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Anti-Interleukin-15 Prevents Arthritis in *Borrelia*-Vaccinated and -Infected Mice

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We showed previously that interleukin-17 (IL-17) plays a significant role in the induction of arthritis associated with *Borrelia* vaccination and challenge. Little information, however, is available about the chain of immunologic events that leads to the release of IL-17. The production of IL-17 has been linked to stimulation of memory cells by IL-15. Therefore, we hypothesized that IL-15 is involved in the induction of arthritis associated with *Borrelia* vaccination and infection of mice. Here we present evidence that treatment of *Borrelia*-vaccinated and -infected mice with anti-IL-15 antibody prevents swelling of the hind paws. More importantly, both anti-IL-15 antibody- and recombinant IL-15 receptor alpha-treated *Borrelia*-vaccinated and -infected mice were free of major histopathologic indications of arthritis, including hyperplasia, hypertrophy, and vilus formation of the synovium. Similarly, the synovial space and perisynovium were free of inflammatory cells. By contrast, the synovium of nontreated *Borrelia*-vaccinated and -infected mice had overt hyperplasia, hypertrophy, and vilus formation. Moreover, the synovial space and perisynovium were infiltrated with neutrophils, macrophages, and lymphocytes. Finally, we show that recombinant IL-15 stimulates the release of IL-17 from lymph node cells obtained near the arthritic site. These results suggest that IL-15 plays a major role in orchestrating IL-17 induction of arthritis associated with *Borrelia*-vaccinated and -infected mice.

Arthritis is a major clinical presentation in humans infected with *Borrelia burgdorferi* (39). Arthritis is also detected in humans following vaccination with *Borrelia* outer surface protein A (34) and animals following *Borrelia* infection (5, 6) or *Borrelia* vaccination and infection (10, 27). The immune mechanisms responsible for this arthritis are poorly understood. We showed previously that treatment of *Borrelia*-vaccinated and -infected mice with anti-interleukin-17 (anti-IL-17) or anti-IL-17 receptor antibodies prevented the induction of arthritis (8). This finding is important because it defines a major contributor to the pathogenesis of arthritis. Little is known, however, about the immunologic events that lead to the expression of IL-17 and the development of arthritis. Other precursor cytokines may trigger the release of IL-17.

It is known that production of the proinflammatory cytokine IL-17, especially from memory T cells (43), is stimulated by IL-15 (13, 24, 43). IL-15 is a recently discovered cytokine (16) that is produced by synoviocytes (26), monocytes (31), neutrophils (19), and bone marrow stromal cells (33) but not primary T cells (12, 16, 17, 20). In addition, IL-15 is produced by dendritic cells and epithelial cells (36) and influences the local infiltration, activation, and proliferation of antigen-driven T cells at the site of infection or inflammation (28, 42). Clinically, abnormalities of IL-15 expression have been reported for several diseases (1, 19, 25), including rheumatoid arthritis (28). Blocking endogenous IL-15 has been effective in reducing pathology (35). Thus, IL-15 may act as a chemotactic and proliferation factor for the attraction and activation of cells, especially memory T cells capable of releasing IL-17.

In this report, we show that treatment of *Borrelia*-vaccinated and -challenged mice with anti-IL-15 or its soluble receptor prevents the development of arthritis. In addition, we show that recombinant IL-15 (rIL-15) can stimulate the release of IL-17 from lymph node cells obtained near the arthritic site.

MATERIALS AND METHODS

Mice. Gamma interferon gene-deficient mice were obtained from W. P. Weidanz (University of Wisconsin) with permission from Genentech (South San Francisco, CA) and housed at the University of Wisconsin Animal Facility. Eight- to 12-week-old male mice weighing 20 to 30 grams were given food and acidified water ad libitum during a light-and-dark cycle of 12 h for the duration of the study. Experimental protocols were approved by the Animal Care and Use Committee of the University of Wisconsin Medical School, Madison.

Organisms. Low-passage-number (<10) *Borrelia burgdorferi* strain 297 (from human spinal fluid) and "*Borrelia bissettii*" (formerly strain C-1-11, from *Microtus pennsylvanicus*) were grown at 32°C in modified Barbour-Stoenner-Kelly (BSK) medium until they reached a concentration of approximately 10^7 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% glycerol (Sigma Chemical Co., St. Louis, MO). The tubes were 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 and enumerated by using dark-field microscopy.

Vaccine preparation. *B. burgdorferi* strain 297 organisms were grown in 1 liter of BSK medium for 6 days before being 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 a 1% formalin solution, incubated at 32°C with periodic mixing for 40 min, and then washed three times by centrifugation with PBS (10,000 × *g*, 15°C, 10 min). Subsequently, the washed pellet was resuspended in 1 ml PBS, dispensed in aliquots, and frozen at −70°C.

