Anti-p19 Antibody Treatment Exacerbates Lyme Arthritis and Enhances Borreliacidal Activity

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Considerable effort has been made to elucidate the mechanism of Lyme arthritis. We focused on p19, a cell cycle-regulating molecule, because it is known to inhibit cell cycle division of T lymphocytes which may be responsible for the induction of arthritis. We show that anti-p19 antibody treatment enhances the inflammatory response normally detected at the tibiotarsal joints of Borrelia burgdorferi-vaccinated and Borrelia bissetti-challenged mice. Specifically, anti-p19 antibody treatment augmented the severity of inflammation within the synovial and subsynovial tissue. Moreover, treatment with anti-p19 antibody caused severe erosion of cartilage and bone with ankle joint destruction. In addition, anti-p19 antibody treatment of Borrelia-vaccinated and -challenged mice enhanced the borreliacidal antibody response, especially against the vaccine isolate. The novel activities of anti-p19 antibody show that p19 may be an important therapeutic site for the treatment of Lyme arthritis.

Arthritis is one of the most frequently reported clinical manifestations associated with humans infected with Borrelia burgdorferi sensu lato (1, 2, 46), the etiologic agent of Lyme borreliosis. Lyme arthritis presents with intermittent attacks of mono- or polyarticular arthritis within months to a few years after tick-transmitted infection in 60% of Lyme borreliosis patients (1, 2, 46). Approximately 10% of these patients develop persistent arthritis, despite extensive treatment with antimicrobial agents (1, 2, 46). In severe cases, chronic inflammatory Lyme arthritis (following a correct diagnosis) can lead to cartilage and bone erosion (46) with permanent joint destruction (46).

Considerable effort has been made to elucidate the immune agents and mechanisms responsible for Lyme arthritis (3, 5–7, 10, 20, 21, 40, 42). It is generally accepted that T cells, particularly CD4+ T cells (5, 7, 20, 51) and CD4+ CD25 + T cells (35–37), participate in the induction, resolution, and prevention of the arthritis. Similarly, accumulating evidence suggests that cytokines, especially interleukin-17 (IL-17) (11) and IL-15 (4), can also influence the activation and maintenance of the immune responses that cause Lyme arthritis. Other cellular immune mechanisms may also be involved (5, 7, 10, 20, 21). Moreover, a paradigm for the molecular regulation of arthritis involving CD4+ T cells, CD4+ CD25 + T cells, and cell-associated cytokine production has emerged in recent years. This involves cell cycle-regulating molecules (13, 22, 26) like protein (p) 19, also referred to as p19INK4D (22, 50). This 165-amino-acid protein participates in the G1 phase arrest of T cells in the cell cycle (13, 16, 29). Disruption of p19 activity has been associated with the development of acute T-cell lymphoblastic leukemia (15, 18, 44) and other cellular diseases (30, 48). This protein may also be important for controlling T-cell proliferation, differentiation, and activation and the release of cytokines that promote the pathology associated with Borrelia-induced arthritis.

We hypothesized that treatment with anti-p19 antibody of Borrelia-vaccinated and -challenged mice would enhance arthritis. In this report, we show that treatment of Borrelia-vaccinated and -challenged mice with anti-p19 antibody augments the arthritic response. In addition, anti-p19 antibody treatment caused an increase in borreliacidal antibody production.

MATERIALS AND METHODS

Mice. Six- to 12-week-old inbred male and female C57BL/6 mice were obtained from William F. Dove (University of Wisconsin). The mice were bred at the animal facility located at the University of Wisconsin Medical School. All mice were kept in a climate-controlled environment at 21°C with 12-h light-dark cycles, and food and water were provided ad libitum. The experimental procedures performed on the mice were approved by the Animal Care and Use Committee for the University of Wisconsin Medical School.

