Interleukin-6 Promotes Anti-OspA Borreliacidal Antibody Production In Vitro

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Determination of the immunological mediators responsible for promoting the production of borreliacidal antibody may facilitate the development of an improved borreliosis vaccine for human and veterinary use. Previously, we developed an in vitro assay to determine if borreliacidal antibody production could be augmented by treatment with different cytokines. In this study, in vitro treatment of lymph node cells producing borreliacidal antibody with recombinant interleukin-6 (rIL-6) resulted in a fourfold enhancement of anti-OspA borreliacidal antibody. Moreover, rIL-6 enhanced Western immunoblot titers and increased the number of B lymphocytes. In contrast, treatment of anti-OspA borreliacidal antibody-producing cells with anti-IL-6 resulted in a fourfold reduction in borreliacidal activity. Treatment with anti-IL-6 also inhibited enhanced borreliacidal antibody production induced by anti-gamma interferon. These data suggest that IL-6 plays a significant role in the production of anti-OspA borreliacidal antibodies.

The increased worldwide prevalence of Lyme borreliosis has prompted the development of subunit vaccines to prevent infection by Borrelia burgdorferi. Recombinant outer surface protein A (rOspA) vaccines have been licensed for administration to humans (32, 34) and canines (22, 35). While OspA preparations induced significant anti-OspA antibody (10, 15, 18, 23, 26, 32, 33, 37), they failed in humans to concomitantly induce a strong and long-lived anti-OspA borreliacidal antibody response (23). The production of anti-OspA borreliacidal antibodies is essential for the efficacy of the recombinant vaccine (2, 6, 7, 29, 30). Unfortunately, the weak and short-lived borreliacidal antibody response may have contributed to the withdrawal of the recombinant vaccine for usage in humans.

Obviously, more needs to be known about the events that promote the production of sustained high levels of anti-OspA borreliacidal antibody. Therefore, we developed an in vitro assay to investigate the cytokine mechanisms that influence borreliacidal antibody production (5, 8, 20). An attempt to augment borreliacidal activity by the addition of interleukin-4 (IL-4), a known B-lymphocyte stimulator (25), to cultures of borreliacidal antibody-producing cells with recombinant interleukin-6 (rIL-6) resulted in a fourfold enhancement of anti-OspA borreliacidal antibody. Moreover, rIL-6 enhanced Western immunoblot titers and increased the number of B lymphocytes. In contrast, treatment of anti-OspA borreliacidal antibody-producing cells with anti-IL-6 resulted in a fourfold reduction in borreliacidal activity. Treatment with anti-IL-6 also inhibited enhanced borreliacidal antibody production induced by anti-gamma interferon. These data suggest that IL-6 plays a significant role in the production of anti-OspA borreliacidal antibodies.

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 30 g were housed at 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 (36). The low-passage (<10) organism was cultured in modified Barbour-Stoenner-Kelly (BSK) medium (3) containing screened lots of bovine serum albumin (4) to a concentration of 5 × 10^7 spirochetes per ml. Five-hundred-microliter samples were then dispensed into 1.5-ml screw-cap tubes (Sarstedt, Newton, NC) containing 500 μl 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 using 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 a 1% suspension of aluminum hydroxide (Reheis, Berkeley Heights, NJ) to yield 4 × 10^6 spirochetes/ml.

Vaccination of mice. Sixty-four mice were anesthetized with methoxyflurane contained in a mouth-and-nose cup and vaccinated subcutaneously in the inguinal region with 0.25 ml (−10^6 B. burgdorferi organisms) of the formalin-inacti-
vated vaccine preparation. The suspension contained approximately 100 µg of borrelial protein. Nonvaccinated mice were injected with BSK medium or aluminum hydroxide alone. These mice did not have a borreliacidal antibody response.