On the day of vaccination, an aliquot was thawed and mixed. The spirochetes

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were enumerated by using dark-field microscopy and a volume was added to 3% aluminum hydroxide (alum; Reheis, Berkeley Heights, NJ) to yield 5×10^6 spirochetes/ml.

Vaccination of mice. Ninety mice were anesthetized with ether contained in a nose-and-mouth cup and injected subcutaneously in the inguinal region with 250 μl of the formalin-inactivated whole-cell *B. burgdorferi* 297 vaccine. Thirty sham-vaccinated mice were also injected with 3% alum. The use of whole cells of *B. burgdorferi* for vaccination is not recommended for humans because of concerns associated with whole-cell vaccines (22). However, the ability of whole cells to consistently induce arthritis in our murine model (8, 10) allowed for the evaluation of the immunological mechanisms that induce arthritis.

Infection of mice. Twenty-one days after vaccination, mice were anesthetized with ether contained in a nose-and-mouth cup and injected subcutaneously in the right hind paw with 50 μl of BSK medium containing 10^6 viable *B. bissettii* organisms. Swelling of the hind paws consistently develops 4 to 6 days after *B. bissettii* infection and peaks on day 8 to 12 (8, 10). Swelling of the hind paws can also be induced by infection with the homologous *B. burgdorferi* strain 297. However, *B. burgdorferi* strain 297-vaccinated mice must be challenged before protective antibodies develop (approximately day 7) or after their decline. Swelling of the hind paws of homologous vaccinated and challenged mice is variable. Therefore, we challenged *B. burgdorferi* strain 297-vaccinated mice with *B. bissettii* to obtain consistent swelling of the hind paws. Vaccination of mice with *B. bissettii* and challenge with *B. burgdorferi* strain 297 also yields consistent swelling of the hind paws, as does challenge with other infectious isolates of *B. burgdorferi* (11, 27, 37). Controls included vaccinated mice injected with alum or BSK medium alone.

Administration of anti-IL-15 antibody and rIL-15 receptor alpha. Lyophilized goat anti-mouse immunoglobulin G polyclonal IL-15 antibody (200 μg), normal goat immunoglobulin G (100 μg), and mouse rIL-15 receptor alpha (100 μg) were obtained from R&D Systems (Minneapolis, MN). The antibodies and rIL-15 receptor were resuspended in filter-sterilized (0.2-μm-pore-size filter) (Acrodisk; Gilman Sciences, Ann Arbor, MI) PBS (pH 7.2) or PBS containing 0.1% bovine serum albumin (Fisher Scientific, Pittsburgh, PA), respectively, to yield concentrations of 50 μg/ml. Twenty-one days after vaccination, three groups of eight mice each were infected with 10^6 *B. bissettii* organisms in the right hind paw. Less than 1 h after infection, the mice were injected subcutaneously in the right hind paw with 50 μl of the anti-IL-15 antibody or rIL-15 receptor alpha preparation. Anti-IL-15 antibody or rIL-15 receptor alpha was injected daily for 6 or 8 days, respectively. In other experiments, anti-IL-15 antibody was injected on day 7 after infection and daily thereafter for 6 days. Control groups received injections with the normal goat isotype antibody or with BSK medium.

Measurement of IL-17 produced by immune lymph node cells. Twenty-one days after vaccination, six mice were euthanized with ether contained in a nose-and-mouth cup and the inguinal lymph nodes were removed. The nodes were teased apart with a forceps, and single-cell suspensions were obtained by passing the cells through a sterile Falcon 100-μm nylon cell strainer (Fisher Scientific) into cold RPMI medium containing 10% fetal calf serum (Sigma, St. Louis, MO) with penicillin and streptomycin (Fisher Scientific). The cells were counted by using a hemacytometer and dispensed at a concentration of 5×10^6 cells per well into a 24-well microtiter plate (Fisher Scientific) in 1 ml of supplemented RPMI medium. Mouse rIL-15 (<1.0 endotoxin unit of endotoxin per 1 μg cytokine; R&D Systems) was reconstituted in PBS containing 0.1% bovine serum albumin and added to wells at a concentration of 50, 100, 200, or 500 ng/well. Wells not receiving rIL-15 were treated with PBS containing 0.1% bovine serum albumin. Viable *B. bissettii* organisms (5×10^6) were added to some wells. Microtiter plates were incubated at 37°C in 5% CO_2 for 48 h before supernatants were removed and analyzed for production of IL-17 using an enzyme-linked immunosorbent assay kit (R&D Systems) according to the manufacturer's instructions.

