Gamma Interferon Inhibits Production of Anti-OspA Borreliacidal Antibody In Vitro

Erik L. Munson,1,2 Brian K. Du Chateau,1,3† Jani R. Jensen,1,2 Steven M. Callister,4 David J. DeCoster,1,2 and Ronald F. Schell1,2,3,*

Wisconsin State Laboratory of Hygiene1 and Departments of Medical Microbiology and Immunology2 and Bacteriology3
University of Wisconsin, Madison, Wisconsin 53706, and Microbiology Research Laboratory and Department of Infectious Diseases, Gundersen Lutheran Medical Center, La Crosse, Wisconsin 546012

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The ability of a Lyme borreliosis vaccine to induce and maintain sustained levels of borreliacidal antibody is necessary for prolonged protection against infection with *Borrelia burgdorferi*. Vaccination against infection with *B. burgdorferi* could be improved by determining the mechanism(s) that influences the production of protective borreliacidal antibody. Borreliacidal antibody was inhibited in cultures of lymph node cells obtained from C3H/HeJ mice vaccinated with formalin-inactivated *B. burgdorferi* and cultured with macrophages and *B. burgdorferi* and treated with recombinant gamma interferon (rIFN-γ). The suppression of production of outer surface protein A (OspA) borreliacidal antibody by rIFN-γ was not affected by the time of treatment. In addition, treatment with rIFN-γ inhibited the production of other anti-*B. burgdorferi* antibodies. By contrast, treatment of cultures of immune lymph node cells with anti-IFN-γ marginally increased the production of borreliacidal antibody and enhanced the production of other antibodies directed against *B. burgdorferi*. These results show that IFN-γ does not play a major role in the production of anti-OspA borreliacidal antibody. Additional studies are needed to determine which cytokine(s) will enhance production of borreliacidal antibody.

Public health concerns about the morbidity associated with Lyme borreliosis have prompted the development of several vaccines to prevent infection with *Borrelia burgdorferi* (34, 38, 39). The efficacies of the vaccines are based on the ability of a major outer surface protein (Osp) of *B. burgdorferi*, specifically OspA, to induce antibody that can kill the Lyme spirochete in vaccinees (7, 25, 35) or ticks (13). In an extensive field trial involving 10,936 participants, the vaccine was 76% effective in preventing infection with *B. burgdorferi* after three inoculations (38). However, the duration of protection had not been determined (38). In addition, we showed previously that vaccination of humans with recombinant OspA (rOspA) induced only low levels of anti-OspA borreliacidal antibody and that the borreliacidal response waned rapidly (25). Only one individual had detectable anti-OspA borreliacidal antibody after 180 days. A similar anti-OspA borreliacidal antibody response was detected in hamsers vaccinated with rOspA (25). The poor antibody response induced by vaccination may have contributed to the withdrawal of the vaccine.

The ability of rOspA or other protective immunogens to induce high and sustained levels of borreliacidal antibody is necessary to ensure prolonged protection against infection with *B. burgdorferi*. Recently, we developed an in vitro system to determine the effects that immunomodulatory mediators have on the production and regulation of borreliacidal antibody. Anti-OspA borreliacidal antibody was readily produced when lymph node cells obtained from vaccinated mice were cultured with macrophages and *B. burgdorferi* (23). When OspA borreliacidal antibody-producing cells were exposed to a known B-lymphocyte-stimulating factor (27), interleukin 4 (IL-4), borreliacidal-antibody production was inhibited. Furthermore, treatment of the immune lymph cell cultures with anti-murine IL-4 did not alter the production of anti-OspA borreliacidal antibody. These results suggested that IL-4 plays a minor role in the production and up-regulation of borreliacidal antibody.

The inability of IL-4-stimulated immune lymph node cells to increase production of borreliacidal antibody may be due to down-regulation of gamma interferon (IFN-γ). It is known that IL-4 strongly down-regulates functions promoted by IFN-γ (26), especially class switching to immunoglobulin G2a (IgG2a) by B lymphocytes (36). Since *B. burgdorferi* organisms are killed by IgG2a and complement (23), we sought evidence of whether IFN-γ augments anti-OspA borreliacidal-antibody production. Such information could provide insight into the mechanism of borreliacidal-antibody production and contribute to the development of a more efficacious Lyme borreliosis vaccine.

