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## Research Article

The effect of anti-interleukin (IL-12) treatment on Lyme borreliosis in C3H/HeN (C3H) mice was assessed because other studies have implicated CD4<sup>+</sup> T cell helper (Th) type 1 responses in the genesis of disease caused by *Borrelia burgdorferi*. Infection of inbred mice with *B. burgdorferi* results in varying degrees of arthritis: BALB/c mice develop mild disease and C3H mice develop severe arthritis that is most pronounced 2-4 wk after infection. Since IL-12 is a major inducer of Th1 responses, we blocked this cytokine in vivo in *B. burgdorferi* infected C3H mice, and evaluated the effects of treatment on the development of arthritis at the peak of acute joint inflammation (14 d) and in the resolution phase (60 d) of disease. As expected, intraperitoneal administration of an anti-IL-12 monoclonal antibody (mAb) to C3H mice resulted in a decrease in both IFN-gamma and *B. burgdorferi*-specific IgG2a in serum, indicative of diminished Th1 responses. No IL-4 production was detected in serum of anti-IL-12 mAb treated or control mice. IgG1 and IgG2b levels did not increase in *B. burgdorferi* infected mice treated with anti-IL-12 mAb compared with controls suggesting that Th2 responses were not affected. Nevertheless, CD4<sup>+</sup> T cells from both control and anti-IL-12 mAb treated mice had similar in vitro responses to *B. burgdorferi* antigens. Treatment with anti-IL-12 mAb produced a significant reduction in [...]

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# Effect of Anti-Interleukin 12 Treatment on Murine Lyme Borreliosis

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## Abstract

The effect of anti-interleukin (IL)-12 treatment on Lyme borreliosis in C3H/HeN (C3H) mice was assessed because other studies have implicated CD4<sup>+</sup> T cell helper (Th) type 1 responses in the genesis of disease caused by *Borrelia burgdorferi*. Infection of inbred mice with *B. burgdorferi* results in varying degrees of arthritis: BALB/c mice develop mild disease and C3H mice develop severe arthritis that is most pronounced 2–4 wk after infection. Since IL-12 is a major inducer of Th1 responses, we blocked this cytokine in vivo in *B. burgdorferi* infected C3H mice, and evaluated the effects of treatment on the development of arthritis at the peak of acute joint inflammation (14 d) and in the resolution phase (60 d) of disease. As expected, intraperitoneal administration of an anti-IL-12 monoclonal antibody (mAb) to C3H mice resulted in a decrease in both IFN- $\gamma$  and *B. burgdorferi*-specific IgG2a in serum, indicative of diminished Th1 responses. No IL-4 production was detected in serum of anti-IL-12 mAb treated or control mice. IgG1 and IgG2b levels did not increase in *B. burgdorferi* infected mice treated with anti-IL-12 mAb compared with controls suggesting that Th2 responses were not affected. Nevertheless, CD4<sup>+</sup> T cells from both control and anti-IL-12 mAb treated mice had similar in vitro responses to *B. burgdorferi* antigens. Treatment with anti-IL-12 mAb produced a significant reduction in peak arthritis severity, and an increase in the number of spirochetes in ear tissue. These data show that treatment of *B. burgdorferi* infected mice with anti-IL-12 mAb results in a reduction of the Th1 and/or innate immune responses in vivo and a reduction in the severity of acute murine Lyme arthritis. (*J. Clin. Invest.* 1996; 97:1028–1034.) Key words: *Borrelia burgdorferi* • arthritis • cytokines • Th1 response • mice

## Introduction

*Borrelia burgdorferi* is the causative agent of Lyme disease, the most common tick-borne infection in the United States (1). The illness typically begins with a skin lesion (*erythema migrans*) and may be accompanied by flu-like symptoms (2). Late-stage disease may involve the cardiovascular, nervous and/or musculoskeletal systems (3, 4). The pathogenesis of

Lyme arthritis is multifactorial and may be related to the host's major histocompatibility haplotype, humoral and cellular immune responses directed towards *B. burgdorferi*, and/or spirochetal virulence (5–7). Several studies have correlated the presence of human CD4<sup>+</sup> T cell helper (Th1) type 1 responses with the pathogenesis of persistent arthritis (8–10).