Assessment of arthritis. Hind-paw swelling was used to determine the level of the inflammatory response in the study mice. Prior to vaccination, random age-matched mice were chosen and their right hind paws were measured to determine the baseline of paw size. Paws were measured 48 h after infection and every other day thereafter for 14 days by using a Vernifer caliper with a digital readout to 0.01 mm. To obtain the measurements, mice were anesthetized with ether and the widths and thicknesses of the right hind tibiotarsal joints were carefully measured. All caliper values within a group were summed and divided by the number of measurements taken in the group to obtain the daily mean value.

Preparation of tissues for histologic examination. At 9 and 14 days after infection, mice were euthanized with ether and their hind paws were amputated at mid-femur. Paws were fixed in 10% neutral buffered zinc formalin for 24 h. Subsequently, the paws were placed in decalcifying solution (Lerner Laboratories, Pittsburgh, Pa.) for 24 h, followed by the addition of fresh decalcifying solution for an additional 48 h. Following decalcification, the legs were placed in tissue-embedding cassettes (Fisher Scientific), embedded in paraffin, and cut into 6-μm-thick sections. These sections were cryptically encoded and placed on glass slides for staining with hematoxylin and eosin. Unbiased histopathologic examinations were performed by a board-certified pathologist (T. F. Warner).

Statistical analysis. All results were tested by analysis of variance. The Fisher least significant test was used to examine pairs of means when a significant F ratio indicated reliable mean differences (38). The alpha level was set at 0.05 before the experiments were started.

RESULTS

Effects of anti-IL-15 antibody treatment on development and progression of hind-paw swelling. Two groups of eight vaccinated mice each were infected with 10^6 viable *B. bissettii* organisms 21 days after vaccination. Concomitantly, one of the two groups of vaccinated and infected mice was treated with anti-IL-15 antibody on the day of the challenge and daily thereafter for 6 days. Significant ($P < 0.05$) swelling of the right hind paw was detected in vaccinated and infected mice 4 days after infection. The swelling peaked on day 8 and rapidly decreased (Fig. 1). In contrast, vaccinated and challenged mice treated with anti-IL-15 antibody had a delayed onset of swelling of the hind paw and considerably less severity of swelling. When treatment with anti-IL-15 was discontinued on day 6 after infection, swelling of the hind paw occurred rapidly (day 10). Thereafter, the swelling gradually decreased. No swelling of the hind paw occurred in vaccinated, nonchallenged mice treated with anti-IL-15 antibody or in untreated vaccinated mice. Finally, nonvaccinated mice challenged with *B. bissettii* also failed to develop swelling of the hind paw. Similar results were obtained when these experiments were repeated with four mice per group.

Histopathologic confirmation that anti-IL-15 antibody treatment inhibited development of arthritis. Vaccinated mice challenged with *B. bissettii* showed severe inflammation and edematous changes throughout the paw, including the synovial space, synovium, perisynovium, and the small bones of the paw 9 days after infection (Fig. 2). Moreover, the synovium had significant hyperplasia and hypertrophy along with vilus formation. However, no evidence of histopathologic changes, except edema and minor infiltration of neutrophils, macrophages, and lymphocytes in the synovium, was detected in vaccinated mice infected with *B. bissettii* and treated with anti-IL-15 antibody (Fig. 2). The controls, including vaccinated mice and vaccinated mice treated with anti-IL-15 antibody, were also free of significant histopathology (Fig. 3). Nonvaccinated but infected mice developed only marginal pathology of the synovial space, synovium, and perisynovium (Fig. 3).

Fourteen days after challenge, considerable infiltration of neutrophils, macrophages, and lymphocytes was observed in the synovial space, synovium, and perisynovium of *Borrelia*-vaccinated and -infected mice with or without prior treatment with anti-IL-15 antibody (Fig. 4). Hyperplasia and vilus formation were also detected in the synovium of *Borrelia*-vaccinated and -infected mice treated with anti-IL-15. Treatment with anti-IL-15 antibody, however, was terminated in this group of mice 8 days previously. No significant histopathologic changes were detected in the remaining controls, including vaccinated mice with or without treatment with anti-IL-15 antibody, or in mice challenged with *B. bissettii* alone.