Materials and Methods

Mice. Eight- to 12-week-old inbred C3H/HeJ mice were obtained from our breeding colony located at the Wisconsin State Laboratory of Hygiene. Mice weighing 20 to 40 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 spinal fluid (37). Low-passage (<6) organisms were cultured once in modified Barbour-Stoenner-Kelly (BSK) medium (3) containing screened lots of bovine serum albumin (6) 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 resuspended in fresh DMEM, the pellets were resuspended in 1 ml of cold DMEM. Cell viability was determined by trypan blue exclusion with 10% heat-inactivated fetal bovine serum, L-glutamine, and 2-mercaptoethanol (2.92 mg/ml; Sigma). Aliquots of the cell suspension were then poured over 100 by 20 mm polystyrene tissue culture dishes (Corning Glass Works, Corning, N.Y.) and uniformly distributed throughout the fields on the dish. Cells were resuspended in 1 ml of cold DMEM. Cell viability was determined by trypan blue exclusion.

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. Three washes were performed 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 (alum; Reheis, Berkeley Heights, N.J.) to yield 4 × 10^6 spirochetes/ml.

Vaccination of mice. We showed previously (23) that mice vaccinated with whole cells of B. burgdorferi with or without alum yielded higher levels of anti-OspA borreliacidal antibody than those vaccinated with rOspA in the presence or absence of alum. Therefore, whole cells of B. burgdorferi were used in this investigation. Whole cells are not recommended as a vaccine for human usage. The ability of whole cells to consistently induce anti-OspA borreliacidal antibody in mice (23) permits evaluation of cytokine mechanisms responsible for induction of anti-OspA borreliacidal ability.

Sixty mice were mildly anesthetized with methoxyflurane contained in a mouth-and-nose cup and vaccinated subcutaneously in the inguinal region with 0.25 ml (10^6 spirochetes/ml) of B. burgdorferi organisms of the formalin-inactivated vaccine preparation. The suspension contained approximately 100 µg of borreliacidal protein. Sham-vaccinated mice were injected with BSK medium or alum alone.

Reciprocal of spirochetes. Five to 10 mice per experimental protocol were mildly anesthetized with methoxyflurane contained in a mouth-and-nose cup and injected intraperitoneally with 2 ml of 3% 3-week-old thioglycolate in PBS. Four days after injection, the mice were euthanized by CO2 asphyxiation, and 8 ml of peritoneal exudate cells were removed from vaccinated and nonvaccinated mice and placed in cold 10% glycerol (Sigma Chemical Co., St. Louis, Mo.), sealed, and stored at −70°C. When resuspended in fresh DMEM, the pellets were resuspended in 1 ml of cold DMEM. Cell viability was assessed by trypan blue exclusion with 10% heat-inactivated fetal bovine serum, L-glutamine, and 2-mercaptoethanol (2.92 mg/ml; Sigma). Aliquots of the cell suspension were then poured over 100 by 20 mm polystyrene tissue culture dishes (Corning Glass Works, Corning, N.Y.) and uniformly distributed throughout the fields on the dish. Cells were resuspended in 1 ml of cold DMEM. Cell viability was determined by trypan blue exclusion.

Production of antibody in vitro. Sterile six-well flat-bottom tissue culture dishes (Becton Dickinson, Lincoln Park, N.J.) were inoculated with lymph node cells (5 × 10^6) obtained from vaccinated and nonvaccinated mice, macrophages (10^6), and 10 live B. burgdorferi organisms. DMEM was added to the suspensions of cells to bring the final volume to 3 ml. On days 3, 6, 9, 12, and 15 after culture, the media were removed at 37°C in the presence of 5.0% CO2, 10-ml samples of the supernatants were removed after gentle agitation and replaced with equal volumes of warm DMEM. In some experiments, rIFN-γ at quantities ranging from 0.1 to 10 µg or rat anti-murine IFN-γ at quantities ranging from 25 to 75 µg (R&D Systems, Minneapolis, Minn.) was added to cultures of immune lymph node cells, macrophages, and B. burgdorferi at 10 min and 2 and 4 days of incubation. In those experiments, one dilution of CD119 at quantities ranging from 100 to 1000 µg (PharMingen, San Diego, Calif.) was added to cultures of immune lymph node cells, macrophages, and B. burgdorferi at 10 min of incubation. In similar fashion, control cultures were treated with a rat isotype-nonspecific antibody. 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 they were used.