Organisms. Low-passage-number (<10) virulent B. burgdorferi sensu stricto isolate 297 (human cerebrospinal fluid) and Borrelia bissetti (isolated by S.M.C. from Microtus pennsylvanicus) were grown at 32°C in modified Barbour-Stoenner-Kelly (BSK) medium to a concentration of 107 spirochetes/ml. Aliquots (500 µl) were then dispensed into 1.5-ml screw-cap tubes (Sarstedt, Newton, NC) containing 500 µl of BSK medium supplemented with 10% glyc erol (Sigma Chemical Co., St. Louis, MO). The tubes were sealed and stored at −70°C.

Vaccine preparation. Frozen aliquots (10 ml) of B. burgdorferi 297 culture were thawed and pelleted by centrifugation (10,000 × g at 25°C for 10 min) and washed three times with phosphate-buffered saline (PBS), pH 7.4 (Grand Island, NY). The washed pellet was then resuspended and mixed with 10% neutral buffered zinc formalin (Sigma Chemical Co.), incubated at ambient temperature with periodic mixing for 30 min, washed three times by centrifugation (10,000 × g at 23°C for 10 min) with PBS, and resuspended in PBS. Subsequently, the formalin-inactivated spirochetes were mixed with a suffi-
cient volume of 3% aluminum hydroxide (Reheis, Berkeley Heights, NJ) to yield 2 × 10^9 spirochetes/ml.

**Vaccination of mice.** Mice were anesthetized with ether (Sigma Chemical Co.) or 15% isoflurane in mineral oil (Sigma Chemical Co.) contained in a nose-and-mouth cup and injected subcutaneously in the inguinal regions with 0.25 ml of the formalin-inactivated whole-cell vaccine preparation. Whole cells of *Borrelia* are not recommended for vaccination of humans, based on concerns associated with other types of whole-cell vaccines (28). However, we have shown that whole cells of *Borrelia* can consistently induce arthritis in gamma interferon-deficient and wild-type C57BL/6 mice after *Borrelia* infection (4, 11, 37).

**Infection of mice.** A frozen aliquot (1 ml) of *B. bissettii* culture was thawed and added to 4 ml of fresh BSK medium and incubated at 32°C for 24 h. Twenty-one or 28 days after vaccination of mice with *B. burgdorferi* 297 in alum, mice were anesthetized with ether or 15% isoflurane in mineral oil contained in a nose-and-mouth cup and were injected subcutaneously in both hind paws with 50 μl of BSK medium containing 10^9 viable *B. bissettii* organisms. Vaccinated mice were also challenged the following day with 10^9 viable *B. bissettii* organisms that had been incubating at 32°C in BSK medium.

It is necessary to infect *B. burgdorferi* 297-vaccinated mice with *B. bissettii* because vaccination induces protective antibodies that prevent a homologous infection from eliciting arthritis (17, 32, 43). Other infectious *Borrelia* isolates, besides *B. bissettii*, can also elicit the arthritis (32). Controls included vaccinated mice injected with only BSK medium, which does not induce arthritis.

**Administration of anti-p19 antibody.** Purified mouse anti-p10<sup>NK4a</sup> monoclonal antibody (BD Pharmingen, San Diego, CA) and nonspecific isotype immunoglobulin G1 (IgG1) antibody (BD Pharmingen) were diluted in filter-sterilized (0.2-μm-pore-size Acrodisc filter; Gelman Sciences, Ann Arbor, MI) BSK (pH 7.4) to a final concentration of 20 μg/ml. Comassic blue-stained gels and Western blot analysis showed the solution of anti-p19 antibody to be free of contaminating protein. The salts and preservatives present were identical to those found in the isotype control. One hour after infection of vaccinated mice, mice were anesthetized with ether or 15% isoflurane in mineral oil contained in a nose-and-mouth cup and were injected subcutaneously in both hind paws with 50 μl of anti-p19 antibody or isotype antibody. Treatment with anti-p19 antibody and controls without treatment of anti-p19 antibody was administered 7 or 15 days after challenge and continued daily for 6 days.