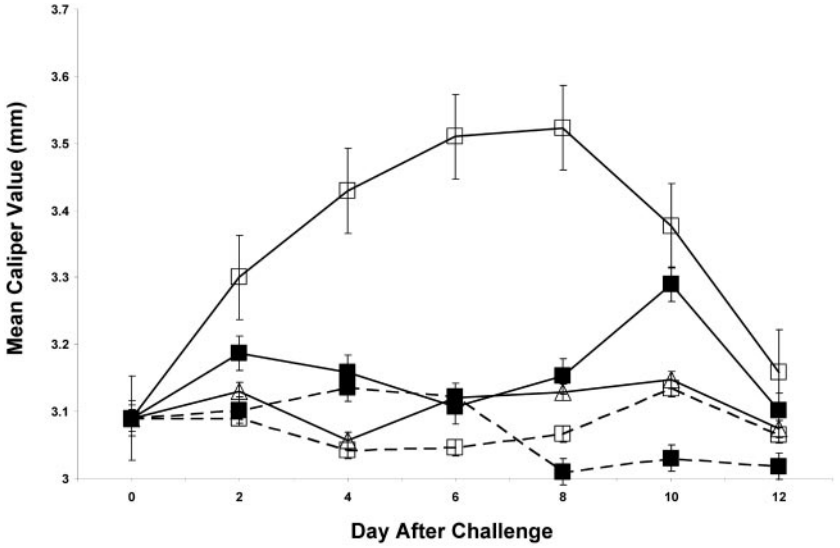


FIG. 1. Development of swelling of the hind paws of vaccinated mice with (solid lines) or without (broken lines) challenge with *B. bissettii* and with (■) or without (□) treatment with anti-IL-15 antibody. The remaining nonvaccinated challenge group (△) did not receive treatment with anti-IL-15 antibody. Data are the means ± standard errors for the experiment.

In a separate set of experiments, *Borrelia*-vaccinated and -infected mice were administered anti-IL-15 antibody 7 days after infection and daily thereafter for 6 days. Significant pathology was detected in the tibiotarsal joints of these mice. In fact, no differences in the swelling of the hind paw or histopathologic changes, compared to non-anti-IL-15 antibody-treated vaccinated and infected mice, were detected.

Effects of rIL-15 receptor alpha on arthritis. Two groups of five vaccinated mice each were infected with 10^6 *B. bissettii* organisms. One of these two groups was administered rIL-15 receptor alpha at the time of infection and daily thereafter for 8 days. Controls included vaccinated mice injected with rIL-15 receptor alpha. Hind-paw swelling was detected in vaccinated mice challenged with *B. bissettii* on day 4 after infection, peaked on day 9, and gradually decreased. Treatment of vaccinated and challenged mice with rIL-15 receptor alpha delayed the onset of swelling of the hind paw and significantly ($P < 0.05$) decreased its severity at all intervals until day 8 after infection. No swelling of the hind paw was detected in vaccinated, but not infected, mice treated with rIL-15 receptor alpha. Histopathologic examination of the paws from each group of five mice confirmed that rIL-15 receptor alpha prevented severe inflammation of the hind paw.

rIL-15 induces IL-17 production from mouse immune cells. Inguinal lymph nodes were obtained from *Borrelia*-vaccinated mice at day 21 after vaccination. The lymph node cells were cocultured with or without 5×10^6 viable *B. bissettii* organisms for 48 h in the presence or absence of rIL-15. Figure 5 shows that immune lymph node cells incubated with 0.5 pg/ml of rIL-15 in the presence or absence of *B. bissettii* induced the production of 18 or more pg/ml of IL-17. Likewise, lower concentrations, 0.1 and 0.2 pg/ml, of rIL-15 in the presence of spirochetes also stimulated immune lymph node cells to release 14 and 13 pg/ml of IL-17, respectively. Immune lymph node cells cultured in RPMI medium alone or RPMI medium with BSK medium released less than 7 pg/ml of IL-17 (similar to background levels in RPMI medium alone), as did cells cultured with PBS containing 0.1% bovine serum albumin. When these experiments were repeated, comparable findings were obtained. Similar experiments conducted using the lymph nodes of nonvaccinated mice yielded no detectable IL-17 (data not shown).

DISCUSSION

In the present study, we hypothesized that IL-15 is a primary mediator involved in the induction of arthritis associated with *Borrelia* vaccination and infection of mice. In support of this hypothesis, we showed that treatment of *Borrelia*-vaccinated and -challenged mice with anti-IL-15 antibody prevented swelling of the hind paws. In addition, treatment of *Borrelia*-vaccinated and -infected mice with rIL-15 receptor alpha also prevented hind-paw swelling. More importantly, both anti-IL-15 antibody and rIL-15 receptor alpha-treated *Borrelia*-vaccinated and -challenged mice were free of major histopathologic indications of arthritis, including hyperplasia, hypertrophy, and vilus formation of the synovium. Similarly, the synovial space and perisynovium were free of inflammatory cells. By contrast, the synovium of non-anti-IL-15-treated *Borrelia*-vaccinated and -infected mice had overt hyperplasia, hypertrophy, and vilus formation. Moreover, the synovial space and perisynovium were infiltrated with neutrophils, macrophages, and lymphocytes. These results suggest that IL-15 plays a major role in the induction of arthritis associated with *Borrelia*-vaccinated and -infected mice.