Models of murine Lyme borreliosis also suggest that the host immune response can influence the severity of arthritis. Several groups have demonstrated that BALB/c mice develop mild arthritis, whereas C3H/HeN (C3H) mice develop more severe arthritis (11, 12). In both cases, disease is most prominent 14–30 d and resolves 60–90 d after infection (11). All the mice remain persistently infected. Studies involving inbred strains of mice have suggested that disease is more severe in genotypes of mice with high numbers of spirochetes and in mice that develop dominant Th1-like cytokine responses and increased IgG2a production (13, 14). In addition, Matyniak and Reiner have correlated the production of IL-4 and IFN- $\gamma$  with resistance and susceptibility, respectively, to the degree of joint swelling in *B. burgdorferi* infected mice. They also showed that treatment of mice with anti-IL-4 antibodies or anti-IFN- $\gamma$  antibodies can result in modulation of joint swelling (15). C3H mice (disease-susceptible, with a predominant Th1 response and IFN- $\gamma$  production) treated with anti-IFN- $\gamma$  antibodies developed less severe joint swelling than untreated C3H mice. Moreover, BALB/c mice (disease-resistant, with a predominant Th2 response and IL-4 production) treated with anti-IL-4 antibodies had more severe joint swelling than their untreated counterparts (15).

IL-12 is a cytokine composed of two subunits (p40 and p35) that is produced by monocyte-macrophages, B cells, and other accessory cell types (16–18). IL-12 production is enhanced by bacterial and parasite stimulation (19, 20), including lipoproteins of *B. burgdorferi* (21), causes the induction of IFN- $\gamma$  production (20, 22), and produces strong Th1-inducing effects (23, 24), as well as augments NK cell cytotoxicity and cytotoxic T cell proliferation and function (16). These characteristics make IL-12 a major modulator of inflammation and immune responses and likely therefore to play a significant role in the pathogenesis of murine Lyme borreliosis. We therefore postulated that blocking the effect of IL-12—by using anti-IL-12 neutralizing antibodies—would influence Lyme borreliosis in the mouse model. The following study describes the evolution of Lyme borreliosis in C3H mice treated with a neutralizing anti-IL-12 monoclonal antibody (mAb) and indicates that anti-IL-12 mAb treatment results in a decrease in the severity of acute murine Lyme arthritis.

## Methods

**Mice.** Six-week-old female C3H/HeN<sup>Cr</sup> mice were obtained from the Frederick Cancer Research Center, Frederick, Maryland. Mice were housed in filter frame cages and euthanized with CO<sub>2</sub>.

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*B. burgdorferi*. A clonal low passage (third passage) of *B. burgdorferi* strain N40 with proven infectivity and pathogenicity in mice was used throughout the studies (25). Spirochetes were grown in Barbour-Stoener-Kelly (BSK) II medium (26). *B. burgdorferi* sonicate was prepared using a 7-d culture of *B. burgdorferi* in BSK II medium. Cells were centrifuged at 10,000 *g* and washed three times with phosphate buffered saline (PBS). After the final wash, bacteria were resuspended in 1 ml of PBS and pulsed three times with a cell sonicator for 20 s, with 25-s intervals.

*Infection of mice with B. burgdorferi*. Individual mice were challenged with a dose of 10<sup>4</sup> spirochetes administered by intradermal injection in the back, according to our published protocols (27). The studies used groups of 3–5 mice. Two or three different experiments were performed to assess the reproducibility of the results.

Mice were sacrificed at selected time points that represent the stages of peak, severe disease (14 d) and resolution of disease (60 d) (28). Mice were assessed for infection by culturing selected organs including the spleen, blood, bladder, and skin (at the inoculation site) (28). To compare the relative number of spirochetes in the spleen and blood of the anti-IL-12 mAb and control mice, three serial (1/10) dilutions were performed on splenic and blood cultures. Cultures were read after 14-d incubations at 33°C, a time period sufficient for a spirochete to grow to stationary phase.

*Pathology*. Hearts and joints (both knees and tibiotarsi) were fixed in formalin, embedded in paraffin and blindly examined microscopically for evidence of disease (29). Arthritis severity was scored on a scale of 0 (no disease) to 3 (severe disease) according to our published protocols (29). Grades 1 and 2 represent mild and moderate arthritis, respectively. Active arthritis was scored, tabulated, and distinguished from resolving lesions (no active exudation). Active carditis had aortic and coronary endarteritis, with inflammation of surrounding connective tissue (25, 30).

*Quantitation of numbers of spirochetes in ear tissue from infected mice by PCR*. One whole ear pinna was aseptically collected from each mouse at sacrifice time to minimize both bacterial and DNA contamination (31) and processed using the QIAmp Tissue kit (Qiagen, Chatsworth, CA). Five microliter aliquots of protease-digested DNA extracts were twofold serially diluted, and each dilution was tested for *B. burgdorferi* plasmid and chromosomal targets, using *ospA* or *flagellin* gene primers (32). The number of spirochetes per ear from each mouse was estimated on the basis of the most conservative assumptions of a single genome copy per spirochete and single-copy sensitivity of the terminal dilution, as described (33).