**Flow cytometry.** The popliteal and inguinal lymph nodes were obtained from four groups of three mice vaccinated with or without challenge and with or without treatment with anti-p19 antibody. In addition, popliteal and inguinal lymph nodes were obtained from non-anti-p19 antibody-treated nonvaccinated and noninfected mice. Single-cell suspensions of the lymph node cells were prepared by teasing apart the lymph nodes with forceps and passing them through a sterile nylon mesh screen (Fisher, Hanover Park, IL) into cold filter-sterilized PBS. The total number of viable lymphocytes was determined. Cells (5 × 10<sup>6</sup>) were then dispensed into chilled centrifuge tubes and mixed with 2.5 μl of fluorescein isothiocyanate-conjugated rat anti-mouse CD4 antibody (BD Pharmingen) and 2.5 μl of phycoerythrin-conjugated rat anti-mouse CD25 antibody (BD Pharmingen) and incubated at 4°C for 30 min under dark conditions. Subsequently, the cells were washed with 1% fetal calf serum (heat inactivated; Sigma Chemical Co.) in PBS at 4°C (500 × g) for 5 min, and the pellets were resuspended in 300 μl of cold 1% fetal calf serum in PBS. The cells were then fixed in 1% methanol-free formaldehyde (Polysciences, Warrington, PA) for 24 h. Data were acquired using a FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ) and diluted serially (1:40 to 1:40,960) with BSK. Serum samples were then transferred to sterile 1.5-ml screw-cap microcentrifuge tubes (Sarstedt, Newton, NC) and diluted serially (1:40 to 1:40,960) with BSK. Serum samples were heat inactivated at 56°C for 10 min, and a 100-μl aliquot of the spirochetes and 10 μl of sterile guinea pig serum (50% hemolytic component of ≥200 units/ml) were added. The assay mixtures were mixed thoroughly and incubated for 16 to 24 h at 35°C.

Following incubation, 100 μl of each assay suspension was transferred to a 12- by 75-mm polystyrene tube (Becton-Dickinson, Franklin Lakes, NJ) containing 400 μl of PBS and 1 μg of acridine orange (Sigma) per ml. A FACScan flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA) was then used to detect borreliacidal activity. Spirochetes were isolated by gating (CellQuest software; Becton Dickinson) and analyzed for 1 to 2 min with the flow rate set at low. Borreliacidal antibodies kill the spirochete by inducing a complement cascade that disrupts the outer membrane and causes the membrane to bleb. Borreliacidal antibodies were detected indirectly by monitoring the increased fluorescence intensity that occurs when the acridine orange intercalates into blebbed, nonviable spirochetes. A shift of ≥13% in the mean fluorescence intensity compared to a normal serum control was considered positive (12). The presence of blebbed, nonmotel spirochetes was then confirmed by dark-field microscopy.

**Assessment of arthritis.** Measurements of hind paw swelling were used to determine the level of the inflammatory response induced in *Borrelia*-vaccinated mice with or without treatment with anti-p19 antibody and challenge with *B. bissettii*. Paws were measured on day of challenge and every other day for approximately 3 weeks using a Vernier digital caliper (Fisher Scientific, Pittsburgh, PA) with readout to 0.01 mm. Mice were anesthetized with ether or 15% isoflurane in mineral oil contained in a nose-and-mouth cup and were injected subcutaneously in both hind paws. Mean paw volume was calculated as: mean paw volume = 0.5 × (length × width × depth). In addition, popliteal and inguinal lymph nodes were obtained from a group of mice were summed and divided by the number of measurements to obtain the mean value of the size of the joint for the day.

**Preparation of tissues for histologic examination.** Twenty-one days after infection, mice were euthanized with ether or 15% isoflurane in mineral oil, and their hind paws were amputated at mid-femur. The paws were fixed in 10% neutral buffered zinc formalin solution for 24 h. Sections were placed in 70% ethanol daily thereafter for 6 or 9 days. For each paw, sections were cut in 6 μm-thick sections. These sections were placed on glass slides and stained with hematoxylin and eosin. Sections were cryoprotected, and an unbiased histopathological examination was performed by a board-certified pathologist (T. F. Warner). The following word scale was used to define the neutrophil infiltration in the tibiotarsal joint: mild (neutrophil infiltration in the subsynovial tissues), moderate (neutrophil infiltration of the subsynovial tissues, synovium, and synovial space), severe (profuse neutrophil infiltration of the subsynovial tissues, synovium, and synovial space), and severe destructive (profuse neutrophil infiltration of all tibiotarsal tissues along with bone and/or cartilage destruction).