Recovery of macrophages. Three to five mice per experimental protocol were anesthetized with methoxyflurane contained in a mouth-and-nose cup and injected intraperitoneally with 2 ml of 3% thioglycollate in PBS. Four days after injection, mice were euthanized by CO2 asphyxiation, and 8 ml of cold Hank’s 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) that was free of antimicrobial agents but supplemented with 10% heat-inactivated (56°C, 45 min) fetal bovine serum (FBS; HyClone Laboratories, Logan, UT), 5 × 10^-5 M 2-mercaptoethanol (Sigma), and 1-glutamine (2.92 mg/ml; Sigma). Aliquots of the cell suspension were then poured into polytylene tissue culture dishes (100 × 20 mm; Corning Glass Works, Corning, NY) and incubated at 37°C in a humidified atmosphere of 5.0% CO2 for 4 to 6 h. After incubation, nonadherent cells were aspirated from the tissue culture dishes. The dishes were gently rinsed twice with 8-ml portions of warm Hank’s buffered salt solution to further eliminate nonadherent cells. Five milliliter of cold, nonenzymatic cell lifter (Sigma) was then added to each tissue culture dish and incubated at 4°C for 30 min. Macrophages were detached from the surfaces of the dishes by vigorously tapping and gently scraping the inside of the tissue culture dishes with a sterile rubber policeman. Suspensions of macrophages from several tissue culture dishes were aspirated, pooled, and centrifuged at 1,500 rpm for 10 min at 4°C. The supernatant was discarded, and the pellet DMEM supplemented with 25% fetal bovine serum was added. Cell viability was determined by trypan blue exclusion. The preparations of macrophages obtained by this method were 98% free of lymphocyte contamination (20).

Isolation of lymph node cells. Mice were euthanized by CO2 inhalation 17 days after vaccination with formalin-inactivated B. burgdorferi. Inguinal lymph nodes were removed from vaccinated and nonvaccinated mice and placed into cold DMEM. Single 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 FBS, 1-glutamine, and 2-mercaptoethanol. Lymph node cells were washed twice by centrifugation (1,500 rpm, 4°C, 10 min) with cold DMEM. Supernatants were decanted, and pellets were resuspended in 1 ml of cold DMEM. Cell viability was assessed by trypan blue exclusion.

Production of antibody in vitro. Sterile six-well flat-bottomed tissue culture dishes (Becton Dickinson, Lincoln Park, NJ) were inoculated with lymph node cells (5 × 10^5) obtained from vaccinated or nonvaccinated mice, 1 × 10^5 macrophages, and 1 × 10^6 live B. burgdorferi cells. DMEM was added to the suspensions of cells to bring the final volume to 3 ml. In some experiments, 0.2 µg of rIL-6 or 75 µg of goat anti-murine IL-6 (R&D Systems, Minneapolis, MN) was added to each culture. Immune lymph node lymphocytes and B. burgdorferi after 10 min of incubation. In a similar fashion, control cultures were treated with sterile PBS or 75 µg of a non-isotype-specific antibody (R&D Systems), respectively. In other experiments, cultures of immune lymph node cells were treated with 70 µg anti-murine IFN-γ (R&D Systems) after 10 minutes of cultivation and subsequently incubated with 75 µg of anti-murine IL-6 or a non-isotype-specific antibody. Separate cultures of immune lymph node cells were also treated with 0.1 µg of rIFN-γ (R&D Systems).

Collection of cell-free supernatants from cultures of lymph node cells. On day 9 of cultivation at 37°C in the presence of 5.0% CO2, 1.0-ml samples of the supernatants were removed after gentle agitation and replaced with an equal volume of warm DMEM. We showed previously (20) that day 9 of cultivation yielded the peak anti-OspA borreliacidal antibody response. 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.

Flow cytometric analysis of in vitro immune cell suspensions. Suspensions of immune lymph node cells containing macrophages and B. burgdorferi in the presence or absence of a non-isotype-specific antibody, anti-murine IL-6, or rIL-6 were analyzed in triplicate for numbers of viable cells, B lymphocytes, and T lymphocytes on day 9 of incubation by using flow cytometry. Briefly, suspensions (1.0 ml) of immune lymph node cells were placed in chilled centrifuge tubes, and the total number of lymphocytes was enumerated. The suspensions of cells were then mixed with both phycoerythrin-conjugated anti-murine CD3e (0.5 mg; 5 µl of a 1:5 dilution) (Pharmingen, San Diego, CA) and fluorescein isothiocyanate-conjugated anti-murine CD45RB/220 (0.5 mg; 10 µl of a 1:40 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 analysis by flow cytometry.

Other controls included supernatants from nonimmune lymph node cells, macrophages alone, and DMEM.

