Neutralization of Gamma Interferon Augments Borreliacidal Antibody Production and Severe Destructive Lyme Arthritis in C3H/HeJ Mice

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Development of a high level of sustained borreliacidal antibody is paramount for maintaining protection against infection with Borrelia burgdorferi. We show that production of borreliacidal antibody can be enhanced by preventing the effects of gamma interferon (IFN-γ). When lymph node cells capable of producing borreliacidal antibody were cultured with anti-murine IFN-γ, an eightfold increase in borreliacidal antibody production was obtained. However, anti-IFN-γ treatment of these cells also enhanced their ability to adaptively induce arthritis. When anti-IFN-γ-treated lymph node cells producing borreliacidal antibody were infused into C3H/HeJ mice and the mice were then challenged with B. burgdorferi, the mice developed severe destructive Lyme arthritis. Additional studies are needed to delineate the immune response responsible for the induction of arthritis and production of borreliacidal antibody. These studies are needed to ensure an effective and safe vaccine against infection with B. burgdorferi.

Vaccination with Borrelia burgdorferi or its components can induce antibodies that prevent infection with the Lyme disease spirochete (1, 6, 8–10, 13, 27, 34). These vaccine-induced antibodies kill B. burgdorferi in the presence of complement (17, 24). Specifically, it has been shown that antiseras raised against recombinant outer surface protein OspA can prevent borreliacidal infection by passive immunization (8, 17, 31, 34), kill B. burgdorferi in vitro (13, 17, 18, 20, 24, 27, 29, 30), and sterilize B. burgdorferi-infected ticks (11). However, anti-OspA borreliacidal activity wanes rapidly after active immunization. Padilla et al. (24) reported that vaccination of experimental animals with recombinant OspA induced a borreliacidal antibody response that peaked 6 weeks after vaccination and declined rapidly. Moreover, human volunteers administered several doses of recombinant OspA produced borreliacidal antibody that rapidly decreased (24). Only one vaccinee had antibody 6 months after vaccination. The poor OspA-borreliacidal antibody response may have been a factor in prompting withdrawal of the vaccine for use in humans.

Very little is currently known about the immunologic events that might promote and maintain a sustained borreliacidal antibody response after vaccination. Therefore, we developed an in vitro system to determine the effects of immunological mediators on production of borreliacidal activity. Several cytokines have been evaluated, including interleukin (IL)-4, a known B-lymphocyte stimulator (26), and its antagonist, gamma interferon (IFN-γ) (25). However, these cytokines failed to induce high levels of borreliacidal antibody (21, 22).

Here, we report that treatment of lymph node cells containing borreliacidal antibody-producing cells with anti-murine IFN-γ augments the production of anti-B. burgdorferi antibody. Unfortunately, treatment of these cells with anti-murine IFN-γ also enhances their ability to induce arthritis.

MATERIALS AND METHODS

Mice. Inbred C3H/HeJ mice 8 to 12 weeks old were obtained from our breeding colony located at the Wisconsin State Laboratory of Hygiene. Mice weighing 20 to 30 g were housed four per cage at an ambient temperature of 21°C. Food and acidified water were provided ad libitum.

Organism. B. burgdorferi sensu stricto isolate 297 was originally isolated from human cerebrospinal fluid (35). Low-passage (<6) organisms were cultured once in modified Barbour-Stoenner-Kelly (BSK) medium (2) containing screened lots of bovine serum albumin (5) to a concentration of 5 × 107 spirochetes per ml. Five-hundred-microliter samples were then dispensed into 1.5-ml screw-cap tubes (Sarstedt, Newton, N.C.) containing 500 μl of BSK supplemented with 10% glycerol (Sigma Chemical Co., St. Louis, Mo.), sealed, and stored at −70°C. When necessary, a frozen suspension of spirochetes was thawed and used to inoculate fresh BSK medium. Spirochetes were viewed by dark-field microscopy and enumerated with a Petroff-Hausser counting chamber.

