Selective Anti-Inflammatory Action of Interleukin-11 in Murine Lyme Disease: Arthritis Decreases while Carditis Persists

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The role of interleukin (IL)-11, a cytokine with potent anti-inflammatory properties, in murine Lyme disease was investigated. *Borrelia burgdorferi*-infected mice treated with IL-11 developed less arthritis than did control animals. In contrast, IL-11 blocking antibodies increased Lyme arthritis. Murine Lyme carditis was not affected by either IL-11 or IL-11 antibodies. Administration of IL-11 was associated with increased production of mRNA for IL-12 and inducible nitric oxide synthase but not interferon-γ or IL-4 in *B. burgdorferi*-infected mice, suggesting a predominant effect of IL-11 on the innate immune response. These data show that IL-11 selectively reduced joint but not cardiac inflammation caused by *B. burgdorferi* in mice.

*Borrelia burgdorferi* causes Lyme disease, an illness that can affect the skin and the cardiovascular, neurologic, and musculoskeletal systems [1]. *B. burgdorferi* is the most common cause of persistent bacterial joint inflammation in the United States. *B. burgdorferi*-infected immunocompetent mice develop arthritis and carditis that is most severe at 2–3 weeks and then resolves over several months, thereby partially mimicking human disease [2].

*B. burgdorferi*-specific antibody responses are important in controlling infection [3]. Acute arthritis resolves in immunocompetent mice, whereas disease persists in severe combined immunodeficient (SCID) animals [4]. Furthermore, antibodies elicited during infection can induce the regression of joint inflammation when passively administered to *B. burgdorferi*-infected SCID mice [5]. Reports have also associated Th1 cell responses with the severity of Lyme arthritis, in both humans [6] and mice [7, 8]. Increased serum levels of IL-12 and interferon-γ (IFN-γ) have been observed in inbred mice that develop severe joint inflammation [8]. Moreover, the administration of blocking monoclonal antibodies (MAbs) against IFN-γ or interleukin (IL)-12 resulted in the reduction of clinical joint swelling [7, 8] and histopathologic arthritis [9] in immunocompetent mice.

The pathogenesis of murine heart and joint disease due to *B. burgdorferi* infection differ. The development of acute murine Lyme carditis is not dependent on major histocompatibility complex (MHC) class II or CD4 T cells [10, 11]. CD4 T cells may, however, be involved in the resolution of carditis. *B. burgdorferi*-infected, MHC class II transactivator (CIITA)-deficient mice, which lack the normal CD4 repertoire, develop acute carditis that regresses more slowly than in control animals [11]. In contrast, the evolution of arthritis is similar in CIITA-deficient and control mice [11]. In addition, the administration of IL-12 or B7-1 antibodies also hastens the resolution of murine Lyme arthritis but not carditis [9, 12].

IL-11 is a cytokine with potent anti-inflammatory actions that belongs to the IL-6-type cytokine family. In addition to its well-defined hematopoietic effects, IL-11 can also stimulate tissue remodeling and is protective in a variety of models of tissue inflammation and injury. Recombinant human (rh) IL-11 reduced acute and chronic inflammation in a number of animal models of human disease. It can also regulate macrophage production of proinflammatory cytokines. However, the role of IL-11 in modulating infection-induced inflammatory disease has not been tested. We therefore investigated the role of IL-11 in the development of murine Lyme arthritis and carditis.

Materials and Methods

**Mice.** Six-week-old female C3H/HeNCr (C3H) mice were purchased from the Frederick Cancer Research Center (Frederick, MD). Mice were housed in filter frame cages and euthanized with CO2.