After incubation, 100 µl of each suspension was removed and placed into a 15-ml sterile tube and cultured 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 a 0.22-µm-pore-size Nuclepore polycarbonate membrane filter (47-mm diameter; Whatman Nuclepore, Clifton, NJ) under negative pressure with a single-place sterile test manifold (Millipore Corporation, Bedford, MA) attached to a vacuum manifold. Membrane filters were prepared with ~8 ml of Cell Groves in a sterile PBS was added. The suspensions were then washed five times with 0.15 M sodium chloride, 50 mM sodium bicarbonate, 0.7 M sodium phosphate, 3.5 mM sodium bicarbonate, and 0.01 M sodium nitrate (ddH2O), removed from the vacuum apparatus, allowed to dry, and then placed onto glass microscope slides. Coverslips were placed on the filters before viewing with a Laborlux S fluorescence microscope (Leitz, Wetzlar, Germany), using a 50× oil immersion objective.

The number of spirochetes on each filter was quantitated 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 in the control. Generally, individual spirochetes with a few clumps were uniformly distributed throughout the fields on filters of the control supernatants.

IL-6 EIA. Sterile 96-well flat-bottomed plates were coated with 50 µl of purified rat anti-murine IL-6 monoclonal antibody (MP5-20F3 clone; Pharmingen) to a concentration of 4 µg/ml. Following incubation at 21°C for 1 h, wells were washed three times with PBS containing 0.05% Tween 20 (PBS-T). Wells were blocked for 2 h with 200 µl of PBS containing 10% FBS (Serum Supreme; BioWhittaker, Inc., Walkersville, MD) (PBS-FBS). After three washes with PBS-T, wells were incubated in triplicate with 50 µl of supernatant samples for 4 h at 21°C. Wells were then washed five times with PBS-T and subsequently incubated for 45 min at 21°C with 50 µl of biotinylated rat anti-murine IL-6 (MP5-32C11 clone; Pharmingen) diluted in PBS-FBS to a concentration of 1 µg/ml. After seven washes with PBS-T, wells were incubated (30 min, 21°C, dark conditions) with 75 µl of a 1:10,000 dilution of alkaline phosphatase-conjugated streptavidin (Kirkegaard & Perry Laboratories, Gaithersburg, MD) in PBS-FBS. Following nine washes with PBS-T, wells were incubated for 1 h with 100 µl of a solution of p-nitrophenyl phosphate (Sigma) and 2 µl of the substrate solution in PBS-T. Wells were then washed five times with PBS-T and subsequently incubated for 45 min at 21°C with 50 µl of 3-bromoindolyl phosphate (Sigma). Reactions were stopped by the addition of 100 µl of 1 M sodium carbonate, and the absorbance at 450 nm was immediately determined. Optical density readings of supernatants were converted into picograms per milliliter through the use of a standard curve obtained from an enzyme immunoassay (EIA) performed on rIL-6 (Pharmingen) that was serially diluted twofold in DMEM-FBS from an initial concentration of 1,000 pg/ml to ~7.8 pg/ml.

Western immunoblotting. 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 PBS at pH 7.4. The washed pellet was resuspended in 1% formalin, incubated at 32°C for 30 min with periodic mixing, washed three times with centrifugation with PBS (12,000 × g, 10°C, 15 min), and resuspended in...
PBS. The borrelial protein content was determined by using a bicinchoninic acid assay (Sigma). 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 into a preparative 12% acrylamide gel, and the proteins were resolved by overnight electrophoresis with an ~7 mA constant current with the buffer system of Laemmli (14). The proteins were transferred onto a nitrocellulose membrane for 1 h at 20 V, using a semidyed blotting apparatus (Bio-Rad Laboratories, Hercules, CA). The nitrocellulose membrane was incubated overnight at 4°C in 5% milk dissolved in Tris-buffered saline (TBS) 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. Strips were then incubated for 1 h with samples of the supernatants (diluted in 5% milk in TBS-T). 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 immunoglobulin G (IgG [heavy and light chain specific]; Kirkegaard & Perry) in 5% milk in TBS-T. This was followed by four washes with TBS. Strips were developed by the addition of 5-bromo-4-chloro-3-indolylphosphate–nitroblue tetrazolium substrate (Kirkegaard & Perry). Reactions were stopped after 2 min with the addition of several large volumes of chilled ddH2O.