Although IL-15 is involved in the induction of arthritis associated with *Borrelia* vaccination and infection, we showed previously that IL-17 is also a major participant in the induction of this arthritis (8). When *Borrelia*-vaccinated and -infected mice were administered anti-IL-17 antibody, development of arthritis was prevented (8, 32). Likewise, treatment of *Borrelia*-vaccinated and -infected mice with anti-IL-17 receptor

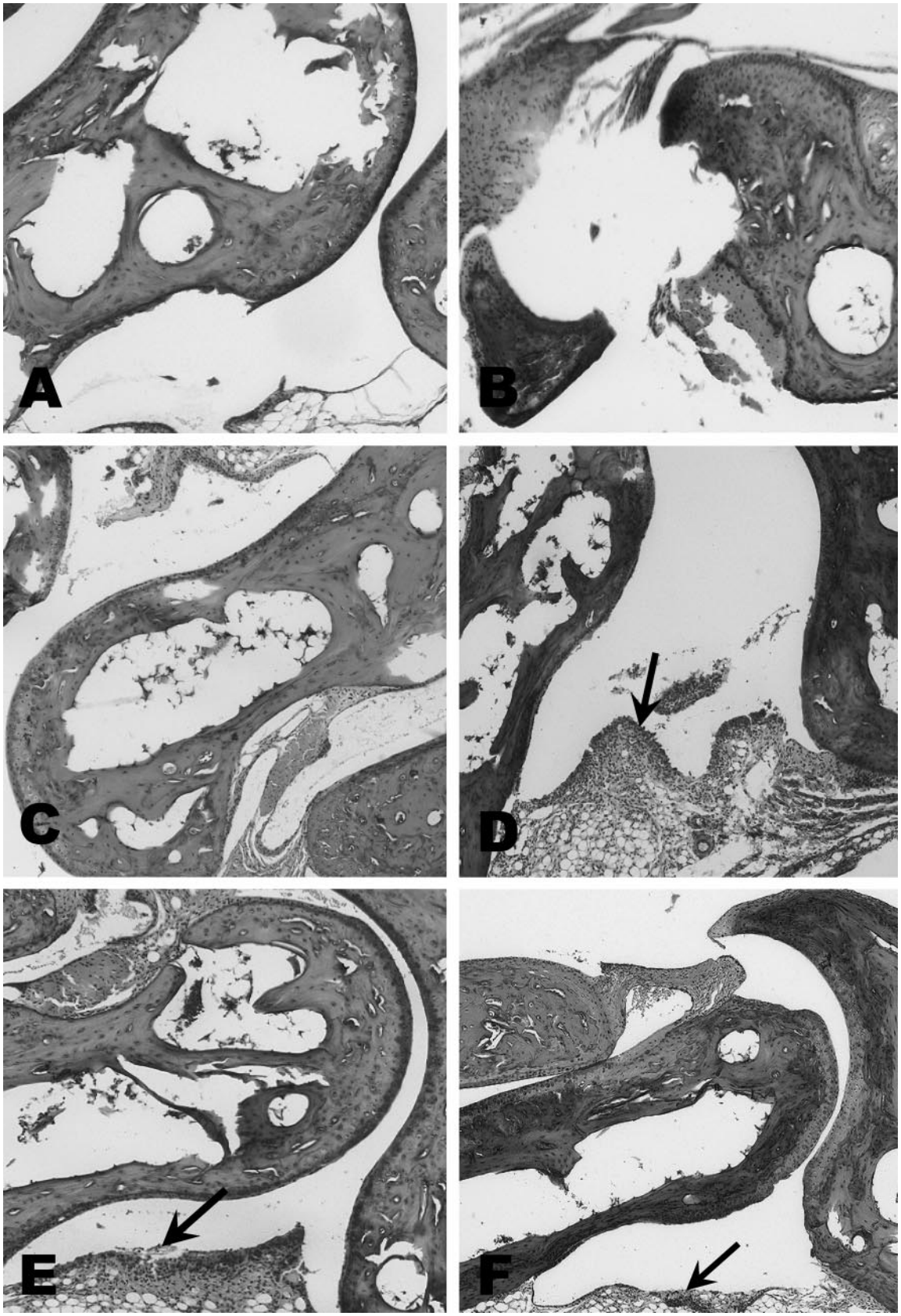
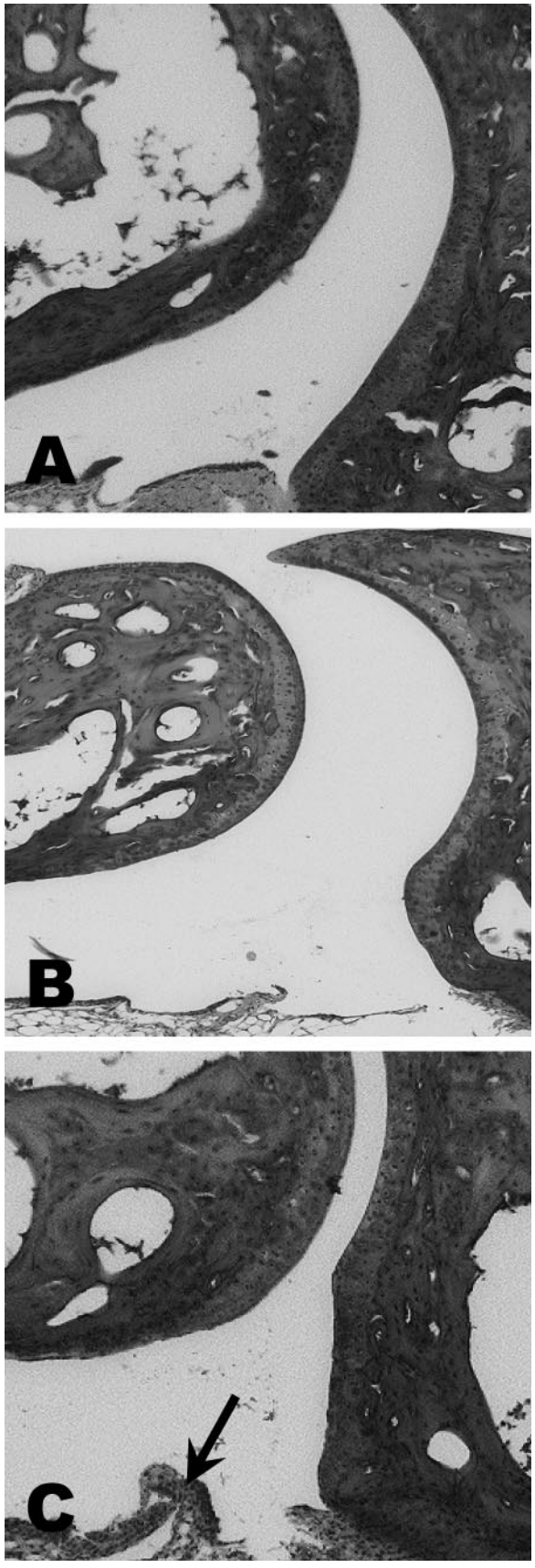


