. An IL-12 dose of 100 ng/d i.p. was used. Numbers of eggs/gram of feces and adult worms and worm fecundity were determined as described in Fig. 8.

Figure 11. IL-12 treatment during 1° and 2° *N. brasiliensis* infections inhibits protective immunity. The effects of IL-12 treatment on *N. brasiliensis* adult survival and egg production were studied in the experiment shown in Fig. 6. Eggs per gram of feces were determined 8 d after the time of the second infection. Mice were killed and adult worm numbers and worm fecundity were determined 3 d later. Numbers of eggs per gram of feces and adult worms and worm fecundity were determined as described in Fig. 8. Pyrantel pamoate treatment was used to terminate primary infections even in mice that were receiving IL-12.

worms were present in mouse intestines 11 d after inoculation (Fig. 11). Treatment with IL-12 during a primary infection prolonged egg production during a challenge infection but did not prevent worm expulsion by 11 d after challenge inoculation. IL-12 treatment that was restricted to a second *N. brasiliensis* infection prolonged adult worm survival but only slightly increased egg production, whereas IL-12 treatment during both primary and second *N. brasiliensis* infections allowed adult worms to survive and produce large numbers of eggs during the second infection.

Discussion

The results of our experiments demonstrate that the IgE, intestinal MMC, and eosinophil responses to *N. brasiliensis* are all completely suppressed by IL-12 during a primary infection. IL-12 also completely suppresses *N. brasiliensis* induction of increased gene expression of the Th2-type cytokines that are associated with these responses: IL-4, IL-5, and IL-9. In addition, gene expression of IL-3, a cytokine associated with both Th1 and Th2 responses (1), but which contributes to the generation of intestinal mucosal mastocytosis (18), is considerably inhibited in mesenteric lymph node by IL-12 treatment. IL-12 suppression of allergy-associated responses is not diminished when IL-12 administration is initiated 4 d after *N. brasiliensis* inoculation. However, the IgE and mast cell responses are no longer suppressed if IL-12 treatment is
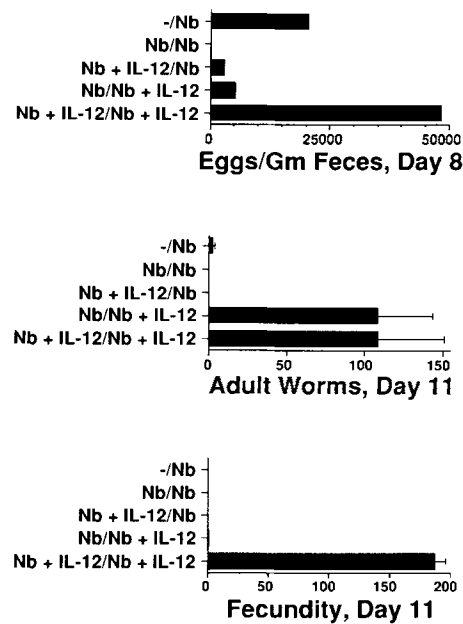
initiated at 6 d and suppression of the eosinophil responses is considerably reduced when IL-12 treatment is delayed for 8 d. These observations and the findings that considerable increases in IL-3, IL-4, and IL-9 mRNA levels in gut-associated lymphoid organs are first observed 4 d after *N. brasiliensis* inoculation and that IL-5 mRNA levels peak ∼8 d after inoculation (Madden, K., unpublished data) suggest that IL-12 loses its ability to suppress the production of allergy-associated cytokines since the secretion of these cytokines has become established. This suggestion is consistent with the inability of IL-12 to suppress the secretion of allergy-associated cytokines during a second *N. brasiliensis* infection. Although IL-12 fails to suppress Th2-associated cytokine production under these circumstances, it induces a large increase in IFN-γ mRNA and IFN-γ secretion, as demonstrated by increased spleen cell expression of an IFN-γ–induced surface marker (Ly6A/E [36], data not shown). These observations are all consistent with the observation by Manetti et al. (37) that IL-12 does not inhibit the secretion of IL-4 by cloned human Th2 cells, but induces these cells to secrete IFN-γ.

An alternate possible explanation for the failure of IL-12 to suppress Th2-associated cytokine production during a second *N. brasiliensis* infection is that autocrine IL-4 production during the second infection blocks the inhibitory effects of IL-12. This possibility is compatible with evidence that exogenous IL-4 prevents IFN-γ from suppressing the differentiation of mouse T cells into Th2 cells in vitro (5, 6), but

probably does not explain our observations, because treatment of mice with anti-IL-4 receptor mAb during a challenge infection with the nematode parasite *H. polygyrus* does not promote IL-12 inhibition of IL-4, IL-5, and IL-9 gene expression (Finkelman, F., J. Urban, and W. Gause, unpublished data). An alternate explanation for the failure of IL-12 to suppress Th2-associated cytokine production during a second *N. brasiliensis* infection is that these cytokines are not being produced by T cells but by FcεRI-bearing cells that have had *N. brasiliensis*-specific IgE on their Fcε receptors cross-linked by *N. brasiliensis* antigens (38–40). FcεRI$^+$ cells that secrete Th2-associated cytokines in response to FcεRI cross-linking increase in number during *N. brasiliensis* infections (39); IL-12 is not known to have any suppressive effects on cytokine production by these cells. We cannot rule out this possibility but believe that it is less likely than the possibility that T cells are the primary producers of Th2-associated cytokines during a second *N. brasiliensis* infection because: (*a*) IL-12 failed to suppress Th2-associated cytokine gene expression in mesenteric lymph node in our experiments and cytokine-producing FcεRI$^+$ cells have not been identified in this organ (39); and (*b*) CD4$^+$ T cells are known to become activated during a second *N. brasiliensis* infection and are required for the generation of an IgE response (41).