*Antibody production and in vivo neutralization studies*. A mAb against murine IL-12 was produced using the hybridoma C17.8 (provided by G. Trinchieri, Wistar Institute, Philadelphia, PA) in pristane-primed severe combined immunodeficient (*scid*) mice. The ascitic fluid containing anti-IL-12 mAb was precipitated with ammonium sulfate at 50% saturation followed by extensive dialysis and filtration through a 0.45- $\mu$ m filter. The protein content was quantitated and adjusted to 10 mg/ml.

In blocking experiments the mAb was injected intraperitoneally in the treated group at a dose of 1 mg/ml in 100  $\mu$ l 1 d before infection, on the day of infection and every 4 d after infection until day 12, and every 8 d thereafter (G. Trinchieri, personal communication). The control group was injected in an identical fashion with an irrelevant control antibody (mouse IgG; Sigma Chemical Co., St. Louis, MO).

*T cell restimulation assays*. Splenic CD4<sup>+</sup> T cells were purified by negative selection using mouse anti-IA<sup>k</sup> (clone 10–3.6) and rat anti-mouse CD8a (clone 53–6.7) (Pharmingen, San Diego, CA) and goat anti-mouse and goat anti-rat IgG bound to magnetic beads (Perspective Biosystems, Cambridge, MA). Typically, the purity of CD4<sup>+</sup> T cells obtained were > 94%, as measured by FACS<sup>®</sup> analysis. 10<sup>6</sup> CD4<sup>+</sup> enriched T cells per well were plated in 24-well plates with cultured *B. burgdorferi* (10<sup>6</sup> spirochetes/ml) or no stimulus, plus 10<sup>6</sup> cells/ml of mitomycin C treated (50  $\mu$ g/ml) (ICN Pharmaceuticals, Costa Mesa, CA) syngeneic splenic APCs at a final volume of 1 ml.

Supernatants were collected after 20 and 72 h for IL-4 and IFN- $\gamma$  quantitation, respectively.

*Evaluation of cytokine production*. The amount of IL-4 and IFN- $\gamma$  present in pooled sera and restimulation supernatants was measured by ELISA using antibodies specific for murine IL-4 and IFN- $\gamma$ , respectively, (Pharmingen), following the manufacturer's instructions with some modifications. Briefly, 96-well ELISA plates (ICN) were coated with the capture antibody (2  $\mu$ g/ml) overnight at 4°C. After blocking with PBS plus 10% FCS (PBS/FCS) for 2 h at room temperature, samples were applied and incubated 1 h at 37°C. The biotinylated detection antibody (1  $\mu$ g/ml) was added after washing the plates with PBS plus Tween 20 (0.5% vol/vol) (PBS/Tween). Quantitation of cytokine levels was made after incubating the plates with horseradish-conjugated avidin and adding the substrate for the enzyme (TMB; Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD). Plates were read at 450 nm after stopping the color developing reaction (TMB 1 Component Stop Solution; Kirkegaard and Perry Laboratories, Inc.). The values indicated are calculated comparing the values obtained with those derived using standard concentrations of recombinant mouse IL-4 and IFN- $\gamma$  (Pharmingen). The sensitivity of the assays was evaluated to be < 0.5 U/ml and 1 U/ml for IL-4 and IFN- $\gamma$ , respectively.

*B. burgdorferi-specific antibody isotype production*. Sera from animals infected with *B. burgdorferi* were used for the quantitation of different antibody isotypes by ELISA using rabbit anti-mouse subclass specific anti-serum to mouse IgG1, IgG2a, and IgG2b (Mouse Typer sub-isotyping panel; Bio-Rad Laboratories, Hercules, CA) as follows. An ELISA plate (ICN) was coated overnight at 4°C with 0.5  $\mu$ g/ml of *B. burgdorferi* sonicate in coating buffer (Bicarbonate buffer, pH 9.6), and blocked for 2 h at room temperature with PBS plus 10% FCS. After two washes with PBS/Tween 20, sera (1/100 or 1/500 dilutions of sera from animals infected for 14 and 60 d, respectively) were applied and incubated 1 h at room temperature. The wells were washed and the specific antiserum was applied and incubated 1 h at room temperature. After four washes, HRP-conjugated goat anti-rabbit IgG was applied and incubated 1 h at room temperature. Quantitation of the different isotypes was carried out after adding peroxidase substrate and reading at 450 nm, as above. Normal (noninfected) mouse serum was used as a control.