Preparation of vaccine. B. burgdorferi organisms were grown in 1 liter of BSK medium for 6 days, pelleted by centrifugation (10,000 × g, 15°C, 10 min), and washed three times with phosphate-buffered saline (PBS; pH 7.4). The washed pellet was resuspended in 1% formalin, incubated at 32°C for 30 min with periodic mixing, washed three times by centrifugation with PBS (12,000 × g, 10°C, 15 min), and resuspended in PBS. Subsequently, the formalin-inactivated spirochetes were mixed in a volume of 1% suspension of aluminum hydroxide (Reheis, Berkeley Heights, N.J.) to yield 4 × 106 spirochetes/ml.

Vaccination of mice. Forty mice were mildly anesthetized with methoxyflurane contained in a mouth-and-nose cup and vaccinated subcutaneously in the inguinal region with 0.25 ml (~107 B. burgdorferi) of the formalin-inactivated vaccine preparation. The suspension contained approximately 100 μg of borrelial protein. Nonvaccinated mice were injected with BSK medium or aluminum hydroxide alone.

Recovery of macrophages. Three to five nonvaccinated mice per experimental protocol were mildly anesthetized with methoxyflurane contained in a mouth-and-nose cup and injected intraperitoneally with 2 ml of 3% thioglycolate in PBS. Four days after injection, mice were euthanized by CO2 asphyxiation, and 8 ml of cold Hanks’ balanced salt solution (Sigma) was injected intraperitoneally. The
peritoneal cavity was massaged for ~1 min, and the exudate cells were recovered by aspiration with a syringe. The suspension of peritoneal exudate cells was centrifuged at 1,500 rpm for 10 min at 4°C. The supernatant was decanted, and the cells were resuspended in Dulbecco's modified Eagle's medium (DMEM; Sigma) with 5% antimicrobial antibiotic solution (10,000 U/ml penicillin, 10,000 U/ml streptomycin, and 2.5 µg/ml amphotericin). The cell suspension was inactivated (56°C, 45 min) fetal bovine serum (HyClone Laboratories, Logan, Utah), 5 × 10^{-5} M 2-mercaptoethanol (Sigma), and 1-glutamine (2.92 mg/ml; Sigma). Aliquots of the cell suspension were then poured over polystyrene tissue culture dishes (100 by 20 mm; Corning Glass Works, Corning, N.Y.) and incubated at 37°C in a humidified atmosphere of 5.0% CO_2 for 4 to 6 h. After incubation, nonadherent cells were aspirated from the tissue culture flasks and washed free of debris, suspended in 0.2 ml of warm DMEM, and viability was enumerated by trypan blue exclusion.

### Isolation of lymph node cells

Twelve to fifteen mice were euthanized by CO_2 inhalation 17 days after vaccination with formalin-inactivated *B. burgdorferi* (22). Inguinal lymph nodes were removed from each back and nonvaccinated mice, minced, and placed separately into cold DMEM. Single-cell suspensions of lymph node cells were prepared by teasing apart the lymph nodes with forceps and gently pressing them through a sterile stainless steel 60-mesh screen into antimicrobial-free cold DMEM supplemented with 10% heat-inactivated fetal bovine serum, 1-glutamine, and 2-mercaptoethanol. Lymph node cells were washed twice by centrifugation (1,500 rpm, 4°C, 10 min) with DMEM. Supernatants were decanted, and pellets were resuspended in 1 ml of cold DMEM. Cell viability was determined by trypan blue exclusion. The preparations of macrophages obtained by this method were 98% free of lymphocyte contamination, as determined by flow cytometry.