Although IL-12 failed to inhibit Th2-associated cytokine gene expression, IgE secretion, and intestinal mucosal mastocytosis during a second *N. brasiliensis* infection, it strongly blocked the blood and tissue eosinophil responses. This suggests that IL-12 inhibits eosinophil responses even when eosinophil-stimulating cytokines are present. This effect of IL-12 is not most likely mediated by IFN-γ, because: (*a*) IFN-γ blocks cytokine stimulation of the in vitro generation of eosinophils from precursors (42); (*b*) IFN-γ inhibits the eosinophil response to a primary *N. brasiliensis* infection (29) and to a secondary response to OVA (43); and (*c*) the ability of IL-12 to prevent the eosinophil response to a second *N. brasiliensis* infection is blocked, to a considerable extent, by anti-IFN-γ mAb. IL-12 inhibition of eosinophilia during a primary *N. brasiliensis* infection, on the other hand, may be IFN-γ independent, since it is not inhibited by anti-IFN-γ mAb. This apparent IFN-γ independence of IL-12 inhibition of the eosinophil response differs from the IFN-γ dependence of IL-12 inhibition of the IgE and MMC responses to a primary *N. brasiliensis* infection. It may be more apparent than real, inasmuch as studies in another in vivo system have suggested that anti-IFN-γ mAb neutralization of IL-12–induced IFN-γ is incomplete (16) and IFN-γ is more potent at suppressing the eosinophil response than either the IgE or MMC response to a primary *N. brasiliensis* infection (29). Alternatively, IL-12 may directly suppress IL-5 expression or eosinophil production, or stimulate the production of mediators other than IFN-γ that have these effects. Experiments with mice that lack a functional IFN-γ (44) or IFN-γ receptor gene (45) will be needed to settle this issue definitively.

The IFN-γ dependence of IL-12 inhibition of IgE and mast cell responses during a primary *N. brasiliensis* infection suggests that IL-12 inhibition of the cytokines that induce these responses, IL-3, IL-4, and IL-9, is also IFN-γ dependent. This is in apparent conflict with the demonstration that IL-12 inhibition of the IL-4 and IgE responses induced in mice by anti-IgD antibody appears to be partially IFN-γ independent (16), as well as the demonstration of IFN-γ–independent IL-12 inhibition of human in vitro IgE responses (15). Further studies will be necessary to determine whether the apparent ability of IL-12 to directly inhibit Th2 cytokine responses and allergy-associated responses in the absence of IFN-γ in some systems is real or an artifact of incomplete neutralization of IFN-γ.

Our observations have practical significance. Most importantly, they indicate that IL-12 inhibits the production of Th2-associated cytokines and stimulates Th1-associated cytokine production in vivo even for immunogens that normally induce powerful Th2 responses. Furthermore, our observations suggest that Th2-associated cytokine responses to a second exposure to the same immunogen can be at least partially suppressed if IL-12 is administered during a primary immunization and completely suppressed if IL-12 is administered during both primary and secondary immunizations. However, IL-12 fails to inhibit Th2-associated cytokine responses once they have developed, and, with the important exception of eosinophil responses, fails to inhibit secondary allergy-associated responses. Thus, IL-12 may be more useful as an adjuvant for the induction of Th1 responses during initial immunization than for converting established Th2 responses to Th1 responses. However, inasmuch as IL-12 induces IFN-γ secretion by Th2 cells and established in vivo IgE responses have been reversed by IFN-γ in other systems (46), IL-12 may be able to modify established Th2 or IgE responses that are induced by less potent stimuli than *N. brasiliensis* infection.

In addition to defining the limits of IL-12 modulation of in vivo cytokine responses, our results indicate that IL-12, which has been shown to make mice less susceptible to some intracellular parasites, can increase their susceptibility to intestinal helminths. This effect of IL-12 is probably mediated by IFN-γ, since it was greatly inhibited by anti-IFN-γ mAb. Treatment of *N. brasiliensis*-inoculated mice with IFN-γ increases worm fecundity and survival (29) and endogenous IFN-γ production suppresses protective immunity to the nematode parasite *Trichuris muris* (47) in some mouse strains. Studies performed with *N. brasiliensis*-inoculated mice suggest that IFN-γ suppression of immunity to helminths is not mediated only by inhibition of the production of known Th2-associated cytokines, since antibodies to IL-3, IL-4, IL-5, and IL-9 have little or no effect on the course of a *N. brasiliensis* infection (18, 48, and Urban, J. F., Jr., unpublished data). Regardless of the mechanisms involved, our observations suggest that IL-12 would be dangerous to use in rodents that have been exposed to parasitic helminths. Additional studies are needed to determine if the same concerns are valid about use of this cytokine in humans.

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