*FACS analysis*. 10<sup>6</sup> cells from single cell suspensions of spleens were stained using phycoerythrin (PE)- or fluorescein isothiocyanate (FITC)-labeled antibodies against CD4, CD8a and B220 (Pharmingen), for 30 min at 4°C. Cell populations were analyzed using a FACS<sup>®</sup> and the LYSYS program (Becton Dickinson, Franklin Lakes, NJ).

## Results

*Treatment with anti-IL-12 mAb causes a decrease in the production of B. burgdorferi-specific IgG2a and IFN- $\gamma$* . The functional effect of anti-IL-12 mAb treatment in C3H mice infected with *B. burgdorferi* was assessed by measuring IFN- $\gamma$ , IL-4 and *B. burgdorferi*-specific IgG1, IgG2a, and IgG2b production in mouse sera compared to sera from infected mice that were not treated with anti-IL-12 mAb. IFN- $\gamma$  and IgG2a were used as two markers for Th1 responses against the spirochete while IL-4 production and IgG1 and IgG2b would reflect Th2 responses. We first analyzed possible changes in *B. burgdorferi*-specific antibody isotypes. Sera from infected control mice had significantly greater amounts of *B. burgdorferi*-specific IgG2a than the anti-IL-12 mAb treated group ( $P < 0.05$ ), both at 14 and 60 d of infection (Table I). When we measured other *B. burgdorferi*-specific antibody isotypes (IgG1 and IgG2b) no differences were observed between the two groups of mice ( $P > 0.05$ ) (Table I). Since IgG2a has been correlated with the production of IFN- $\gamma$ , a cytokine produced during Th1

Table I. *B. burgdorferi*-specific Antibodies in Sera of Anti-IL-12 mAb-treated and Control Mice, after 14 and 60 d of Infection

Mice		Antibody isotype (mean OD <sub>450</sub> ±STDV)		
Treatment	Day of Death	IgG1	IgG2a	IgG2b
Anti-IL-12 mAb	14	0.272±0.03	0.114±0.01*	0.242±0.03
Control	14	0.221±0.05	0.214±0.04*	0.213±0.06
Anti-IL-12 mAb	60	0.950±0.15	0.580±0.01*	0.744±0.06
Control	60	0.745±0.00‡	0.919±0.11*	0.763±0.09
Normal mouse serum		0.155±0.01	0.074±0.01	0.49±0.02

Sera were pooled and tested in ELISA plates coated with 0.5 µg/ml of a *B. burgdorferi* extract. Sera were assessed at dilutions of 1/100 and 1/500 at 14 and 60 d, respectively. Data represent results from 10–16 individual mice in each group. Normal mouse serum was used as a control (1/100 dilution). Statistically significant differences ( $P < 0.05$ ), between treated and control groups are indicated with an asterisk. ‡STDV < 0.005.

responses, we determined IFN-γ production by measuring the amount of IFN-γ present in the sera of infected animals. The amount of IFN-γ present in the sera of the mice treated with anti-IL-12 mAb was lower than in the control group, both at 14 and 60 d (Fig. 1) and correlated with the decreased production of IgG2a. In addition, no IL-4 production was detected in either group of mice (Fig. 1). These results indicate that anti-IL-12 mAb treatment reduced the specific Th1 response against *B. burgdorferi* in vivo.

*Anti-IL-12 mAb treatment does not affect CD4<sup>+</sup> T cell priming in vivo.* To determine if the lack of detectable IL-4 in both treated and untreated mice and the lower production of IFN-γ in the anti-IL-12 mAb treated mice could reflect a lack of activation of CD4<sup>+</sup> T cells in vivo, we checked the in vitro

lymphokine response of CD4<sup>+</sup> T cells of the infected animals after 14 d of infection following restimulation with *B. burgdorferi* antigens. No significant differences were detected in the ability of CD4<sup>+</sup> T cells to produce in vitro IFN-γ in response to *B. burgdorferi* antigens (Fig. 2). Moreover, as observed in the sera of the mice, no IL-4 production was detected upon specific restimulation (Fig. 2). These data indicate that anti-IL-12 mAb treatment does not block the differentiation of precursor CD4<sup>+</sup> T cells to effector Th1 cells induced by *B. burgdorferi* and does not favor a Th2 response.