### Inoculation of in vitro cultures

Sterile six-well flat-bottomed tissue culture dishes (Becton Dickinson, Lincoln Park, N.J.) were inoculated with lymph node cells (5 × 10^5) obtained from vaccinated or nonvaccinated mice, macrophages from nonvaccinated mice (1 × 10^7), and 10^2 live *B. burgdorferi*. DMEM was added to the suspensions of cells to bring the final volume to 3 ml. Cells were cultured at 37°C in the presence of 5.0% CO_2. In some experiments, 10 µg of recombinant IFN-γ (rIFN-γ) or 75 µg of rat anti-murine IFN-γ (R&D Systems, Minneapolis, Minn.) was added to cultures of immune lymph node cells, macrophages, and *B. burgdorferi* at 10 min of incubation. These concentrations of rIFN-γ and anti-murine IFN-γ yielded maximum responses. In similar fashion, control cultures were incubated with a rat isotype-nonspecific antibody. One set of cultures was inoculated for determination of borrelial activity, while another set was inoculated for subsequent infusion into recipient mice and flow cytometric analysis.

### Infusion of in vitro culture contents into recipient mice

Twenty-four hours after cultivation, nonimmune or immune lymph node cells treated with rIFN-γ, anti-murine IFN-γ, or an isotype-nonspecific antibody were aspirated and dispensed into separate centrifuge tubes. The cells were washed four times by centrifugation with warm DMEM (1,000 rpm, 22°C, 10 min). The cells were then resuspended in ~250 µl of warm DMEM, and viability was enumerated by trypan blue exclusion. Subsequently, the concentrations of cells were adjusted with warm DMEM to yield 10^7 lymphocytes/ml. Four groups of three mice each were mildly anesthetized with methoxyflurane and then injected subcutaneously in the hind paw with 0.1 ml of the lymphocyte suspensions. A fifth group of mice were administered 0.1 ml of warm DMEM.

### Flow cytometric analysis for CD4+ and CD8+ cells

Twenty-four hours after in vitro cultivation, 5 × 10^6 cells were transferred to chilled centrifuge tubes containing 500 µl of cold DMEM. Cells were then incubated (15 min, 4°C, dark conditions) with 5 µl of phycoerythrin-conjugated rat anti-murine CD4 or rat anti-murine CD8 (PharMingen, San Diego, Calif.). Separate populations of cells were also incubated with phycoerythrin-conjugated rat IgG2a (PharMingen) as an isotype control. Cells were then washed two times by centrifugation with PBS containing 0.1% bovine serum albumin (1,500 rpm, 4°C, 10 min), fixed with 1% paraformaldehyde, and kept in the dark until flow cytometric analysis. Data were acquired on a FACSCalibur flow cytometer (Becton Dickinson, San Jose, Calif.) with CellQuest acquisition and analysis software (Becton Dickinson). Cells were detected by forward scatter, side scatter, and phycocerythrin fluorescence. Data from 10,000 events were analyzed by histogram profiles of phycoerythrin fluorescence. Gates were established with samples stained with the isotype control antibody. The percentage of CD4+ and CD8+ cells was determined by the shift in phycoerythrin fluorescence of the stained cells.

### Collection of cell-free culture supernatants from cultures of lymph node cells

At day 9 of cultivation at 37°C in the presence of 5.0% CO_2, 1.0 ml samples of the supernatants were removed after gentle agitation and replaced with an equal volume of warm DMEM. Supernatants were collected after centrifugation at 13,000 rpm for 8 min to remove spirochetes and other cellular debris. Supernatants were stored at ~70°C until used.

### Assessment of arthritis

Swelling of the left hind paws of mice was used to evaluate the inflammatory response. Before experimentation, mice were chosen randomly and their hind paws were measured before they were assigned to cages. The percentage of nonimmune lymph node cells or macrophages alone and DMEM, the left hind paws of recipient mice were measured periodically for 21 days with a dial-type vernier caliper (Fisher Scientific, Pittsburgh, Pa.) graduated in 0.1-mm increments. Severe swelling was assessed by mildly anesthetizing each mouse, carefully measuring the width and thickness of each left hind paw, combining these values, and determining mean dimensions within a given group. The standard error of the mean was also calculated for each mean caliper value.