FIG. 2. Histopathology of the tibiotarsal joints of three *Borrelia*-vaccinated and -infected mice (day 9 after infection) with (panels A, B, and C) and without (panels D, E, and F) treatment with anti-IL-15 antibody. No histopathology was found in the three *Borrelia*-vaccinated and -infected mice (panels A, B, and C) treated with anti-IL-15 antibody. In the absence of anti-IL-15 antibody treatment, the three vaccinated and challenged mice (panels D, E, and F) showed substantial inflammation at the tibiotarsal joint. Arrows indicate inflammation. Magnification, ×40.



antibody prevented arthritis, including cartilage and bone destruction (8). Taken together, these results show that both IL-15 and IL-17 play a significant role in arthritis induced by *Borrelia* vaccination and infection of mice.

What is the mechanism by which IL-15 and IL-17 drive the arthritis? Presumably, at the time of infection, some spirochetes are processed by neutrophils (14, 30, 41) and macrophages (15, 29), leading to the release of IL-15 from these cells (19, 31). Since IL-15 is rarely detected in culture supernatants or tissues (4, 31), it probably binds rapidly to the IL-15 receptor alpha, the IL-2 receptor beta, and the common gamma-chain located especially on memory T cells (4, 16, 17, 20). The memory cells, or *Borrelia*-vaccinated T lymphocytes, then release IL-17 that triggers the production of other proinflammatory cytokines, such as IL-1, IL-6, and IL-8 (3, 9, 21), that contribute to the development of arthritis. In support of this theory, we showed that rIL-15 caused the release of IL-17 in ex vivo cultures of immune lymph node cells isolated from a site adjacent to the hind paws. Ziolkowska et al. (43) and Kim et al. (24) also showed that IL-15 triggers the release of IL-17. Our results, as well as those of Ziolkowska et al. (43) and Kim et al. (24), show a definite connection between IL-15 and IL-17. Additional studies are needed to determine if IL-15 directly causes IL-17 production. This linkage is important for the potential therapy of *Borrelia*-induced arthritis as well as other inflammatory diseases (1, 9, 25). Clearly, therapy with anti-IL-15 or anti-IL-17 (8) antibody benefits vaccinated mice when it is started at the time of challenge with *Borrelia* organisms.