*Effects of anti-IL-12 mAb treatment on the course of murine Lyme arthritis.* The evolution of murine Lyme arthritis was evaluated in mice treated with anti-IL-12 mAb compared to a control infected group. Fourteen and 60 d after infection with 10<sup>4</sup> spirochetes, the knees and both tibiotarsi were blindly examined for microscopic evidence of arthritis. An index of the degree of arthritis for each individual mouse was determined by scoring arthritis severity in both tibiotarsal joints

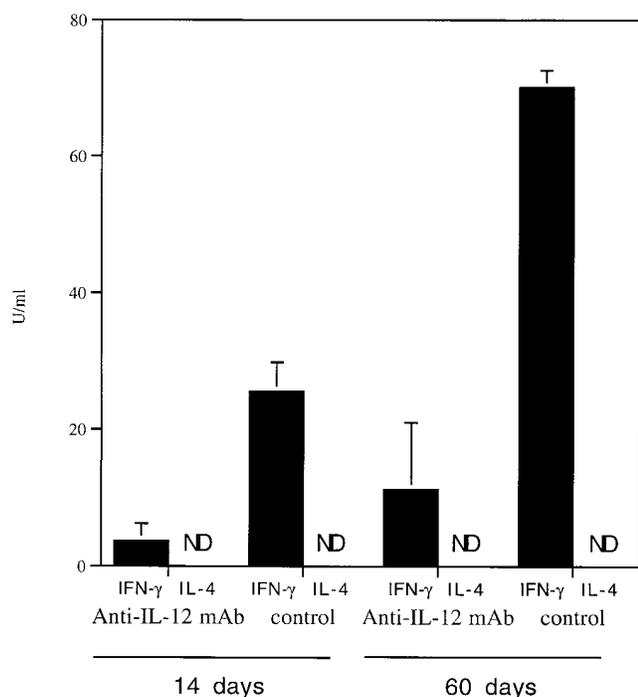


Figure 1. IFN-γ and IL-4 detection in the sera of the infected mice. Fourteen and 60 days after infection, sera from anti-IL-12 mAb treated and control mice (10 mice each, in two separate experiments) were collected and pooled, and the levels of IFN-γ (■) and IL-4 (□) were determined by ELISA. ND, not detected.

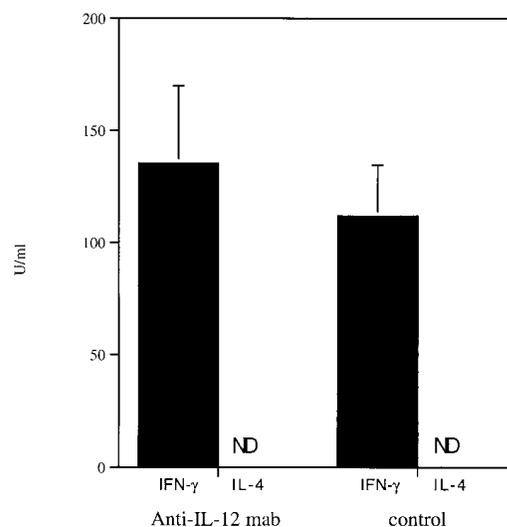


Figure 2. IFN-γ (■) and IL-4 (□) produced by pooled splenic CD4<sup>+</sup> T cells from anti-IL-12 mAb treated and control mice at 14 d of infection, restimulated in vitro with *B. burgdorferi*. Splenic CD4<sup>+</sup> T cells were purified by negative selection and restimulated (10<sup>6</sup> cells/ml) with cultured *B. burgdorferi* (10<sup>6</sup> spirochetes/ml), using syngeneic APCs (10<sup>6</sup> cells/ml). ND, not detected. The values represented correspond to a representative experiment from three performed.

from each mouse, because this is the joint in which arthritis is most consistently discerned (29). Probably due to age of inoculation, arthritis severity was generally mild (Fig. 3), but was significantly less severe 14 d after challenge in mice treated with anti-IL-12 mAb compared to control mice (Student *t* test  $P < 0.05$ ). In addition to the decrease in arthritis severity, the number of joints with arthritis was reduced following treatment with anti-IL-12 mAb: 77% of the knees in the control group had some degree of disease versus 54% in the treated group, and 38% versus 13% of the tibiotarsal joints ( $P < 0.05$ ) (Table II). No differences in carditis were found at this time point (Table II). When the mice were sacrificed at 60 d no differences in the degree of arthritis were observed between anti-IL-12 mAb-treated and control mice (Fig. 2). The number of tibiotarsi with arthritis, however, was lower in the treated group (28 vs 40% in the control group, 5 of 18 and 8 of 20, respectively, Table II). Carditis was also somewhat less prevalent in anti-IL-12 mAb treated mice than in control animals at 60 d after infection. All 10 mice in the control group had carditis, whereas 7 of 9 (88%) in the anti-IL-12-treated group had evidence of carditis (Table II). We attempted to correlate the degree of arthritis, as determined by histopathology, with swelling of the tibiotarsus of infected mice, measured in a double blind fashion using calipers. No differences in tibiotarsal swelling (data not shown) were observed between the two groups.