Detection of borreliacidal antibody by membrane filtration. The frozen supernatants were thawed, heat inactivated (56°C, 30 min), sterilized with a 0.22-µm-pore-size filter (Aercodisk; Gelman Sciences, Ann Arbor, Mich.), and serially twofold diluted (from undiluted to 1/8,192) 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 10^5 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 suspensions of nonimmune lymph node cells with macrophages and B. burgdorferi. Other controls included supernatants from nonimmune lymph node cells, macrophages alone, and DMEM.

After incubation, 100 µl of each suspension was removed and placed in 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 sterile 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 sterile test manifold (Millipore Corporation, Bedford, Mass.) attached to a vacuum pump. The membrane filters were washed with ∼8 ml of sterile double-distilled H2O (ddH2O), removed from the vacuum apparatus, allowed to dry, and placed onto glass microscope slides. Coverslips were placed on the filters before they were viewed with a Labolorax S fluorescence microscope (Leitz, Wetzlar, Germany) using an ×50 oil immersion objective.

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

Western immunoblotting. B. burgdorferi 297 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 PBS at pH 7.4. The washed pellet was resuspended in 1% formalin and incubated at 32°C for 30 min with periodic mixing and then washed three times by centrifugation with PBS (12,000 × g; 10°C; 15 min) and resuspended in PBS. The spirochetes were suspended in sodium dodecyl sulfate-polyacrylamide gel electrophoresis sample buffer and boiled for ∼3 min. One hundred twenty micrograms of B. burgdorferi lysate was loaded onto a preparative 5% polyacrylamide gel. The supernatant was removed, and the gel was stained with Coomassie blue. Electrophoresis was performed at 70 mA constant current with the buffer system of Laemmli (17). The proteins were transferred onto a nitrocellulose membrane for 1 h at 20 V, using a semidry blotting apparatus (Bio-Rad Laboratories, Hercules, Calif.). The nitrocellulose membrane was incubated overnight at 4°C in 5% milk dissolved in Tris-buffered saline with 0.05% Tween 20 (TBS-T; pH 7.4) to block nonspecific reactivity, washed two times each with TBS-T and ddH2O, allowed to dry, and finally cut into strips. The strips were washed three times with TBS and subsequently incubated for 1 h with a 1:1,000 dilution of an alkaline phosphatase-conjugated goat anti-murine IgG (heavy and light chain specific; Kirkegaard & Perry Laboratories, Gaithersburg, Md.) in 5% milk in TBS-T. This was followed by four washes with TBS. The strips were developed by the addition of 5-bromo-4-chloro-3-indolylphosphate–nitroblue tetrazolium substrate (Kirkegaard & Perry). The reactions were stopped after 2 min with several large volumes of chilled ddH2O.

Flow cytometric analysis of immune supernatants. Suspensions of immune lymph node cells containing macrophages and B. burgdorferi in the presence or absence of IFN-γ, anti-IFN-γ, or isotype-nonspecific antibody were analyzed for the numbers of cells, B lymphocytes, and T lymphocytes on day 4 of incubation by using flow cytometry. Brieﬂy, suspensions (1 ml) of immune lymph node cells were mixed with chilled centrifuge tubes, and the total number of lymphocytes was determined. The suspensions of cells were then mixed with both phycoerythrin-conjugated anti-murine CD3 (5 µl of a 1:5 dilution; PharMingen) and fluorescein isothiocyanate-conjugated anti-murine CD45R/B220 (10 µl of a 1:50 dilution; PharMingen) and incubated at 4°C for 15 min. The cells were washed twice by
centrifugation with PBS containing 0.1% bovine serum albumin (1,500 rpm; 4°C; 10 min). The pellets of cells were resuspended in 250 µl of cold DMEM and kept in the dark at 4°C until they were analyzed by flow cytometry.