*B. burgdorferi* and murine infection. A clonal low-passage isolate of *B. burgdorferi* N40 was used throughout the studies. Spirochetes were grown in Barbour-Stoenner-Kelly II medium at 34°C. Mice were infected with an inoculum of 10^8 spirochetes administered by intradermal injection in the back. Mice were killed
after 3 weeks of infection, a time point that represents the acute phase of the disease. Infection was assessed by culturing the blood, urinary bladder, spleen, and skin (at the inoculation site). Cultures were examined for *B. burgdorferi* after 14 days, a time period sufficient for a spirochete to grow to stationary phase. Hearts and joints (both knees and tibiotarsi) were fixed in formalin, embedded in paraffin, and examined microscopically for evidence of inflammation. Arthritis severity was scored on a scale of 0 (indistinguishable from uninfected controls, no disease) to 3 (severe disease) [9]. Grades 1 and 2 represent mild and moderate arthritis, respectively.

Cytokines, antibodies, and in vivo treatments. rhIL-11 and anti–IL-11 MAbt were provided by Genetics Institute (Cambridge, MA). *B. burgdorferi*-infected mice were treated with rhIL-11 every 24 h for 5 consecutive days, followed by a 2-day resting period, until sacrifice. Mice were given 0.1, 1.0, or 2.0 µg of rhIL-11 per injection throughout the period of infection. rhIL-11 was administered in PBS supplemented with 1% normal mouse serum (NMS). PBS plus 1% NMS was used as a control. Uninfected mice (controls) were treated with rhIL-11 in an identical fashion. Anti–IL-11 was given 24 h before infection, then every 24 h for 4 days, and then every 4 days until sacrifice. The dose of anti–IL-11 was 850 mg/injection. The same amount of rat IgG (Sigma, St. Louis) was used as a control.

Reverse transcriptase–polymerase chain reaction (RT-PCR). Whole splenocytes, pooled from the mice in each group and depleted of red cells, were used to extract RNA using the Micro RNA Isolation Kit (Stratagene, La Jolla, CA). RNA (5–10 µg) was used to obtain cDNA with an RT-PCR kit (Stratagene). Quantitative competitive PCR was performed as described [12] using the polycotopmer cDNA construct, pPQRS [12]. Oligonucleotide primers were synthesized as described [12]. Conditions of the PCR were 94°C for 20 s, 60°C for 40 s, and 72°C for 20 s, for 35 cycles. All reactions were performed in a Peltier Thermal Cycler-200 (MJ Research, Watertown, MA) using reagents (Taq polymerase, dNTPs) from Boehringer Mannheim (Indianapolis). Hypoxanthine-guanine phosphoribosyl transferase was used as a control to ensure equal loads of cDNA.

Statistical analysis. Arthritis incidence and severity data for rhIL-11 treatment groups were analyzed with Kruskal-Wallis 1-way analysis of variance. For pairwise comparisons, the Wilcoxon rank sum test was used. Significance was assessed at *P* < .05.

Results

The effect of IL-11 on experimental murine Lyme borreliosis was investigated because IL-11 has potent anti-inflammatory capabilities. C3H mice were treated with different doses of rhIL-11 (0.1, 1.0, and 2.0 µg). The administration of rhIL-11 resulted in a decrease in the severity of murine Lyme arthritis compared with PBS-treated controls when rhIL-11 was given at 2.0 µg/ injection (table 1). Mice administered 2.0 µg of rhIL-11 per injection had milder arthritis than controls (average ± SE = 0.5 ± 0.1 vs. 1.1 ± 0.1, respectively; *P* < .05). Conversely, *B. burgdorferi*-infected, anti–IL-11 MAb–treated mice developed significantly more severe arthritis than did rat IgG–treated controls (average degree of arthritis ± SE = 1.3 ± 0.3 vs. 0.7 ± 0.2, respectively; Wilcoxon rank sum test, *P* < .05, table 1). Arthritis incidence was, nevertheless, unaffected by the treatments (table 1, *P* > .05). Murine Lyme carditis was not affected by IL-11 or anti–IL-11.