The arthritic mechanism, however, may be more complex than the simple linear stimulation of IL-17 production by IL-15. Histopathologic examination showed that treatment of *Borrelia*-vaccinated and -infected mice with anti-IL-15 antibody or rIL-15 receptor alpha dramatically decreased the infiltration of inflammatory cells at the tibiotarsal joint, especially neutrophils. In contrast, neutrophils dominated the cell infiltrate in non-anti-IL-15-treated *Borrelia*-vaccinated and -infected mice. It is known that resting neutrophils, along with infiltrating monocytes, express IL-2 receptor beta and IL-2 receptor gamma, which can readily bind high concentrations of IL-15 (43). We speculate that initially resting neutrophils exposed to the infectious challenge release IL-15. Subsequently, IL-15 binds to these IL-2 receptors as well as the high-affinity IL-15 receptor alpha (23). Interactions between IL-15 and its receptor complex lead to increased signaling and activation of neutrophils (19). Further activation of these neutrophils by IL-15 also increases their phagocytic ability (19). Therefore, IL-15 may have a dual role in the response to infection. It promotes the proinflammatory cytokine cascade via IL-17 production and may oversee the elimination of the initial spirochete challenge. In support of this theory, termination of anti-IL-15 treatment 6 days after challenge was followed immediately by

FIG. 3. Histopathology of the tibiotarsal joints of vaccinated mice (A), vaccinated mice treated with anti-IL-15 antibody (B), and nonvaccinated mice infected with *B. bissettii* (C). No histopathologic changes were detected in the vaccinated mice or vaccinated mice treated with anti-IL-15 antibody. Only minor inflammation (day 9) was observed in nonvaccinated infected mice. The arrow indicates an area of inflammation. Magnification, ×40.

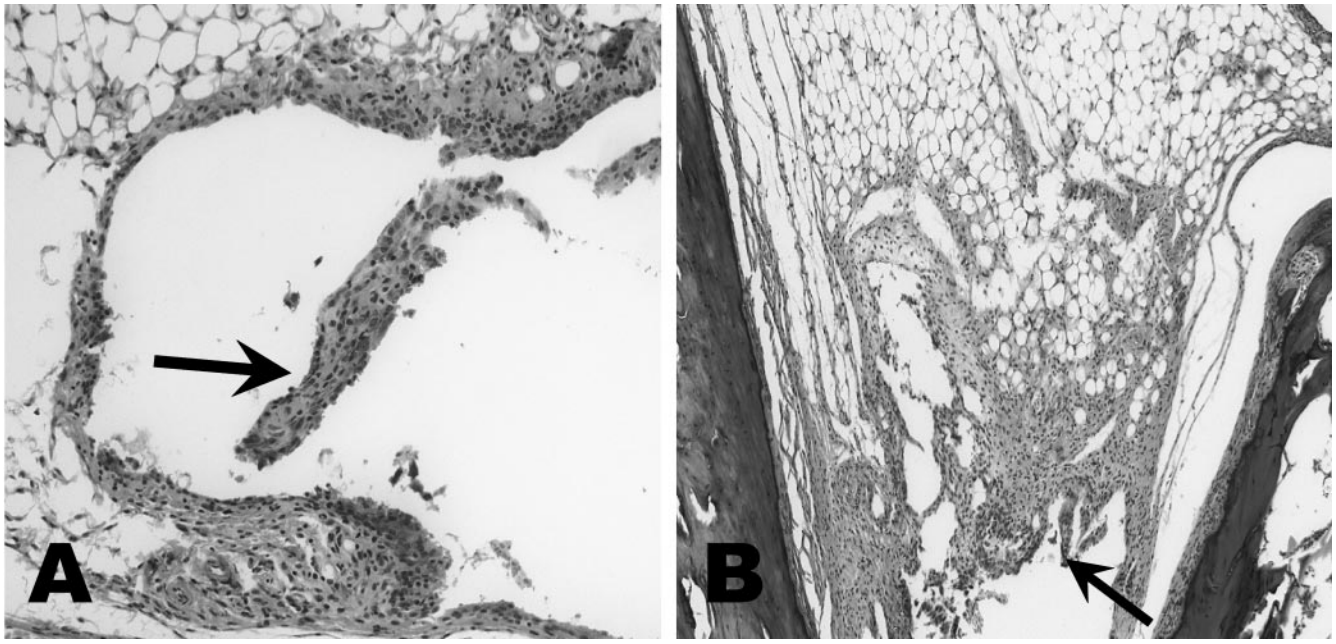


FIG. 4. Histopathology of the tibiotarsal joint of *Borrelia*-vaccinated and -infected mice (day 14 after infection) with (A) and without (B) treatment with anti-IL-15 antibody. (A) Anti-IL-15 antibody treatment was discontinued 8 days previously or 6 days after infection. Inflammation of the tibiotarsal joint is present in both mice. Arrows indicate areas of inflammation. Magnification, ×28 (original magnification, ×40).

an increase in hind-paw swelling in *Borrelia*-vaccinated and -challenged mice. Histological samples from these animals taken 14 days after challenge (8 days after treatment cessation) revealed dramatic infiltration of immune cells as well as vilus formation and destruction of the synovial lining. In addition, others (18) have shown that IL-15 can affect the migration and phagocytosis of neutrophils in patients with Lyme disease.