*Increase of the number of spirochetes as a result of in vivo blocking of IL-12.* To determine whether anti-IL-12 mAb treatment altered the number of spirochetes within infected mice we quantified *B. burgdorferi* using PCR (32). Quantitative PCR, using ears from the infected animals as an indication of spirochete load, showed that a greater number of spiro-

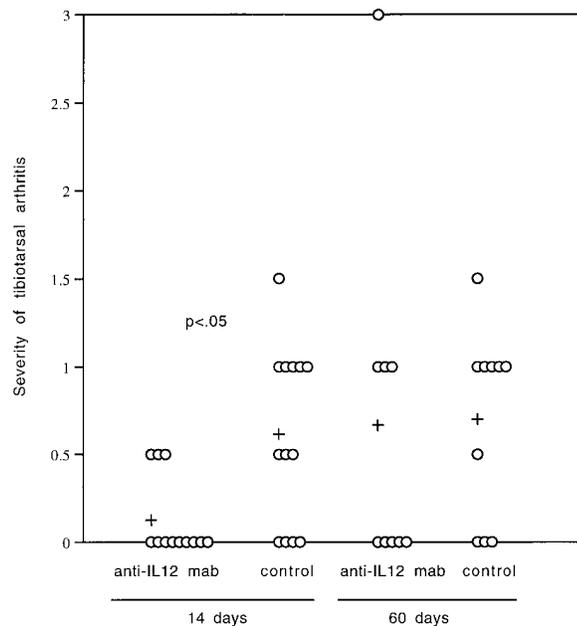


Figure 3. Histopathologic arthritis of the tibiotarsal joints in anti-IL-12 mAb (12 mice) and control mice (13 mice) after 14 and 60 d of infection. Tibiotarsal joints were fixed in formalin, embedded in paraffin and blindly examined for histopathological signs of arthritis. Each joint was scored in a scale from 0 to 3, and the values for both hind-legs were added in each mouse (o). The average for each group is also represented (+).

Table II. Arthritis and Carditis in Anti-IL-12 mAb-treated and Control Mice after 14 and 60 d of Infection with  $10^4$  *B. burgdorferi* N40

Mice		Arthritis and carditis Number of joints or hearts with disease/number of joints examined		
Treatment	Day of Death	Knee	Tibiotarsus	Heart
Anti-IL-12 mAb	14	13/24	3/23	11/12
Control	14	20/26	10/26	13/13
Anti-IL-12 mAb	60	0/18	5/18	7/9
Control	60	0/20	8/20	10/10

Hearts and both knee and tibiotarsal joints were examined for arthritis by histopathology of the fixed and embedded tissue.

chetes were present in ears of anti-IL-12 mAb treated mice than in control animals (Fig. 4) at both time points. More spirochetes were present in ears of anti-IL-12 mAb treated or control mice at 14 d than at 60 d. At 14 d ears of control mice had an average of  $8.6 \times 10^4$  spirochetes, whereas ears of anti-IL-12 mAb treated mice had  $6 \times 10^5$ . At 60 d, the average number of spirochetes in ears of control mice had decreased to  $4.6 \times 10^3$ , and the number of spirochetes in ears of anti-IL-12 mAb treated mice had been reduced to  $1.3 \times 10^4$ , a level similar to that found in control mice at 14 d. Furthermore, when blood and spleens were cultured using serial dilutions to compare relative bacterial numbers in both groups, we detected spirochetes at higher dilutions in the spleens of the anti-IL-12 mAb treated mice after 14 d of infection (Table III). At 60 d, 3 of 7 spleens from the anti-IL-12 mAb treated mice were culture positive and 1 of 10 mice in the control group had a culture-positive spleen.

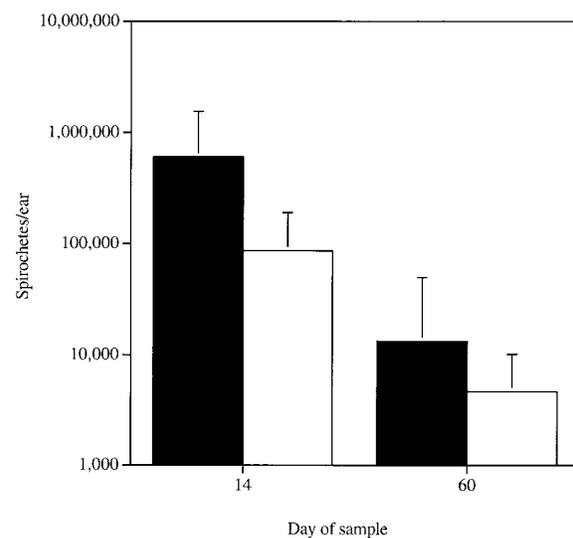


Figure 4. Number of spirochetes in anti-IL-12 mAb (■) and control mice (□) measured using quantitative PCR of one ear from individual mice (5–10 mice per group), both at 14 and 60 d following *B. burgdorferi* infection.