One hundred microliters of 50-µg/ml propidium iodide (Sigma) was then added to each tube just prior to acquisition by the flow cytometer to discriminate viable and nonviable cells. Data were acquired on a FACSCalibur flow cytometer (Becton Dickinson, San Jose, Calif.) using CellQuest acquisition and analysis software (Becton Dickinson). Twenty thousand events were detected by forward and side angle light scatter and by propidium iodide, phycoerythrin, and fluorescein isothiocyanate fluorescence. A dot blot profile of forward angle light scatter and propidium iodide fluorescence enabled identification and gating of live lymphocyte populations. The gated events were subsequently analyzed by quadrant dot blots of phycoerythrin and fluorescein isothiocyanate fluorescence for enumeration of CD3+ and B220+ lymphocytes in a given sample.

Statistical analysis. A t-test (41) was used to determine significant differences in the titers of borreliacidal antibody and in lymphocyte counts among supernatants. In addition, titers that were determined during kinetic studies of in vitro borreliacidal antibody production were tested by analysis of variance, utilizing the Minitab statistical analysis program. The Fisher least-significant-difference test (41) was used to examine pairs of means when a significant F ratio indicated reliable mean differences. The alpha level was set at 0.05 before the experiments were started.

RESULTS

Modulation of in vitro production of anti-OspA borreliacidal antibody by IFN-γ. We showed previously (23) that anti-OspA borreliacidal antibody is readily detected in supernatants of lymph node cells obtained from mice vaccinated with formalin-treated *B. burgdorferi* in adjuvant and cultured with macrophages and *B. burgdorferi* for several days. The data presented in Fig. 1 confirms and extends these findings. High levels of anti-OspA borreliacidal antibody (titer, 256) were detected in supernatants on day 6 of culture of immune lymph node cells with macrophages and *B. burgdorferi*. The peak anti-OspA borreliacidal activity (titer, 1,024) was detected with supernatants obtained on days 9 to 15 of culture. When cultures of immune lymph node cells with macrophages and *B. burgdorferi* were exposed to 0.1, 1.0, or 10 µg of rIFN-γ, the levels of anti-OspA borreliacidal antibody were significantly reduced 16-fold or more. Maximum suppression of anti-OspA borreliacidal antibody occurred in cultures of lymph node cells exposed to 0.1 µg of rIFN-γ followed by 1.0 and 10 µg of rIFN-γ. The borreliacidal activity was due to the production of anti-OspA antibody. Adsorption of the supernatant from immune lymph node cells with rOspA reduced the borreliacidal-antibody titer. No borreliacidal antibody was detected in supernatants obtained from nonimmune lymph node cells cultured with macrophages and *B. burgdorferi*. When these studies were repeated three times, similar results were obtained.

In other studies, immune lymph node cells were treated with 25, 50, or 75 µg of anti-murine IFN-γ. Anti-OspA borreliacidal-antibody production was increased two- to fourfold in supernatants obtained from immune lymph node cell cultures treated with 50 or 75 µg of anti-IFN-γ for 9 days or more compared to the levels of anti-OspA borreliacidal antibody detected in cultures of untreated immune lymph node cells (Fig. 2). However, treatment of immune lymph node cells with 25 µg of anti-IFN-γ did not affect the production of borreliacidal antibody. No borreliacidal antibody was detected in supernatants obtained from nonimmune lymph node cells cultured with macrophages and *B. burgdorferi*. Similar results were obtained when these studies were repeated.

Temporal effects of rIFN-γ and anti-IFN-γ on the produc-

![Temporal effects of rIFN-γ and anti-IFN-γ on the production of anti-OspA borreliacidal antibody](image)

**FIG. 1.** Effect of 0.1 (●), 1 (▲), or 10 µg (○) of rIFN-γ on production of borreliacidal antibody by lymph node cells obtained from 17-day-vaccinated mice cultured with macrophages and *B. burgdorferi* for 3, 6, 9, 12, and 15 days. Controls included lymph node cells obtained from vaccinated (■) and nonvaccinated (▲) mice cultured with macrophages and *B. burgdorferi*. The error bars indicate standard deviations.

**FIG. 4A** shows that supernatant obtained from untreated immune lymph node cell cultures treated with rIFN-γ or anti-IFN-γ at 10 min or 2 or 4 days after cultivation. Production of anti-OspA borreliacidal antibody was inhibited by rIFN-γ at all treatment intervals, although less effect was detected with rIFN-γ treatment on day 4 of cultivation (Fig. 3A). Treatment of immune lymph node cell cultures with anti-IFN-γ slightly enhanced production of anti-OspA borreliacidal antibody at all treatment intervals (Fig. 3B). When these studies were repeated, similar results were obtained.