**RESULTS**

**Effect of anti-murine IL-6 and rIL-6 on in vitro borreliacidal activity.** Immune lymph node cells were obtained from 32 *Borrelia*-vaccinated C3H/HeJ mice, pooled, and cocultured with macrophages and viable *B. burgdorferi* cells for 9 days. In addition, immune lymph node cells were cultured with macrophages and spirochetes and incubated with anti-IL-6 antibody, a non-isotype-specific antibody, and rIL-6. Significant borreliacidal activity (titer, 256) was detected in the supernatants of cultures of immune lymph node cells incubated with macrophages and *B. burgdorferi* in the presence of a non-isotype-specific antibody (Fig. 1). Similar responses were also obtained in the absence of a non-isotype-specific antibody or with sham-vaccinated mice. When these borreliacidal antibody-producing cells were treated with 75 μg of anti-murine IL-6, in vitro anti-OspA borreliacidal activity was reduced fourfold (titer, 64). Conversely, treatment of borreliacidal antibody-producing cells with 0.2 μg of rIL-6 resulted in a fourfold increase of in vitro borreliacidal activity (titer, 1,024). When these studies were replicated with 32 immunized mice, similar results were obtained.

**Effects of anti-murine IL-6 and rIL-6 on anti-OspA antibody production.** Figure 2 shows the results of Western immunoblotting for the detection of anti-OspA antibody with supernatants obtained from cultures of immune lymph node cells incubated with macrophages and *B. burgdorferi* (19) in the presence or absence of anti-IL-6 antibody or rIL-6. Supernatants obtained from immune lymph node cells incubated with macrophages and *B. burgdorferi*, with or without anti-IL-6, had an anti-OspA titer of 2,560 (Fig. 2A and B). In contrast, supernatants obtained from cultures of immune lymph node cells incubated with macrophages, *B. burgdorferi*, and rIL-6 had an eightfold increase in anti-OspA titer (20,480; Fig. 2C). Similar results were obtained when these studies were replicated.

**Flow cytometric analysis of immune lymph node cell cultures.** Immune lymph node cells cultured with macrophages and *B. burgdorferi* for 9 days in the presence or absence of anti-murine IL-6 or rIL-6 were analyzed by flow cytometry for viability and numbers of B and T lymphocytes. Figure 3 shows that the numbers of viable cells detected in cultures treated with anti-murine IL-6 (Fig. 3B) and rIL-6 (Fig. 3C) were relatively equal. Approximately 20% fewer viable cells were detected in cultures of immune lymph node cells treated with a non-isotype-specific antibody (Fig. 3A). The lower viability may be due to the lack of IL-6 interaction with the IL-6 receptor. IL-6 is a growth-promoting cytokine (9, 28). Moreover, treatment of immune lymph node cells with anti-murine IL-6 or rIL-6 did not significantly alter the numbers of T lymphocytes (CD3 marker) between the two treatment groups (Fig. 3B and C). However, the number of B lymphocytes (B220 marker) detected in cultures of immune lymph node cells was significantly increased (approximately 49%; *P ≤ 0.05*) upon treatment with either anti-murine IL-6 (Fig. 3B) or rIL-6 (Fig. 3C) compared to cultures of immune lymph node cells treated with a non-isotype-specific antibody (Fig. 3A). A similar trend was observed in replicate experiments.

**Effects of anti-murine IL-6 on anti-IFN-γ enhance in vitro borreliacidal antibody production.** We previously demonstrated that the production of in vitro anti-OspA borreliacidal antibody is augmented upon treatment of borreliacidal antibody-producing cells with anti-murine IFN-γ (22). Here we confirmed that a significant (16-fold) enhancement of borreliacidal activity was found upon treatment of borreliacidal antibody-producing cells with 70 μg anti-murine IFN-γ (Fig. 4). Furthermore, treatment with rIFN-γ inhibited the borreliacidal response. When borreliacidal antibody-producing cells were treated concomitantly with 70 μg anti-murine IFN-γ and...
75 μg of anti-murine IL-6, the borreliacidal activity was reduced fourfold (Fig. 4).

Quantitation of IL-6 in lymph node cell culture supernatants. The concentration of IL-6 was determined in supernatants obtained from cultures of immune lymph node cells incubated with macrophages and *B. burgdorferi* and treated with non-isotype-specific antibody, anti-murine IL-6, anti-murine IFN-γ, anti-murine IFN-γ and anti-murine IL-6, or rIFN-γ. Cultures treated with a non-isotype-specific antibody or with anti-murine IFN-γ produced significant levels of IL-6 (Table 1). Treatment of cultures with anti-murine IL-6 or the combination of anti-murine IL-6 and anti-IFN-γ yielded low levels of IL-6. Interestingly, immune lymph node cells treated with 0.1 μg rIFN-γ, which resulted in a marked suppression of borreliacidal activity (Fig. 4), had significant levels of IL-6 (Table 1).

