Ablation of Interleukin-12 Exacerbates Lyme Arthritis in SCID Mice

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The administration of interleukin-12 (IL-12) antibodies to Borrelia burgdorferi-infected C3H/HeN-scid mice increased the severity of acute Lyme arthritis. These results contrasted with the reduction of Lyme arthritis by IL-12 antibodies in immunocompetent animals. These data suggest that downregulation of innate immunity in SCID mice in the absence of B- and T-cell responses leads to an exacerbation of joint inflammation.

The murine model of Lyme disease is useful for studying pathogenesis and immunity to Borrelia burgdorferi (3). Antibodies play a major role in protection and disease modulation in C3H/HeN (C3H) mice (12, 14). In contrast to immunocompetent mice, in which acute arthritis regresses, severe combined immunodeficient (SCID) mice are unable to control infection and develop progressive, unremitting arthritis (8, 20). Arthritis resolves when SCID mice are administered sera from B. burgdorferi-infected immunocompetent mice (5) and when B and T cells together, not T cells alone, are given during the course of infection (21), indicating that antibodies are sufficient to modulate disease.

Murine Lyme arthritis is influenced by both the spirochete and the host response. Disease severity correlates with the presence and number of B. burgdorferi spirochetes in the joint (7, 19, 24). Furthermore, Th1-polarized responses are associated with arthritis in immunocompetent mice. The disease is more severe in strains of mice that produce high levels of gamma interferon (IFN-γ), interleukin-12 (IL-12), and B. burgdorferi-specific immunoglobulin G2a (IgG2a) antibodies (17, 18). Moreover, IFN-γ and IL-12 antibodies reduce disease (1, 17, 18). Because IL-12 is also produced by cells of the innate immune system, such as macrophages (9, 11), and Lyme arthritis develops in SCID mice, we examined the production of this cytokine and the effect of IL-12 antibodies on the genesis of Lyme arthritis in SCID mice.

Mice were given IL-12 antibodies, challenged with B. burgdorferi, and then examined for infection and arthritis. C3H mice were purchased from the Frederick Cancer Research Center (Frederick, Md.), and C3H-scid mice were obtained from Jackson Laboratories (Bar Harbor, Maine). All mice were housed in isolator cages. Mice were intradermally inoculated on the dorsal thoracic midline with 10⁴ B. burgdorferi N40 spirochetes (6, 15). A group of C3H-scid mice were inoculated with 10⁴ spirochetes (6, 15). A group of C3H-scid mice were infected.

Infection was assessed by culturing selected organs in Barbour-Stoenner-Kelly II medium. +, B. burgdorferi infected.

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Each mouse was infected with 10⁴ spirochetes.

Arthritis was documented by blinded examination of formalin-fixed, paraffin-embedded tibiotarsal joints. Tibiotarsal arthritis was scored on a scale from 0 to 3 as previously described (13).

![Graph showing IL-12 levels in sera of B. burgdorferi-infected C3H and C3H-scid mice. Sera from individual mice were pooled and examined by enzyme-linked immunosorbent assay for IL-12.](Image)

![Table 1. Arthritis in C3H-scid mice treated with IL-12 MAb](Table)
treated with an ammonium sulfate-purified ascitic fluid preparation (passed over a protein G column) of IL-12 monoclonal antibody (MAb) C17.8 (1). Control mice were given nonspecific antibodies (rat IgG; Sigma Chemical Co., St. Louis, Mo.). One milligram of antibody in 100 μl was administered per injection 1 day prior to spirochetal inoculation on days 1, 2, and 3 and then every 4 days until animals were sacrificed as previously described (1). Animals were killed on day 21 and analyzed for infection by culture of selected organs (blood, urinary bladder, spleen, and skin at the inoculation site) in Barbour-Stoenner-Kelly II medium (2). Both knees and tibiotarsal joints from each mouse were fixed in formalin, embedded in paraffin, sectioned, and stained for blinded histopathologic analysis (13). The prevalence of arthritis among the four joints examined from each mouse was tabulated, and tibiotarsal arthritis severity was scored on a scale of 0 to 3, as previously described (5).

The serum IL-12 levels in infected C3H-scid mice were measured by enzyme-linked immunosorbent assay (Pharmingen, San Diego, Calif.) because this cytokine is associated with disease in immunocompetent animals (1). B. burgdorferi-infected C3H-scid mice had low (but detectable) levels of IL-12 compared to C3H mice (Fig. 1). The IL-12 levels in the sera of disease-resistant BALB/c mice were below the detection limit (not shown). These reduced levels of IL-12 may have been because of a lack of B cells that are a source of IL-12 or, alternatively, because of enhanced IL-12 production by macrophages during infection with the cooperation of T cells, probably through the interaction between CD40 on macrophages and CD40-ligand on B. burgdorferi-activated T cells (10, 16, 23). Indeed, B. burgdorferi-infected CD40-ligand-deficient mice produce less IL-12 than do controls (1a).

The effect of anti-IL-12 treatment on arthritis in C3H-scid mice was examined. As expected (22), C3H-scid mice developed more severe disease (mean tibiotarsal arthritis score ± standard deviation [SD], 2.2 ± 0.2) than did immunocompetent C3H mice (mean score ± SD, 0.6 ± 0.7) (Table 1). When C3H-scid mice were treated with a blocking MAb against IL-12, the increase in arthritis was highly significant (mean score ± SD, 2.8 ± 0.3; by the Student t test, P < 0.0001, compared to untreated C3H-scid mice) (Fig. 2 and 3 and Table 1). Arthritis prevalence was unaffected by this treatment.

The SCID mouse model provides a means of assessing the influence of IL-12 on Lyme arthritis in the absence of T-helper-cell development. C3H-scid mice developed more severe Lyme arthritis than did controls in part because of the inability to generate B. burgdorferi-specific, disease-modulating antibodies. Our data suggest that anti-IL-12, as expected, downregulates the innate immune response in an environment without T or B cells. Moreover, the increase in disease severity in mice treated with a blocking MAb to IL-12 suggests that innate immunity has a role in the modulation of disease. This interpretation agrees with the observed increase in arthritis in mice with the beige (bg) mutation, which have defects in innate immunity, including NK cell and granulocyte functions (4). We postulate that IL-12 induces inflammation through the stimulation of IFN-γ and other proinflammatory cytokines (tumor necrosis factor alpha and IL-1β), as well as chemokines that can be important in the chemotaxis of granulocytes toward the joint.

We previously reported that arthritis was reduced in immunocompetent B. burgdorferi-infected C3H mice treated with the same IL-12 MAb (1). These data suggest that the control of infection and inflammation, with subsequent tissue destruction, differs in immunocompetent and SCID mice. In immu-
T cells can lead to an exacerbation of murine Lyme arthritis. The downregulation of TH1 responses and their inflammatory action resulted in a decrease in joint disease. In contrast, in C3H-scid mice, anti-IL-12 appeared to affect the innate immune response in the absence of proinflammatory T cells without affecting the number of spirochetes in the ear tissues of infected animals (not shown). Therefore, the mechanisms of innate defense important in B. burgdorferi control and arthritis development may be detectable only in SCID mice, although they probably act in both immunocompetent and SCID mice. Indeed, the differential effect of anti-IL-12 treatment on Lyme arthritis in immunocompetent and SCID mice is observed in C3H beige mice. J. Infect. Dis. 162:492–500.

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