Effects of rIFN-γ and anti-IFN-γ on production of anti- *B. burgdorferi* antibody. *B. burgdorferi* antigens induce production of antibodies to multiple *B. burgdorferi* antigens (Fig. 4C). Furthermore, supernatant from anti-IFN-γ-treated lymph node cell cultures had an anti-OspA Western immunoblot titer of 40,960 compared to a titer of 2,560 for supernatant obtained from untreated immune lymph node cell cultures.
Flow cytometric analysis of lymph node cell cultures. Immune lymph node cells cultured with macrophages and *B. burgdorferi* in the presence or absence of rIFN-γ/H9253 or anti-IFN-γ/H9253 for 4 days were analyzed for viability and numbers of T and B lymphocytes by using flow cytometry. Figure 5 shows that the number of viable cells in lymph node cell cultures treated with rIFN-γ (Fig. 5B) decreased approximately 25% from the number of viable cells detected in cultures of lymph node cells treated with an isotype-nonspecific antibody (Fig. 5A) or anti-IFN-γ (Fig. 5C). In addition, the number of B lymphocytes (B220 marker) decreased approximately 50% in lymph node cell cultures treated with rIFN-γ (Fig. 5B) compared to the control (Fig. 5A). By contrast, a 10% increase in B lymphocytes was detected in lymph node cell cultures treated with anti-IFN-γ (Fig. 5C). Similarly, the number of T lymphocytes (CD3 marker) decreased approximately 25% in cultures of lymph node cells treated with rIFN-γ (Fig. 5B), while a 6% decrease was detected in cultures treated with anti-IFN-γ (Fig. 5C) compared to control cultures (Fig. 5A). When these studies were repeated, similar results were obtained.

Effect of rIFN-γ on macrophages. In other studies, the lymph node cell cultures treated with an isotype-nonspecific antibody (control), rIFN-γ, or anti-IFN-γ were gently washed to remove nonadherent cells and viewed by light microscopy. The macrophages cultured with rIFN-γ were rounded and lacked pseudopodia (Fig. 6B), whereas macrophages treated with the isotype-nonspecific antibody or anti-IFN-γ were spindle shaped with long pseudopodia (Fig. 6A).

**DISCUSSION**

*B. burgdorferi* organisms are easily killed when they bind specific antibody that activates complement (8, 31, 32). Several outer surface proteins of *B. burgdorferi*, including OspA, OspB, OspC, and the 39-kDa protein (2, 22, 28–30, 33), induce complement-dependent borreliacidal antibody. This feature makes these outer surface proteins ideal candidates for development of a vaccine to prevent infection with *B. burgdorferi*. Of these proteins, OspA has been used to vaccinate humans (34, 38, 42) and animals (11, 13, 19).

Although rOspA is immunogenic, the antibody response is dominated by production of antibody that is not protective (10, 12). Most importantly, vaccinated animals challenged with *B. burgdorferi* during periods with concomitant high levels of nonbactericidal anti-OspA and low levels of anti-OspA borreliacidal antibodies develop arthritis (11, 19, 20). Recently, we showed that hamsters vaccinated with 30, 60, or 120 μg of rOspA with or without alum developed severe destructive arthritis when challenged with *B. burgdorferi* (11). Similarly, hamsters vaccinated with a commercially available canine rOspA vaccine developed severe destructive arthritis after challenge with the Lyme spirochete (11). Only the presence of high levels of borreliacidal antibody prevented infection with *B. burgdorferi* from inducing arthritis in vaccinated animals (19). Maximum protection due to borreliacidal antibody, however, is restricted to 7 to 9 weeks after vaccination.
One approach to increase the duration of protection and lessen the potential for adverse effects is to determine the immune mediators responsible for the production and maintenance of borreliacidal antibody. We showed previously (23) that IL-4 does not augment the production of anti-OspA borreliacidal antibody, even though IL-4 is known to up-regulate B-lymphocyte growth and differentiation (27). In fact, IL-4 inhibited the production of anti-OspA borreliacidal antibody, including IgG2a, by lymph node cells obtained from vaccinated mice (23). It is known that IgG2a is negatively regulated by IL-4 but up-regulated by IFN-γ (26). Our data and those of others (26, 27) suggested that IL-4 counteracted the effects of IFN-γ on the production of borreliacidal antibody.