**DISCUSSION**

The induction of a sustained high level of borreliacidal antibody is an important component for establishing the efficacy of Lyme borreliosis vaccines. Previously, we developed an in vitro assay to determine means by which borreliacidal antibody production can be augmented (20). Cultivation of lymph node cells obtained from vaccinated (for 17 days) C3H/HeJ mice with macrophages and *B. burgdorferi* resulted in borreliacidal antibody production of the isotypes IgG1, IgG2a, and IgG2b (20). IL-4, a known stimulator of B lymphocytes, drives the production of antibodies of the IgG1 isotype (24). Attempts to increase borreliacidal activity by treatment with exogenous rIL-4 were not successful (20). The IgG2a component of the borreliacidal antibody response suggested that IFN-γ may act upon IL-4 in an antagonistic fashion (24) and consequently induce borreliacidal antibody production. However, treatment of borreliacidal antibody-producing cells with exogenous IFN-γ resulted in a marked suppression of borreliacidal activity (19). Surprisingly, the neutralization of IFN-γ augmented the borreliacidal antibody response (21). Taken together, these data suggest that IL-4 and IFN-γ play only a minor role in the generation of borreliacidal antibodies.

These data also imply that borreliacidal antibody production may not have a large dependency on T-helper lymphocyte influence but may be driven by an immunological mediator, such as IL-6, that is liberated from other immune cells (9). The role of IL-6 in the elicitation of humoral immunity has been characterized in a variety of systems (9). Weis and colleagues (17, 31, 38) also demonstrated that *B. burgdorferi* functions as both a B-lymphocyte mitogen and a stimulus for the release of IL-6 in both in vitro and in vivo models of *B. burgdorferi* infection. Anguita et al. (1, 28) demonstrated the necessity of IL-6 for the development of a complete humoral response upon stimulation by *B. burgdorferi*. However, the effects of IL-6 on the borreliacidal antibody component of the total humoral response was not investigated.

Here we show that borreliacidal antibody-producing cells obtained from 17-day vaccinates and treated with a neutralizing antibody to murine IL-6 caused a significant reduction of borreliacidal activity compared to immune cells treated with an isotype control (Fig. 1). In addition, the incorporation of anti-murine IL-6 into an established cell culture model of augmented borreliacidal activity (20) resulted in a reduction of borreliacidal antibody production (Fig. 4). Other studies performed by EIA showed that IL-6 was effectively neutralized by treatment with anti-IL-6. More importantly, treatment of borreliacidal antibody-producing cells with rIL-6 enhanced anti-OspA antibody production fourfold. Remington et al. (27) also reported that rIL-6 increased the production of another borreliacidal antibody, OspC. These results suggest that IL-6 may
play a major role in the regulation of antibodies that kill *B. burgdorferi*.

Surprisingly, borreliacidal activity was greatly depressed after treatment of borreliacidal antibody-producing cells with rIFN-γ, despite the presence of high concentrations of IL-6 in the cultures. This suggests that other immune mechanisms or cytokines may participate in IL-6-mediated production of anti-OspA borreliacidal activity. It is also likely that IFN-γ can directly suppress borreliacidal activity, even in the presence of IL-6. In support of this statement, we showed previously that treatment of borreliacidal antibody-producing cells with rIFN-γ resulted in the preferential death of B lymphocytes (19). Taken together, our results suggest that IL-6 induces borreliacidal antibodies while IFN-γ down-regulates the response. This may explain why borreliacidal antibodies (5, 8) are not sustained in naturally infected or vaccinated subjects (11, 12, 13, 23).