Although we showed that treatment of *Borrelia*-vaccinated mice at the time of challenge with anti-IL-15 antibody prevented arthritis, treatment with anti-IL-15 antibody failed to ameliorate the arthritis once it was established. Specifically, no resolution of arthritis was detected when *Borrelia*-vaccinated and -challenged mice were administered anti-IL-15 antibody 7 days after infection, as opposed to the case for controls. This was disappointing, and it suggests that anti-IL-15 therapy is not suitable for the treatment of established arthritis associated with *Borrelia* vaccination and infection. In contrast, Baslund et al. (7) have recently shown that treatment of humans with an antibody targeting IL-15 benefited patients with longstanding rheumatoid arthritis. This discrepancy may be related to differences in immune responses that maintain chronic arthritis in rheumatoid arthritis and arthritis induced by *Borrelia burgdorferi*. Additional studies are under way to determine which mediators could be neutralized to hasten the resolution of Lyme arthritis.

The failure of anti-IL-15 antibody to ameliorate established

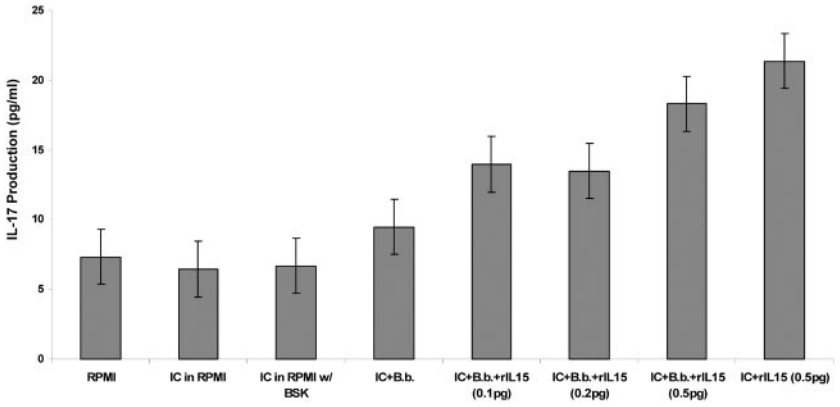


FIG. 5. The effects of rIL-15 (0.1, 0.2, or 0.5 pg) on production of IL-17 from lymph node cells obtained from *Borrelia*-vaccinated mice in the presence or absence of 4×10^6 viable *B. bissettii* organisms (B.b.). Control groups included supernatants from wells containing only RPMI medium or wells in which immune cells (IC) were cultured with RPMI medium, RPMI medium with BSK medium, or RPMI medium with BSK medium containing 4×10^6 viable *B. bissettii* organisms. Data are the means ± standard errors for the experiment.

arthritis does not lessen the cytokine's clinical importance. A major concern with development of a Lyme disease vaccine is the induction of adverse effects, such as arthritis. Once a Lyme disease vaccine has been shown to be protective, it could also be tested for its ability to cause the release of IL-15 or other proinflammatory cytokines from cells (peripheral lymphocytes) obtained from humans or animals exposed to *B. burgdorferi*. If proinflammatory cytokines are detected, the vaccine could be modified to remove epitopes that promote adverse effects but do not influence the ability of the vaccine to induce protection.

Finally, several investigators (2, 31, 40) have shown that gamma interferon can up-regulate the production of IL-15. This suggests that gamma interferon may precede IL-15 in the cytokine cascade for the modulation of arthritis associated with *Borrelia* vaccination and challenge. However, our studies were performed using gamma interferon-deficient mice. Additional studies are needed to define specific cytokines, *Borrelia* antigens, or other immune factors that initiate the production of IL-15. Some of these approaches may provide a new perspective for the treatment of *Borrelia*-associated arthritis.

In conclusion, we show that IL-15 plays a major role in the induction of Lyme arthritis. Although rIL-15 treatment of immune cells led to increased production of IL-17 from these cells, additional studies are needed to determine if IL-15 solely promoted this production. Other studies are also needed to determine mediators that could be neutralized to enhance the resolution of established arthritis.

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