### Acquisition of sera

Mice were anesthetized by inhalation of methoxyflurane contained in a mouth-and-nose cup and bled by intracardiac puncture.Recipient mice were bled 21 days after infusion of cell culture aspirates. The blood was allowed to clot, and serum was separated by centrifugation (1,500, 4°C, 10 min), divided into 0.20-ml aliquots, dispensed into 1.5-ml screw-cap tubes (Sarstedt), and frozen at ~70°C until used.

### Preparation of tissues for histology

Twenty-four hours after infusion of cell culture aspirates, the blood was allowed to clot, and serum was separated by centrifugation (1,500, 4°C, 10 min), divided into 0.20-ml aliquots, dispensed into 1.5-ml screw-cap tubes (Sarstedt), and frozen at ~70°C until used.

### Detection of borrelial antibody by membrane filtration

Frozen supernatants or sera were thawed, heat-inactivated (56°C, 30 min), sterilized with a 0.22-µm filter (Acrordisk; Gelman Sciences, Ann Arbor, Mich.), and serially twofold diluted (neat to 1:8,192 for supernatants; 1:10 to 1:1,280 for sera) with fresh BSK medium. One-hundred-microliter aliquots of each dilution were transferred to 1.5-ml screw-cap tubes (Sarstedt), and 100 µl of BSK containing 10^8 B. burgdorferi organisms per ml was added along with 20 µl of sterile guinea pig complement (Sigma). The tubes were then gently shaken and incubated for 3 days at 32°C. Controls included filter-sterilized supernatants obtained from cultures of nonimmune lymph node cells or macrophages alone and DMEM.

After incubation, 100 µl of each suspension was removed and placed into individual 1.5-ml screw-cap tubes (Sarstedt). Subsequently, 100 µl of a solution of propidium iodide (1.0 mg/ml, Molecular Probes, Eugene, Ore.) diluted 1:20 in 200 µl of PBS was added. The suspensions were briefly mixed before being incubated at 56°C for 30 min to permit intercalation of propidium iodide into the spirochetes. One hundred microliters of each sample was then filtered through 0.22-µm-pore-size Nuclepore polycarbonate membrane filters (47-mm diameter; Whatman Nuclepore, Clifton, N.J.) under negative pressure with a single-place sterility test manifold (Millipore Corporation, Bedford, Mass.) attached to a vacuum pump. Membrane filters were washed with ~8 ml of sterile double-distilled H_2O removed from the vacuum apparatus, allowed to dry, and placed onto glass microscope slides. Coverslips were placed on the filters before viewing with a Laborlux S fluorescence microscope (Leitz, Wetzlar, Germany) with a 50x oil immersion objective.

The number of spirochetes on each filter was quantitated by viewing ~30 fields. The borrelial antibody titer was defined as the reciprocal of the dilution preceding the dilution at which the number of spirochetes or clumping was equal to the control. Generally, individual spirochetes with a few clumps were uniformly distributed throughout the fields on filters of the control supernatants.

### Statistical analysis

A t test (36) was used to determine significant differences in the titers of borrelial antibody. In addition, differences in edema between treatment groups were tested by two-way analysis of variance, utilizing the Minitab statistical analysis program. The Fisher least-significant-difference test (36) was used to examine pairs of means when a significant difference was determined. The standard error of the mean was also calculated for each mean caliper value.
RESULTS

Effect of anti-murine IFN-γ on production of borreliacidal antibody. Ten C3H/HeJ mice were vaccinated with formalin-inactivated *B. burgdorferi* in aluminum hydroxide. Lymph node cells were isolated 17 days after vaccination, cultured with macrophages and *B. burgdorferi*, and treated with anti-murine IFN-γ, rIFN-γ, or an isotype-nonspecific antibody (Fig. 1). An eightfold increase in borreliacidal antibody was detected in supernatant obtained from cultures of lymph node cells treated with anti-murine IFN-γ (titer 4,096) compared with levels of borreliacidal antibody detected in supernatant obtained from lymph node cells treated with an isotype nonspecific antibody (titer 512). In contrast, treatment of lymph node cells with rIFN-γ inhibited the borreliacidal antibody response (titer 4). When these studies were repeated five times with 50 mice, anti-murine IFN-γ augmented borreliacidal antibody production 8- to 16-fold.