In this study, exogenous macrophages were required for IL-6-enhanced production of anti-OspA borreliacidal antibody. In contrast, Remington et al. (27) showed that enhanced in vitro production of anti-OspC borreliacidal activity occurred in the absence of supplementation with macrophages. Since macrophages produce IL-6 (9), these results imply that the level of IL-6 necessary to augment the anti-OspA borreliacidal antibody level is considerably higher than that for anti-OspC borreliacidal activity. In support of this suggestion, the neutralization of IL-6 had its greatest reduction effect on the

- **Fluorescein Thioscyanate Fluorescence**

FIG. 3. Flow cytometric analysis of lymph node cells cultured with macrophages and *B. burgdorferi* in the presence of a non-isotype-specific antibody (A), anti-murine IL-6 (B), or rIL-6 (C) for 9 days. Cells were labeled with both phycoerythrin-conjugated anti-murine CD3e (T cells) and fluorescein isothiocyanate-conjugated anti-murine CD45R/B220 (B cells). Viability was determined by the exclusion of propidium iodide.

- **FIG. 4.** Detection of borreliacidal antibody in cultures of immune lymph node cells cultured with macrophages and *B. burgdorferi* and treated with 70 μg of a non-isotype-specific antibody (black bar), 70 μg anti-murine IFN-γ (diagonally hatched bar), 70 μg anti-murine IL-6 (cross-hatched bar), or 0.1 μg rIFN-γ (striped bar). In most cases (90%), the same titers were obtained when the study was repeated.

### TABLE 1. Concentrations of IL-6 obtained by EIA analysis

| Treatment                                      | IL-6 concn (pg/ml) |
|------------------------------------------------|--------------------|
| Non-isotype-specific antibody                  | 3,292.86 ± 105.14   |
| Anti-murine IL-6                               | 1.00 ± 2.17         |
| Anti-murine IFN-γ                              | 2,740.23 ± 56.31    |
| Anti-murine IFN-γ + anti-murine IL-6            | 8.98 ± 7.77         |
| rIFN-γ                                         | 3,612 ± 58.35       |

*Mean IL-6 concentrations (± standard errors of the means) determined from cultures of immune lymph node cells cultured with macrophages and *B. burgdorferi* and treated with 75 μg of a non-isotype-specific antibody, 75 μg anti-murine IL-6, 70 μg anti-murine IFN-γ, 70 μg anti-murine IFN-γ followed by 75 μg anti-murine IL-6, or 0.1 μg rIFN-γ.
production of anti-OspC borreliacidal antibodies (27). This suggests that the levels of IL-6 present in the host after infection with B. burgdorferi may contribute to the development of anti-OspC and the subsequent production of anti-OspA borreliacidal antibodies. We hypothesize that low levels of IL-6 present during early infection may stimulate anti-OspC production. Once processing and elimination of spirochetes occur by macrophages, IL-6 levels increase along with the production of anti-OspA borreliacidal antibodies.

An interesting observation was made by Western immunoblot analysis of lymph node supernatants. As expected, treatment of borreliacidal antibody-producing cells with rIL-6 resulted in an eightfold increase in anti-OspA titer that coincided with the observed increase in borreliacidal activity. However, when borreliacidal antibody-producing cells were treated with anti-murine IL-6, anti-OspA titers were not significantly decreased, even though a fourfold reduction in anti-OspA borreliacidal antibody titer was recorded. Further studies by flow cytometric analysis revealed an increase in B-lymphocyte populations in cultures of borreliacidal antibody-producing cells treated with rIL-6 compared to control cultures. Moreover, no decrease in the number of B lymphocytes was detected following treatment with anti-murine IL-6, despite a significant decrease in borreliacidal antibody production in these cell cultures.

These results mimic the previously reported bipartite humoral response that is induced by rOspA preparations (22). The vaccination of LSH hamsters with rOspA resulted in borreliacidal antibody titers that peaked 6 weeks after vaccination, with significant waning thereafter. Immunization of human volunteers with rOspA also generated a significant borreliacidal antibody response that peaked at 60 days of immunization and waned rapidly thereafter. Only one individual had detectable borreliacidal activity half a year after immunization (22). In contrast, anti-OspA EIA titers remained elevated in humans and hamsters (23), despite the decline in borreliacidal activity.

These findings are important because they may facilitate the design of future Lyme borreliosis vaccines. Ma et al. (16) reported that OspA possesses epitopes that do not generate borreliacidal antibodies. The data described in this investigation also suggest that macrophages process OspA borreliacidal epitopes and elicit IL-6 production. This results in borreliacidal antibody production without a strong influence from TH2 responses and increased Lyme arthritis. J. Infect. Dis. 178: 1512–1515.

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