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 10^4 *B. burgdorferi* N40 spirochetes (6, 15). A group of C3H-*scid* mice were

FIG. 1. 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.

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TABLE 1. Arthritis in C3H-*scid* mice treated with IL-12 MAb^a

Mouse group and no.	Rat IgG treatment		Anti-IL-12 treatment	
	Infection ^b	Arthritis severity ^c	Infection ^b	Arthritis severity ^c
C3H				
1	+	0		
2	+	0		
3	+	2		
4	+	1		
5	+	0.5		
6	+	0		
7	+	1.5		
8	+	1		
9	+	0.5		
10	+	0.5		
C3H- <i>scid</i>				
1	+	2.5	+	2.5
2	+	2	+	3
3	+	2	+	2
4	+	2	+	3
5	+	2	+	2.5
6	+	2	+	3
7	+	2	+	3
8	+	2	+	3
9	+	2.5	+	3
10	+	2.5	+	3

^a Each mouse was infected with 10^4 spirochetes.

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

^c 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).

FIG. 2. Tibiotarsus of a *B. burgdorferi*-infected SCID mouse treated with control IgG. The bone at the right is the calcaneus, and the Achilles tendon is at the bottom. There is mild inflammation along the Achilles tendon, and there is synovial hypertrophy at the top.

FIG. 3. Tibiotarsus of a *B. burgdorferi*-infected SCID mouse treated with anti-IL-12 MAb. This section is in the same orientation as that in Fig. 2. Note the marked, diffuse inflammation involving the Achilles tendon, synovium, and surrounding adipose tissue.

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 μ l 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 $\pm$ standard deviation [SD], 2.2 ± 0.2) than did immunocompetent C3H mice (mean score $\pm$ 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 $\pm$ 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-1 β), 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-

nocompetent C3H mice, anti-IL-12 treatment was accompanied by a decreased Th1 response, as well as effects on innate immunity, such as that mediated by NK cells, which are primary targets for IL-12 in the initiation of an immune response (1, 11). 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 immunodeficient mice was recently postulated by Seiler and Weis (22). It is clear that antibodies, T cells, and the innate immune response all play important roles in spirochete clearance and the pathogenesis of arthritis. These data show that downregulation of the innate immune response in the absence of B and T cells can lead to an exacerbation of murine Lyme arthritis.