Effect of anti-murine IFN-γ on induction of arthritis. Lymph node cells were obtained from 17-day vaccinated mice and cultured with macrophages and *B. burgdorferi* in the presence or absence of anti-murine IFN-γ, an isotype-nonspecific antibody, or rIFN-γ for 24 h. Subsequently, each group of treated lymph node cells was injected into the hind paws of normal C3H/HeJ mice (Fig. 2). A fourth group of four mice was injected in the hind paws with lymph node cells cultured with macrophages and *B. burgdorferi* obtained from nonvaccinated mice. Swelling of the hind paws was detected in all groups because infection with *B. burgdorferi* alone can induce edema of the hind paws (3, 19, 23, 32). However, the severity of swelling was greater in recipients infused with lymph node cells treated with anti-murine IFN-γ or rIFN-γ. Of these two groups, the hind paws of recipients of lymph node cells treated with anti-murine IFN-γ were more swollen day 18 to 21 after cell transfer. When these experiments were repeated three times with 36 mice, similar results were obtained.

Histopathology of the hind paws. Twenty-one days after cell transfer, destructive arthropathy was detected in the tibiotarsal joints of recipients of lymph node cells treated with anti-murine IFN-γ (Fig. 3B and C). A dense proliferation of synovioocytes and fibroblasts was observed on the periphery of the periosteum and surrounding tendons (Fig. 3B). Focal erosion of bone was also detected (Fig. 3C). By contrast, only a mild synovitis was found in recipients infused with lymph node cells treated with rIFN-γ, despite the presence of edema (Fig. 2). Synoviocyte-fibroblast proliferation was present in the periarticular soft tissue and flexor and extensor tendons (Fig. 3E). However, the synoviocyte-fibroblast proliferation was considerably less than the proliferation detected in recipients of lymph node cells treated with anti-murine IFN-γ. Similar histopathology was detected in recipients of lymph node cells treated with an isotype-nonspecific antibody (Fig. 3D). The synoviocyte-fibroblast proliferation enveloped the flexor and extensor tendons and was present in the periarticular soft tissue. Recipients injected with lymph node cells from nonvaccinated mice also developed a mild synovitis (Fig. 3F), while recipients infused with culture medium (Fig. 3A) had no significant histopathology.

In vivo borreliacidal antibody production after infusion of lymph node cells. Sera were obtained from recipient mice 21 days after infusion of lymph node cells from nonvaccinated mice and immune lymph node cells treated with anti-murine IFN-γ, rIFN-γ, or an isotype-nonspecific antibody (Table 1). No significant differences (*P* > 0.05) in the level of borreliacidal antibody were detected among the groups.

Flow cytometric examination of lymph node cells. Table 2 shows that treatment of lymph node cells with anti-murine IFN-γ, rIFN-γ, or an isotype-nonspecific antibody did not greatly alter the T-lymphocyte populations. An increase in CD8+ T lymphocytes (5%) was detected in lymph node cells treated with anti-murine IFN-γ. No differences in the populations of CD4+ T lymphocytes was detected among these groups.

DISCUSSION

Although vaccination with *B. burgdorferi* or its components induce protective borreliacidal antibody (1, 20, 27, 28, 30, 33), the duration of protection is short, less than 8 weeks (24). The development and maintenance of a high level of sustained borreliacidal antibody is paramount for prolonged protection against infection with *B. burgdorferi*. Previously, we showed (21, 22) that treatment of cells capable of producing borreliacidal antibody with exogenous IL-4 or IFN-γ failed to augment borreliacidal activity.