**Statistical analysis.** Swelling of the hind paws and flow cytometry data among groups were tested by analysis of variance (45). The alpha level was set at 0.05 before the experiments were started. The standard error for the experiment was then determined.

**RESULTS**

Effects of anti-p19 antibody treatment on development and progression of hind paw swelling. Four groups of four mice each were vaccinated with *B. burgdorferi* isolate 297 in alum. Twenty-one days after vaccination, two of the four groups of vaccinated mice were challenged in the hind paw with *B. bissettii*. Concomitantly, one of the two groups of vaccinated and infected mice was administered anti-p19 antibody on the day of challenge and daily thereafter for 6 or 9 days. A group of four vaccinated but noninfected mice was also treated with anti-p19 antibody on day 21 of vaccination and daily thereafter for 6 or 9 days. A remaining group of four vaccinated but not infected mice was not treated with anti-p19 antibody. Additional controls included nonvaccinated and nonchallenged mice and vaccinated and challenged mice treated with an IgG1 isotype antibody.
Similar, significant ($P < 0.05$) swelling of the right and left hind paws was detected in vaccinated and infected mice with or without treatment with anti-p19 antibody (Fig. 1) or with treatment with an IgG1 isotype antibody. The hind paw swelling of untreated and anti-p19 antibody-treated Borrelia-vaccinated and -challenged mice peaked on days 7 and 9, respectively, and gradually decreased. In contrast, no swelling of the hind paws occurred with vaccinated, nonchallenged mice with or without treatment with anti-p19 antibody. Likewise, no swelling was detected in the nonvaccinated and nonchallenged mice. Similar results were obtained when these experiments were repeated four times with three or four mice per group.

**Histopathologic effects of anti-p19 antibody treatment of vaccinated and challenged mice.** Although no differences in swelling of the hind paws were detected between vaccinated and challenged mice with or without treatment with anti-p19 antibody, there were differences in histopathology between the groups, especially 21 days after infection. Anti-p19 antibody-treated Borrelia-vaccinated and -challenged mice showed severe inflammation of the periarticular soft tissue. Moreover, the ankle joint showed severe inflammation of the synovial and subsynovial tissue along with severe neutrophilic infiltration of the synovial space. Most importantly, severe erosion of cartilage and bone occurred, and the ankle joints were destroyed (Fig. 2D). In contrast, vaccinated and challenged mice with or without treatment with a nonspecific isotype IgG1 antibody showed moderate inflammation of the periarticular soft tissue, moderate inflammation of the synovial and subsynovial tissue, and no erosion of cartilage or bone (Fig. 2C). Vaccinated mice with (Fig. 2B) or without (Fig. 2A) treatment with anti-p19 antibody showed only mild or no inflammatory changes of the tibiotarsal joint or surrounding tissue. Likewise, no histopathological changes were observed with the tibiotarsal joints of nonvaccinated and nonchallenged mice (Fig. 2E). When these studies were repeated four times, anti-p19 antibody-treated Borrelia-vaccinated and -challenged mice (total 16) always had more severe pathology than the vaccinated and challenged mice without anti-p19 antibody and the other control animals.

**Effects of delayed administration of anti-p19 antibody.** In a separate set of experiments, Borrelia-vaccinated and -infected mice were administered anti-p19 antibody at the time of infection or at day 7 or day 15 after infection. The anti-p19 antibody treatment was continued daily for 6 days with each group of vaccinated and challenged mice. Controls included non-anti-p19 antibody-treated Borrelia-vaccinated mice. These latter mice developed moderate histopathology involving the tibiotarsal joint (Fig. 2C). Enhanced pathology involving the synovium, subsynovial tissue, and joint space developed only in vaccinated and challenged mice that received anti-p19 antibody at the time of infection. No significant differences in histopathology were detected among non-anti-p19 antibody-treated vaccinated and challenged mice and vaccinated and challenged mice that received anti-p19 antibody at day 7 or day 15 after challenge.