Table III. Isolation of Spirochetes from Selected Organs of Anti-IL-12 mAb-treated and Control Mice at 14 and 60 d after Infection

Mice		Isolation of <i>B. burgdorferi</i> Number of positive cultures/total number of cultures*					
Treatment	Day of Death	Bladder	Inoc. site	Blood 1	Blood 2	Spleen 1	Spleen 2
Anti-IL-12 mAb	14	10/12	12/12	7/12	1/12	6/9	5/11
Control	14	13/13	13/13	7/13	2/13	5/11	1/13
Anti-IL-12 mAb	60	6/9	7/9	0/10	0/10	3/7	0/9
Control	60	4/10	10/10	0/10	0/10	1/10	0/10

Blood 1, blood 2, spleen 1, and spleen 2 indicate serial dilutions of these specimens. Cultures were read after 14 d of incubation of BSK-II medium at 33°C. Inoc. site represents a specimen of skin (5 mm) from the site location where *B. burgdorferi* were injected. \*Only the cultures that were not contaminated were scored and tabulated.

## Discussion

The analysis of *B. burgdorferi*-infected mice and T cell clones obtained from patients with Lyme disease has suggested that the development of Th1 responses influences the severity of arthritis. Mouse strain-related differences in cytokine and IgG2a production in response to spirochete infection, reflecting the differential development of T cell helper responses, suggest a correlation of increased Th1 responses with disease severity (13, 15). Moreover, the administration of neutralizing antibodies directed against IFN- $\gamma$  in vivo, mimicking diminished Th1 responses, resulted in a decrease in joint swelling in C3H mice (15). Therefore, although it may be advantageous to mount Th1 responses to control infections by intracellular pathogens, this strategy is not particularly effective against *B. burgdorferi*, an organism that may be predominantly extracellular. Protection against *B. burgdorferi* infection requires the generation of borreliacidal antibodies directed against certain spirochete antigens, including outer surface protein (Osp) A and B (34, 35), and is therefore influenced by B cell help provided by Th2 type CD4<sup>+</sup> T cells. Thus, the induction of a predominant Th1 response may be a mechanism utilized by the spirochete to reduce, in part, appropriate T cell responses that can facilitate efficient antibody production and therefore avoid host defenses. Moreover, the maintenance of a Th1 inflammatory response, which leads to destruction of certain tissues where the spirochete resides might be an important source of nutrients.

Our results suggest a role for IL-12 in the expression of arthritis. Anti-IL-12 mAb treatment reduced both the incidence and the severity of acute murine Lyme arthritis in C3H mice. The reduction in arthritis correlated with a lower level of IFN- $\gamma$  in the sera of the treated animals. Splenic CD4<sup>+</sup> T cells from *B. burgdorferi* infected mice treated with an anti-IL-12 mAb or a control antibody produced similar amounts of IFN- $\gamma$  when they were restimulated in vitro with *B. burgdorferi*, indicating that the reduced level of IFN- $\gamma$  produced in vivo was not result of lack of T cell activation.

Murine Lyme arthritis in C3H mice can be divided into two different phases. Approximately 2–4 wk after infection with *B. burgdorferi*, disease is most severe, with active joint inflammation and carditis. By 60 d, acute inflammation has significantly resolved. Anti-IL-12 mAb treatment produced a significant decrease in arthritis at the peak of the disease (14 d), but had little or no effect at 60 d, the resolution phase of disease. Thus, IL-12 may be involved in the genesis of acute murine Lyme ar-