We now show that neutralization of IFN-γ does enhance borreliacidal antibody production. However, when anti-murine IFN-γ treated cells were transferred to normal C3H/HeJ recipients, the mice developed severe destructive arthritis. These results show that cell populations capable of producing enhanced borreliacidal antibody also contain cells capable of inducing severe destructive arthritis. Since we used a whole-cell vaccine, these results might be expected because of the numerous outer surface proteins of *B. burgdorferi*. However, subunit vaccines may also contain amino acid sequences capable of inducing adverse effects even though they can induce borreliacidal antibody. Our in vitro assay will be valuable for testing both effects.

This study demonstrates for the first time that borreliacidal antibody can be augmented by preventing the effects of IFN-γ.
When lymph node cells capable of producing borreliacidal antibody were treated with anti-murine IFN-γ, an eightfold increase in borreliacidal antibody production was obtained. These results suggest that the amount of endogenous IFN-γ present in cultures of borreliacidal antibody producing cells affects the level of production of borreliacidal antibody. In support, we showed previously that addition of exogenous IFN-γ to cells capable of producing borreliacidal antibody abrogated the borreliacidal response (21).

The mechanism by which IFN-γ controls the production of borreliacidal antibody is unknown. It is known that IFN-γ is an antagonist for IL-4, a known B-lymphocyte stimulator. Neutralization of IFN-γ may enhance borreliacidal antibody production by up-regulation of IL-4. When rIL-4 was added to cells capable of producing borreliacidal antibody, it also failed to enhance borreliacidal antibody production (22). Moreover, neutralization of endogenous IL-4 failed to alter production of borreliacidal antibody. Collectively, these results suggest that a cytokine(s) other than IFN-γ and IL-4 is responsible for the modulation of borreliacidal antibody. Additional experiments are needed to determine which cytokine(s) augments borreliacidal antibody production in anti-murine IFN-γ-treated borreliacidal antibody-producing cells.

Although treatment of immune cells with anti-murine IFN-γ enhanced in vitro production of borreliacidal antibody, a similar response was not detected in recipients of these cells. In fact, no significant differences in the levels of borreliacidal activity were detected among groups that received nonimmune cells or cells treated with anti-murine IFN-γ, rIFN-γ, or an isotype-nonspecific control antibody. These results suggest that
Borreliacidal antibody producing cells can be rapidly down-regulated by host cells. In support, Jensen et al. (12) showed that production of borreliacidal antibody could be prevented by incubating antibody producing cells with cells incapable of producing cidal antibody. The mechanism of downregulation may include IL-4 (26) and IFN-γ (25). We showed previously that IL-4 and IFN-γ could abrogate the borreliacidal antibody response (21, 22). Another explanation is that borreliacidal antibody production in vivo was delayed. In support, borreliacidal activity is not detected in vitro until 6 days after incubation (22).

These results are encouraging because enhanced production of borreliacidal antibody was achieved. However, treatment of borreliacidal antibody-producing cells with anti-murine IFN-γ also augmented the ability of these cell populations to induce arthritis. When cells containing borreliacidal antibody-producing cells were infused into recipients, histopathologic responses were observed (Fig. 3).

![Histopathologic responses in C3H/HeJ mice infused with DMEM (A) or immune lymph node cells treated with anti-murine IFN-γ (B, C), isotype-nonspecific antibody (D), or rIFN-γ (E). Lymph node cells from nonvaccinated C3H/HeJ mice (F) were also infused into recipients. Magnification, ×400.](image)

**TABLE 1.** Borreliacidal antibody titers in sera from recipient C3H/HeJ mice 21 days after infusion of cultures of immune lymph node cells treated with anti-murine IFN-γ, an isotype-nonspecific antibody, or rIFN-γ

| Treatment group    | Borreliacidal antibody titer |
|--------------------|------------------------------|
| Nonimmune          | 80                           |
| Anti-murine IFN-γ  | 80                           |
| Isotype-nonspecific antibody | 160                   |
| rIFN-γ             | 160                          |