**Effects of anti-p19 antibody treatment on the number of lymphocytes, CD4$^+$ T cells and CD4$^+$ CD25$^+$ T cells in the lymph nodes.** Popliteal and inguinal lymph nodes were obtained from nonvaccinated mice, Borrelia-vaccinated mice with or without treatment with anti-p19 antibody, and Borrelia-vaccinated and -infected mice with or without treatment with anti-p19 antibody on day 8 after infection. Table 1 shows a slight increase in the total number of lymphocytes and CD4$^+$ T cells in all groups of mice except vaccinated mice and vaccinated mice treated with anti-p19 antibody. Likewise, vacci-
FIG. 2. Histopathology of the tibiotarsal joints of vaccinated mice (A), vaccinated mice treated with anti-p19 antibody (B), vaccinated and challenged mice (C), and vaccinated and challenged mice treated with anti-p19 antibody (D) at day 21 after Borrelia infection or day 42 after vaccination. A fifth tibiotarsal joint is shown from a nonvaccinated and nonchallenged mouse (E). Arrows indicate inflammation. Additional controls (not shown) included vaccinated and challenged mice treated with an IgG1 isotype antibody. The histopathology detected in vaccinated and challenged mice treated with the isotype antibody was similar to the histopathology observed in vaccinated and challenged mice, shown in panel C. Magnification, ×100.
TABLE 1. Numbers of lymphocytes, CD4+ T cells, and CD4+ CD25+ T cells in the popliteal and inguinal lymph nodes of vaccinated and treated mice with or without anti-p19 antibody

| Treatment# | No. of lymphocytes/nodea | No. of CD4+ lymphocytes/nodeb | No. of CD4+ CD25+ lymphocytes/nodec |
|------------|--------------------------|-----------------------------|-----------------------------------|
| Nonvaccinated | 1.2 × 10⁶ | 2.2 × 10⁵ | 5.7 × 10⁴ |
| Vaccinated | 1.9 × 10⁶ | 2.1 × 10⁵ | 4.4 × 10⁴ |
| Vaccinated and treated with anti-p19 | 0.9 × 10⁶ | 0.1 × 10⁵ | 2.1 × 10⁴ |
| Vaccinated and challenged | 6.1 × 10⁶ | 3.7 × 10⁵ | 1.1 × 10⁵ |
| Vaccinated, challenged, and treated with anti-p19 | 7.4 × 10⁶ | 5.5 × 10⁵ | 1.3 × 10⁵ |

# Eighteen to 24 popliteal and inguinal nodes were collected from 5 to 6 mice per group 8 days after infection. Single-cell suspensions in PBS were obtained, and cells were counted with a Petroff-Hauser counting chamber.

a Obtained by multiplying the percentage of lymphocytes by the average number of cells/node.

b Obtained by multiplying the percentage of CD4+ or CD4+ CD25+ lymphocytes by the average number of lymphocytes/node.

tated mice treated with or without anti-p19 antibody had decreased numbers of CD4+ CD25+ T cells compared to the number of CD4+ CD25+ T cells in the control group. In contrast, Borrelia-vaccinated and -challenged mice treated with or without anti-p19 antibody had significantly (P ≤ 0.1) increased numbers of CD4+ CD25+ T cells (1.1 × 10⁶ to 1.3 × 10⁷). In summary, anti-p19 antibody treatment of Borrelia-vaccinated and -challenged mice slightly increased the number of lymphocytes, CD4+ T cells, and CD4+ CD25+ T cells compared to the other control groups. However, these results were not statistically significant. There were four control groups (Table 1).

Treatment with anti-p19 antibody enhances borreliacidal activity. Sera were collected from B. burgdorferi isolate 297-vaccinated mice with or without B. bissetti challenge and with or without treatment with anti-p19 antibody 43 days after vaccination or 22 days after infection, respectively. Figure 3A shows that borreliacidal antibody directed against B. burgdorferi isolate 297 was enhanced fourfold or more in vaccinated mice or vaccinated and challenged mice treated with anti-p19 antibody. Likewise, borreliacidal antibody directed against the challenge agent, B. bissetti (Fig. 3B), was enhanced twofold or more in vaccinated and vaccinated and challenged mice after anti-p19 antibody treatment.