thritis, and the development of a Th1 response may be important in the first stage of the infection. Nevertheless, this response has little effect in the clearance of the bacteria and resolution of the disease because the number of spirochetes in ear tissue of anti-IL-12 mAb treated mice at 14 and 60 d is greater than in control mice. Moreover, the disease is similar in both groups at 60 d. The reduction in *B. burgdorferi*-specific antibody production in anti-IL-12 mAb treated mice may allow the bacterium to persist more efficiently, which explains the higher burden of spirochetes found in the ear tissue of the treated group. This would suggest that the development of arthritis in C3H mice is dependent in part on the type of cellular immune response against *B. burgdorferi* antigens, and not only on the number of spirochetes in the host. Indeed, anti-IL-12 mAb treatment decreases the innate immune response, likely including macrophage activation as a result of a lower level of IFN- $\gamma$ , and this may contribute, in part, to the reduction in arthritis at the 14-d time point. In contrast, in the late stage of disease, when arthritis is resolving, the treatment with anti-IL-12 mAb does not result in accelerated reduction of arthritis. Other studies have correlated bacterial burden with severity of disease (13, 30, 32). It is likely that, under normal immune conditions, an increase in the number of spirochetes present in the infected animals produces an increase in the immune response against *B. burgdorferi*, causing an increase in cytokine production and inflammatory responses. In our experiments, in which the response is diminished as a result of the treatment, an increase in the spirochetal burden does not result in increased cytokine production or inflammatory response. It is likely, then, that both factors, the number of spirochetes and the resulting immune response play roles in the pathogenesis of arthritis, and the relative contribution of both factors may differ at various time points in the course of the infection.

In contrast to the arthritis documented by histopathology data, anti-IL-12 mAb treatment did not affect the degree of joint swelling, indicating that gross joint swelling is not indicative of arthritis. Patients with Lyme disease have joint swelling that is disproportionate to the degree of underlying arthritis (36), and it is due to subcutaneous edema. The same is true of mice. They get transient subcutaneous edema, often in the absence of underlying arthritis, or they get arthritis in the absence of subcutaneous edema (gross joint swelling). Thus, the two indices should not be equated to one another. Joint swelling reflects edema and possibly arthritis, but arthritis can only be verified at the microscopic level.

The reduction in IFN- $\gamma$  and IgG2a production as a result of

anti-IL-12 mAb treatment reflects a decrease in the murine Th1 responses in vivo against *B. burgdorferi*. Interestingly, in spite of the Th1 responses produced as a consequence of the treatment, IL-12 neutralization in vivo did not lead to a Th2 response, based on the lack of detection of IL-4 or Th2-mediated IgG isotypes production in the serum of the treated animals. Nevertheless, the lack of difference in cytokine production by primed CD4<sup>+</sup> T cells after in vitro restimulation with *B. burgdorferi* suggests that the differentiation to a Th1 phenotype in both groups of mice was equivalent. Some reports have indicated that the presence of IL-12 is required for the development of Th1 responses (37, 38), while other studies suggest that both IL-12 and IFN- $\gamma$  are required (39). Moreover, mouse strain-specific backgrounds can directly influence Th1 development under neutral conditions (40). The disparity observed in terms of IFN- $\gamma$  production in vitro and in vivo in the anti-IL-12 mAb treated group of mice could be a consequence of the lower amount of IL-12 in vivo in the treated group, causing a decrease in CD4<sup>+</sup> T cell responses (41, 42), while in vitro the APCs used can produce appreciable amounts of IL-12 in response to *B. burgdorferi* that could serve as a costimulatory signal (21). The lower levels of IFN- $\gamma$  in the sera of the treated mice may also be a consequence of the lack of activation of other cell types that can produce this cytokine, such as NK cells or  $\gamma\delta$  T cells (43). These cells could be less activated in the anti-IL-12 mAb treated group, even though TNF- $\alpha$  produced in response to *B. burgdorferi* lipoproteins (21, 44) can produce NK cell activation (45) and the proposed presence of a superantigen-like activity in *B. burgdorferi* (46) could cause IFN- $\gamma$  production by some cell types (47).

In conclusion, treatment with anti-IL-12 mAb during *B. burgdorferi* infection in C3H mice results in a decrease in the severity of acute arthritis, suggesting that a decrease in the Th1 and/or innate cellular inflammatory responses are associated with a reduction in Lyme disease in the early stage of murine infection. Even though murine and human Lyme borreliosis cannot be considered as equivalent, the murine model has provided insights into the immune response to *B. burgdorferi* that has helped lead to the development of an experimental human OspA based vaccine. The finding that the treatment with anti-IL-12 decreases the Th1 responses in vivo and consequently the degree of acute disease, indicates that the development of this type of T cell response is responsible for some of the arthritis that results from spirochetal infection. Th1 responses are produced during human Lyme disease (9, 10), suggesting a correlation between the development of Th1 responses against *B. burgdorferi* and the severity of human Lyme disease. Our results would indicate that immune responses that can be blocked by anti-IL-12 contribute to the pathogenesis of acute murine Lyme arthritis. It is likely, therefore, that *B. burgdorferi* specific Th1 responses influence the evolution of murine and human Lyme borreliosis.

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