**TABLE 2.** Percentages of CD4+ and CD8+ T lymphocytes, as determined by flow cytometry, within populations of borreliacidal antibody-producing cells treated with an isotype-nonspecific antibody, anti-murine IFN-γ, or rIFN-γ

| Treatment group    | Mean % CD4+ ± SE | Mean % CD8+ ± SE |
|--------------------|------------------|------------------|
| Isotype-nonspecific antibody | 55 ± 0.22         | 23 ± 0.31         |
| Anti-murine IFN-γ  | 54 ± 0.22         | 28 ± 0.31         |
| rIFN-γ             | 53 ± 0.22         | 22 ± 0.31         |
ing cells treated with anti-murine IFN-γ were infused into immunocompetent C3H/HeJ recipients, they developed severe destructive Lyme arthritis. Histopathologic examination showed that severe erosion of bone had occurred. The severity of the arthropathy detected in recipients of anti-IFN-γ-treated cells exceeded the histopathology reported previously in other immunocompetent murine models of Lyme borreliosis (3, 19, 23, 32). These studies (3, 19, 23, 32) and ours showed that mice infused with rIFN-γ-treated borreliacidal antibody-producing cells, antibody-producing cells treated with a isotype-nonspecific control antibody, or nonimmune cells developed mild synovitis without erosion of bone.

Brown and Reiner (4) showed that IFN-γ was not required for the development or resolution of arthritis. When IFN-γ-deficient C3H mice were challenged with *B. burgdorferi*, they developed arthritis. By contrast, Keane-Myers and Nickell (14-15) showed in cell depletion experiments that CD8+ T-lymphocyte-derived IFN-γ promoted the development of arthritis while CD4+ T lymphocytes prevented arthropathy. Although we found no difference in CD4+ T lymphocyte populations, we did detect a 5% increase in CD8+ T lymphocytes in lymph node cell cultures treated with anti-murine IFN-γ. It is doubtful, however, that these cells could produce enough IFN-γ in the presence of anti-murine IFN-γ to have an effect in vivo. In support, we showed that rIFN-γ-treated cells failed to induce severe destructive Lyme arthritis. Recipients of rIFN-γ-treated cells developed a mild synovitis similar to the synovitis detected in mice challenged with *B. burgdorferi*.

The inability of anti-murine IFN-γ treatment of immune cells obtained from *B. burgdorferi* vaccinated mice to decrease adverse arthritic effects, despite augmenting production of borreliacidal antibody, does not lessen the significance of this finding. Borrelial vaccines could be easily evaluated with this assay for increased production of borreliacidal antibody and for the ability to induce arthritis. Lymph node cells from vaccinated mice would be cultured in the presence of macrophages, borrelial vaccine, and anti-murine IFN-γ. The level of production of borreliacidal antibody could be readily determined in vitro, while infusion of these anti-murine IFN-γ-treated cells into recipients would assess their ability to induce arthritis. If a vaccine induced both borreliacidal antibody and arthritis, selective deletion of epitopes that did not affect the production of borreliacidal antibody could be determined by retesting the modified vaccine. Presently, we are using this system to determine the arthritogenic epitopes on OspA.

In conclusion, neutralization of IFN-γ in cultures of cells from *B. burgdorferi*-vaccinated mice enhanced both the production of borreliacidal antibody and the development of arthritis. Our in vitro-in vivo system can easily be used to evaluate the potential of borrelial vaccines for induction of borreliacidal antibody and arthritis. Additional studies are needed to delineate the cytokine mechanism(s) that prevents the development of arthritis and yet augments borreliacidal antibody production. These studies are necessary to ensure an effective and safe vaccine against infection with *B. burgdorferi*.

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