In other studies, anti-p19 antibody was administered at day 7 or day 15 after infection of Borrelia-vaccinated mice. Anti-p19 antibody treatment was continued daily for 6 or 9 days. Treatment with anti-p19 antibody enhanced borreliacidal antibody directed against B. burgdorferi isolate 297. A two- to fourfold increase in borreliacidal antibody was detected in sera from B. burgdorferi isolate 297-vaccinated and B. bissetti-challenged mice that received anti-p19 antibody at day 7 or day 15 after infection, respectively. By contrast, no significant increases in borreliacidal antibody against B. bissetti were detected when vaccinated and challenged mice were administered anti-p19 antibody at day 7 or day 15 after infection.

DISCUSSION

Several cytoplasmic proteins are required to inhibit cell division of T cells (13, 22, 26). We focused on p19 because it inhibits cyclin-dependent kinases (CDKs), especially CDK6, the earliest inducible member of the CDK family involved in cell cycle progression of T lymphocytes (13, 15, 22, 25, 26). Increasing amounts of intracellular p19 have also been shown to inhibit the kinase activity of CDK4 (13, 47). Therefore, p19 may restrict the number of T cells that can be produced following antigenic stimulation or infection (13, 15). This characteristic of p19 may also prevent tumor development (9, 30) and T-cell-driven or -associated diseases (18, 48). Little information, however, is available as to whether inhibitors of p19 will augment immune responses associated with Borrelia vaccination and challenge. Selective enhancement or inhibition of p19 could point the way to prevention of Lyme arthritis or enhancement of the quality of the protective borreliacidal response in future Lyme vaccines.

We showed previously that B. burgdorferi-vaccinated mice challenged with B. bissetti develop arthritis at the tibiotarsal joint (4, 11, 35–37). This finding is confirmed in the present report. The synovial space, synovium, and subsynovial tissue of the tibiotarsal joint of Borrelia-vaccinated and -challenged mice showed a severe inflammatory response involving a pro-
fuse infiltration of neutrophils. Subsequently, we hypothesized that the inflammatory arthritic response could be exacerbated by neutralizing p19 in Borrelia-vaccinated and -challenged mice. We speculated that in the absence of the p19 inhibitor, the responding immune cells in the periarticular tissues would continue to proliferate and promote the inflammatory response in the tibiotarsal joint.

Here, we show that anti-p19 antibody treatment did augment the inflammatory response normally detected at the tibiotarsal joints of Borrelia-vaccinated and -challenged mice. Although the synovial space, synovium, and subsynovial tissues contained a profuse infiltration of neutrophils, the most distinctive feature detected in anti-p19 antibody-treated Borrelia-vaccinated and -challenged mice was erosion of cartilage and bone at the tibiotarsal joint. In fact, the joints were destroyed in most of the anti-p19-treated Borrelia-vaccinated and -challenged mice compared to the non-anti-p19-treated Borrelia-vaccinated and -challenged control mice. These results show that treatment with anti-p19 antibody exacerbates the arthritic response.

Treatment with anti-p19 antibody also induced several other effects in Borrelia-vaccinated and -challenged mice. Anti-p19 antibody treatment enhanced the borreliacid antibody response of Borrelia-vaccinated and -challenged mice. Borreliacid antibody increased two- to fourfold against both the vaccine and challenge agent compared to the borreliacid activity detected in non-p19 antibody-treated Borrelia-vaccinated and -challenged mice. In addition, anti-p19 treatment was effective in enhancing borreliacid antibody even when administered several days after Borrelia-vaccinated mice were challenged, especially against the vaccine agent. These results support our claim that anti-p19 treatment can affect the immune response to Borrelia vaccination and challenge.