Acquired Th1 responses against *B. burgdorferi*, with elevated production of IFN-γ and *B. burgdorferi*-specific IgG2a [8, 9], correlate with the degree of arthritis. In contrast, IL-4, an effecter of Th2 responses, has been associated with decreased joint inflammation [8]. To determine a mechanism for the reduction of arthritis in rhIL-11–treated mice, quantitative RT-PCR assessed these cytokines in the spleens of the control and treated infected animals. Although not completely reflective of the situation in the joints and hearts, spleen RNA determinations provide insight into the effects that cytokine administration and spirochetal infection have on the immune system [9]. We also assessed mRNA levels for IL-12, because this cytokine is associated with the innate immune response. Inducible nitric oxide synthase (iNOS) mRNA was examined because nitric oxide has been associated with the killing of *B. burgdorferi* in vitro [13], and its production has been shown to be regulated by IL-11 [14]. mRNA levels for IFN-γ and IL-4 were similar in experimental and control mice (figure 1). Levels of *B. burgdorferi*–specific antibodies (IgG1, IgG2a, and IgG2b), which

<table>
<thead>
<tr>
<th>Treatment (dose)</th>
<th>Infection (no. infected/no. examined)</th>
<th>Average no. of joints affected/ mouse ± SE</th>
<th>Severity ± SE</th>
<th>Carditis (no. with carditis/no. examined)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS 1% NMS</td>
<td>25/25</td>
<td>2.2 ± 0.2</td>
<td>1.1 ± 0.1</td>
<td>25/25</td>
</tr>
<tr>
<td>rhIL-11 (0.1 µg/injection)</td>
<td>10/10</td>
<td>1.6 ± 0.4</td>
<td>1.3 ± 0.2</td>
<td>10/10</td>
</tr>
<tr>
<td>rhIL-11 (1.0 µg/injection)</td>
<td>15/15</td>
<td>2.0 ± 0.3</td>
<td>0.8 ± 0.1</td>
<td>15/15</td>
</tr>
<tr>
<td>rhIL-11 (2.0 µg/injection)</td>
<td>15/15</td>
<td>1.2 ± 0.3</td>
<td>0.5 ± 0.1</td>
<td>15/15</td>
</tr>
<tr>
<td>Rat IgG</td>
<td>5/5</td>
<td>1.6 ± 0.5</td>
<td>0.7 ± 0.2</td>
<td>5/5</td>
</tr>
<tr>
<td>Anti-IL-11 (860 µg/injection)</td>
<td>4/4</td>
<td>2.3 ± 0.6</td>
<td>1.3 ± 0.3</td>
<td>4/4</td>
</tr>
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NOTE. Mice were assessed for infection by culture of selected specimens after sacrifice. Arthritis and carditis were evaluated by histopathologic analysis of joints (both knees and tibiotarsi) and hearts of infected mice. NMS = normal mouse serum.

* a *P* < .05 vs. controls.
can also be associated with Th1 or Th2 responses, were comparable in both groups of animals (not shown). Levels of IL-12 and iNOS mRNA were increased in IL-11–treated mice, the effects of increased IL-12 occur independently of IL-12–mediated INF-γ induction.

These data agree with those of a recent study demonstrating lipopolysaccharide-mediated IL-12 production in mice without increased levels of circulating INF-γ [14]; this suggests that IL-11 may act on different cell types, possibly including NK and T cells, to block the induction of INF-γ or, alternatively, through the effect of this cytokine on coactivators of INF-γ production, such as TNF-α or IL-1β. The increase in iNOS suggests IL-11–dependent activation of macrophages. It is possible that the macrophage activation, while helping to clear the spirochete, also contributes to the persistence of the cardiac infiltrate while the joint lesion becomes less severe.

The anti-inflammatory effect of IL-11 is probably dependent on the balance and interplay between the infectious agent, the cells involved in the defense against the invading agent, and the cytokine response produced by the interaction between them. In the case of *B. burgdorferi* infection, IL-11–mediated activation of innate immunity results in a decrease in the development of murine Lyme arthritis.

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**References**


EM. Early murine Lyme carditis has a macrophage predominance and is independent of major histocompatibility complex class II–CD4+ T cell interactions. J Infect Dis 1995;171:362–70.