When lymph node cells producing borreliacidal antibody were exposed to rIFN-γ, borreliacidal antibody production was inhibited. The time of exposure (10 min to 4 days) of immune lymph node cells to rIFN-γ did not affect the inhibition of production of borreliacidal antibody. In addition, treatment with rIFN-γ inhibited the production of other anti-\textit{B. burgdorferi} antibodies. The suppression of borreliacidal and other anti-\textit{B. burgdorferi} antibodies by rIFN-γ was unexpected. Flow cytometric analysis of rIFN-γ-treated and untreated immune lymph node cells showed that treatment with rIFN-γ reduced the number of viable lymphocytes, especially B lymphocytes. In addition, macrophages cultured in the presence of exogenous rIFN-γ were rounded and exhibited rare pseudopodia. By contrast, macrophages obtained from cultures of untreated lymph node cells producing borreliacidal antibody were spindle shaped with many pseudopodia. IFN-γ has been shown to induce apoptosis in T (1, 21, 24) and B (15, 16, 40) lymphocytes. These events could have affected the activation of macrophages and their ability to process borrelial antigen.

It is possible that inhibition of borreliacidal antibody was due to the use of toxic concentrations of rIFN-γ. However, when more physiologically relevant concentrations (1.0 or 0.1 μg) of rIFN-γ were added to immune lymph node cells, borreliacidal activity also failed to increase. In addition, immune lymph node cells were treated with anti-CD119, which blocks the binding of murine IFN-γ to cellular receptors (9, 18). No significant decrease in borreliacidal-antibody production was

![FIG. 4. Western immunoblots obtained with supernatants from cultures of immune lymph node cells (day 17 after vaccination) cultured with macrophages and \textit{B. burgdorferi} without treatment (A) or treated with IFN-γ (B) or anti-IFN-γ (C). The supernatants were diluted 1:160 (lanes I) or 1:640 (lanes II).]
detected in these cultures compared to immune lymph node cells treated with an isotype-nonspecific antibody (data not shown). These results suggest that IFN-γ is not a major force in driving the production of borreliacidal antibody.

Perhaps the most compelling evidence that IFN-γ is not responsible for production of borreliacidal antibody came from experiments utilizing neutralizing antibody to IFN-γ. Treatment of immune lymph node cells with various concentrations of anti-IFN-γ failed to suppress borreliacidal activity. In fact, borreliacidal antibody production was marginally enhanced, especially when treatment with anti-IFN-γ occurred early in cultivation of the immune lymph node cells. Furthermore, treatment with anti-IFN-γ resulted in polyclonal expansion of the anti-B. burgdorferi antibody response. In support of this idea, by using flow cytometric analysis, we also detected a significant increase in the number of B lymphocytes in cultures of immune lymph node cells treated with anti-IFN-γ.

IFN-γ performs numerous immunologic functions, including T helper lymphocyte differentiation and stabilization (4, 5), enhancement of major histocompatibility complex expression on both B lymphocytes and macrophages (4, 5), antiviral effects (4, 5, 43), and amelioration of production of IgG2a (14). Our results, however, show that IFN-γ suppresses antibody production, including IgG2a borreliacidal antibody (23). An explanation may be that IgG2a antibody expression is not completely dependent upon IFN-γ (14). In addition, IFN-γ may prevent the expression of IL-4 functions (26). However, we showed
previously that IL-4 suppressed the production of borreliacidal antibody (23). This suggests that other cytokines, but not IL-4 or IFN-γ, may be responsible for the induction, production, and maintenance of borreliacidal antibody. In support of this idea, treatment with anti-IFN-γ augmented not only borreliacidal antibody but other antibodies directed against B. burgdorferi. Additional studies are needed to determine which cytokines are responsible for the production of borreliacidal antibody.

In conclusion, we showed that IFN-γ plays a major role in suppression of the production of borreliacidal antibody. Effective neutralization of endogenous IFN-γ slightly augmented the production of borreliacidal antibody and expanded the anti-B. burgdorferi antibody responses. Determination of the mechanism that inhibits the production of borreliacidal antibody by rIFN-γ may lead to the development of a safe and effective Lyme borreliosis vaccine.

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