In an attempt to elucidate the mechanism by which treatment with anti-p19 antibody exacerbated arthritis, we determined the number of lymphocytes, CD4+ T cells, and CD4+ CD25+ T cells in the lymph nodes (popliteal and inguinal) draining the site of the arthritic response. In general, the number of these cells was increased in anti-p19 antibody-treated Borrelia-vaccinated and -challenged mice compared to the controls. This suggests that treatment with anti-p19 antibody prolongs or amplifies the immune response. In support of this hypothesis, it is known that increases in the number of lymph node cells and CD4+ T cells are associated with antigenic stimulation, cell proliferation, and activation (39). Of special interest is the number of CD4+ CD25+ T cells in the anti-p19-treated Borrelia-vaccinated and -challenged mice. Although CD4+ CD25+ T cells may play a major role in suppressing immune reactivity (8, 14, 27, 31, 41), initially CD4+ T cells expressing CD25, the IL-2α receptor, are active participants in developing the immune response (19, 23, 52). The increase (13%) in CD4+ CD25+ T cells found in the lymph nodes of anti-p19 antibody-treated Borrelia-vaccinated and -challenged mice may have aided the arthritis-inducing effector CD4+ T cells by binding the T-cell growth-promoting factor, IL-2, to the CD25 receptor. This would further stimulate proliferation of arthritic reactive CD4+ T cells (19) and account for the enhanced histopathology of the tibiotarsal joint found in anti-p19 antibody-treated Borrelia-vaccinated and -challenged mice.

The ability of anti-p19 antibody treatment to augment the borreliacid antibody response of Borrelia-vaccinated and Borrelia-vaccinated and -challenged mice, especially against the vaccine isolate, was a surprise. Sustained high levels of borreliacid antibody is an important feature for establishing the efficacy of the next generation of Lyme vaccines. A major concern, however, for vaccine manufacturers is how to stimulate production of a strong and long-lived borreliacid antibody response that has minimal side effects. Our results show that considerable enhancement of borreliacid antibody can be achieved with anti-p19 antibody treatment; however, the mechanism of enhancement needs to be determined. By contrast, Borrelia-vaccinated and -challenged mice treated with anti-p19 antibody exhibited enhanced lymphocyte responses and borreliacid activity to the vaccine isolate. Anti-p19 antibody treatment of Borrelia-vaccinated mice and Borrelia-vaccinated and -challenged mice may have caused a restricted clonal expansion of T cells specifically dedicated to stimulate production of anti-297 borreliacid antibody (vaccine isolate). This restriction may have limited the borreliacid antibody response to the challenge agent, B. bissetti. Our results show that anti-19 antibody treatment of vaccinated mice can enhance borreliacid antibody which kills the homologous challenge agent, even when anti-p19 antibody treatment is delayed for 7 or 15 days. Additional studies with anti-p19 antibody are needed to determine the mechanism of antibody enhancement.

The novel activities of anti-p19 antibody are difficult to explain. How was the intracellular protein, p19, neutralized by treatment with anti-p19 antibody? It is generally accepted that antibodies cannot cross the cell membrane of eukaryotic cells (33, 39). However, there are antibodies called intrabodies that can function within a cell (24, 33, 49), and these can inhibit protein function (24, 33, 49). Intrabodies contain the antigen-binding regions and are generally small fragments (light chain) of the antibody molecule (24, 33). Presumably, anti-p19 antibody was altered in Borrelia-vaccinated and -challenged mice to form intrabodies that crossed the cell membrane and neutralized p19. Another explanation may be that p19 is expressed to form intrabodies that crossed the cell membrane and neutralized p19. Another explanation may be that p19 is expressed transiently on the surface of T cells and that interaction with anti-p19 antibody inactivates or down-regulates production of intracellular p19. A similar mechanism has been described for down-regulation or prevention of the production of cytokines (19, 39). Finally, anti-p19 antibody may be cross-reactive and bind to the cell receptors of alpha interferon or IL-10. These cytokines have been shown to increase the CDK inhibitor p19 (34, 38) and inhibit proliferation of immune cells (34, 38).

Blockage of the receptors would prevent production of p19. Presently, we are investigating this latter hypothesis.

In conclusion, we show that treatment with anti-p19 antibody can alter the immune response, especially the severity of the arthritis induced in Borrelia-vaccinated and -challenged mice.

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