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REFERENCES

- Anguita, J., D. H. Persing, M. Rincon, S. W. Barthold, and E. Fikrig. 1996. Effect of anti-interleukin 12 treatment on murine Lyme borreliosis. *J. Clin. Invest.* **97**:1028–1034.
- Anguita, J., and E. Fikrig. Unpublished data.
- Barbour, S. W., M. S. deSouza, J. L. Janotka, A. L. Smith, and D. H. Persing. 1993. Isolation and cultivation of Lyme disease spirochetes. *Yale J. Biol. Med.* **57**:521–525.
- Barthold, S. W., D. S. Beck, G. M. Hansen, G. A. Terwilliger, and D. Moody. 1990. Lyme borreliosis in selected strains and ages of laboratory mice. *J. Infect. Dis.* **162**:133–138.
- Barthold, S. W., and M. de Souza. 1995. Exacerbation of Lyme arthritis in beige mice. *J. Infect. Dis.* **172**:778–784.
- Barthold, S. W., M. de Souza, and S. Feng. 1996. Serum-mediated resolution of Lyme arthritis in mice. *Lab. Invest.* **74**:57–67.
- Barthold, S. W., M. S. de Souza, J. L. Janotka, A. L. Smith, and D. H. Persing. 1993. Chronic Lyme borreliosis in the laboratory mouse. *Am. J. Pathol.* **143**:959–971.
- Barthold, S. W., D. H. Persing, A. L. Armstrong, and R. A. Peeples. 1991. Kinetics of *Borrelia burgdorferi*: dissemination and evolution of disease following intradermal inoculation of mice. *Am. J. Pathol.* **136**:263–273.
- Barthold, S. W., C. L. Sidman, and A. L. Smith. 1992. Lyme borreliosis in genetically resistant and susceptible mice with severe combined immunodeficiency. *Am. J. Trop. Med. Hyg.* **47**:605–613.
- Brunda, M. J. 1994. Interleukin-12. *J. Leukocyte Biol.* **55**:280–288.
- Campbell, K. A., P. J. Ovendale, M. K. Kennedy, W. C. Fanslow, S. G. Reed, and C. R. Maliszewski. 1996. CD40 ligand is required for protective cell-mediated immunity to *Leishmania major*. *Immunity* **4**:283–289.
- Chehimi, J., and G. Trinchieri. 1994. Interleukin-12: a bridge between innate resistance and adaptive immunity with a role in infection and acquired immunodeficiency. *J. Clin. Immunol.* **14**:149–161.
- Fikrig, E., S. W. Barthold, F. S. Kantor, and R. A. Flavell. 1990. Protection of mice against the Lyme disease agent by immunizing with recombinant OspA. *Science* **250**:553–556.
- Fikrig, E., S. W. Barthold, and R. A. Flavell. 1993. OspA vaccination of mice with established *Borrelia burgdorferi* infection alters disease but not infection. *Infect. Immun.* **61**:2553–2558.
- Fikrig, E., S. W. Barthold, N. Marcantonio, K. Deponte, F. S. Kantor, and R. A. Flavell. 1992. Roles of OspA, OspB, and flagellin in protective immunity to Lyme borreliosis in laboratory mice. *Infect. Immun.* **60**:657–661.
- Fikrig, E., H. Tao, F. S. Kantor, S. W. Barthold, and R. A. Flavell. 1993. Evasion of protective immunity by *Borrelia burgdorferi* by truncation of outer surface protein B. *Proc. Natl. Acad. Sci. USA* **90**:4092–4096.
- Kamanaka, M., P. Yu, T. Yasui, K. Yoshida, T. Kawabe, T. Horii, T. Kishimoto, and H. Kikutani. 1996. Protective role of CD40 in *Leishmania major* infection at two distinct phases of cell-mediated immunity. *Immunity* **4**:275–281.
- Keane-Myers, A., and S. P. Nickell. 1995. Role of IL-4 and IFN-gamma in modulation of immunity to *Borrelia burgdorferi* in mice. *J. Immunol.* **155**:2020–2028.
- Matyniak, J. E., and S. L. Reiner. 1995. T helper phenotype and susceptibility in experimental Lyme disease. *J. Exp. Med.* **181**:1251–1254.
- Persing, D. H., B. J. Rutledge, P. N. Rys, D. S. Podzorski, P. D. Mitchell, K. D. Reed, B. Liu, E. Fikrig, and S. E. Malawista. 1994. Target imbalance: disparity of *Borrelia burgdorferi* genetic material in synovial fluid from Lyme arthritis patients. *J. Infect. Dis.* **169**:668–672.
- Schaible, U. E., S. Gay, C. Museteanu, M. D. Kramer, G. Zimmer, K. Eichmann, U. Museteanu, and M. Simon. 1990. Lyme borreliosis in the severe combined immunodeficiency (*scid*) mouse manifests predominantly in the joints, heart, and liver. *Am. J. Pathol.* **137**:811–820.
- Schaible, U. E., R. Wallich, M. D. Kramer, G. Nerz, T. Stehle, C. Museteanu, and M. M. Simon. 1994. Protection against *Borrelia burgdorferi* infection in SCID mice is conferred by presensitized spleen cells and partially by B but not T cells alone. *Int. Immunol.* **6**:671–681.
- Seiler, K. P., and J. J. Weis. 1996. Immunity to Lyme disease: protection, pathology and persistence. *Curr. Opin. Immunol.* **8**:503–509.
- Soong, L., J. C. Xu, I. S. Grewal, P. Kima, J. Sun, B. J. Longley, Jr., N. H. Ruddie, D. McMahon-Pratt, and R. A. Flavell. 1996. Disruption of CD40-CD40 ligand interactions results in an enhanced susceptibility to *Leishmania amazonensis* infection. *Immunity* **4**:263–273.
- Yang, L., J. H. Weis, E. Eichwald, C. P. Kolbert, D. H. Persing, and J. J. Weis. 1994. Heritable susceptibility to severe *Borrelia burgdorferi*-induced arthritis is dominant and is associated with persistence of large numbers of spirochetes in tissues. *Infect. Immun.* **62**:492–500.