

T Helper Phenotype and Genetic Susceptibility in Experimental Lyme Disease

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Infection of inbred mice with *Borrelia burgdorferi* results in strain-specific variation in the severity of pathogen-induced arthritis: BALB/c mice develop only mild disease whereas C3H/HeJ mice develop severe arthritis. The immunologic basis for varying host susceptibility has yet to be defined. We modified experimental Lyme disease to facilitate measurement of antigen-specific cytokine production in resistant and susceptible mice. The analysis revealed highly polarized lymphokine patterns directly linked to differing disease outcomes. Among the inbred strains of mice challenged with *B. burgdorferi*, production of interleukin 4 (IL-4) correlated to resistance whereas production of interferon γ (IFN- γ) correlated to susceptibility. We also demonstrate that production of IL-4 or IFN- γ regulates the severity of arthritis after infection. Neutralization of IL-4 in resistant BALB/c mice resulted in more severe arthritis whereas neutralization of IFN- γ in susceptible C3H/HeJ mice attenuated the severity of disease. These results suggest a primary relationship between T helper cell phenotype and the genetic basis for susceptibility to experimental Lyme borreliosis.

Lyme disease is the most common vector-borne illness in the United States and is increasingly recognized as a source of significant human morbidity due to the secondary and tertiary complications of cardiac, neurologic, and rheumatic involvement (1). Among inbred strains of mice experimentally infected with *Borrelia burgdorferi*, the severity of arthritis is under host genetic control, with BALB/c mice developing only mild disease but C3H/HeJ mice acquiring severe arthritis (2, 3). Severity has been linked to higher spirochete burdens (4), suggesting that susceptibility to pathology represents a deficiency in microbial immunity. In a number of infectious disease models with varying genetic susceptibility, polarized lymphokine responses underlie the discordance in disease outcome (5–7). Direct evidence for a difference in the T helper subsets that susceptible and resistant hosts mount in response to *B. burgdorferi* has been lacking.

To examine the development of T helper cell subsets in experimental Lyme borreliosis, we inoculated the spirochete in BALB/c and C3H/HeJ mice in a manner that would allow removal of a draining lymph node that had focused the immune response to the microbial depot. In addition to characterizing the phenotype of Th1 and Th2 cytokines produced during differential disease responses, we have neutralized IL-4 and IFN- γ in vivo to ascribe a causal relationship between the type of T helper cell response and the genetic basis for susceptibility to Lyme disease.

Materials and Methods

Animals and Bacteria. Female BALB/c and C3H/HeJ 5-wk-old mice were obtained from The Jackson Laboratory (Bar Harbor, ME)

and housed in the University of Chicago Animal Care Facility. The N40 isolate of *B. burgdorferi* was kindly provided by Stephen Barthold (Yale University, New Haven, CT) and used as described by Yang et al. (3) with the following modification: anesthetized mice were inoculated in the right hind footpad with 2×10^5 bacteria in 50 μ l of BSK media (Sigma Chemical Co., St. Louis, MO). Tibiotarsal joints were measured weekly using a metric caliper (Ralmike's Tool-A-Rama, South Plainfield, NJ) through the thickest anteroposterior diameter of the ankle.

Antigen-specific Restimulation Assays. Popliteal lymph nodes and spleens were harvested from killed animals 4–5 wk after infection in various experiments and assayed as pooled groups of two or more animals. Single-cell suspensions were washed twice with balanced salt solution/1% FCS, and plated at 10^6 cells/well in RPMI media/10% FCS in a 96-well plate in duplicate with and without *Borrelia* soluble sonicate (35 μ g/ml final concentration). Supernatants were collected at 72 h and assayed for IL-4 and IFN- γ using mAb pairs (PharMingen, San Diego, CA) in standard sandwich ELISA according to the manufacturer's instructions.

Cytokine Neutralization. 1 mg of 11B11 (rat anti-mouse IL-4) or XMG 1.2 (rat anti-mouse IFN- γ) antibody was administered intraperitoneally on the day of infection and in some cases doses were repeated weekly or twice weekly for 2 wk.

Results

Proximal Arthritis with Genetic Variability Develops after Subcutaneous Footpad Inoculation of *Borrelia burgdorferi*. In animals infected in the right footpad, maximal right ankle arthritis developed over 2–4 wk with differing severity for BALB/c and C3H/HeJ mice. In four separate experiments, the pre-mortem peak ankle diameters were consistently larger in C3H/HeJ mice (mean diameter 3.43 mm, SEM 0.06, $n =$



Figure 1. *Borrelia burgdorferi*-induced proximal arthritis in inbred mice. Right tibiotarsal joints were examined 3 wk after right footpad infection to correlate gross pathological changes with pre-mortem finding of increased ankle diameter in C3H/HeJ (left) compared with BALB/c (right) mice. Periarticular swelling (posterior) with synovial and tendon fibrosis (anterior) in C3H/HeJ mice is similar to that described following distant inoculation of the spirochete (2, 3, 10).

24) compared with BALB/c mice (mean diameter 2.94 mm, SEM 0.04, $n = 18$) and correlated with greater periarticular inflammation and synovial and tendon fibrosis on gross examination of dissected ankles from killed animals (Fig. 1). Left ankle arthritis began to develop after the third or fourth week but was generally absent in BALB/c mice and less severe in C3H/HeJ mice than their own right ankle disease (data not shown).

Polarized Lymphokine Patterns Reflect Differing Disease Severity. Right popliteal lymph node cells from infected animals were cultured in vitro, with and without borrelial antigen preparation, and IFN- γ and IL-4 were measured as an index of Th1 and Th2 development (Fig. 2 A). In four separate experiments, BALB/c mice consistently developed a Th2 pattern of cytokine secretion with high IL-4 and low IFN- γ production during antigen-specific recall, whereas C3H/HeJ mice developed a Th1 pattern of cytokine secretion with absent-to-low levels of IL-4 and high levels of IFN- γ . This pattern of cytokine production was as polarized when

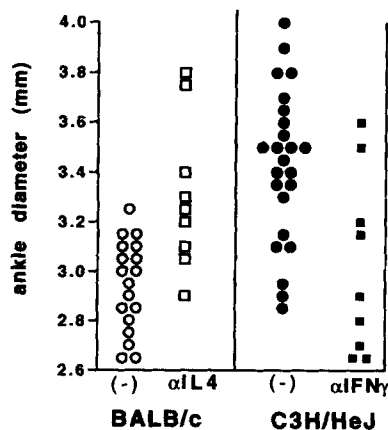


Figure 3. Effect of cytokine neutralization on the course of experimental Lyme disease. Right ankle measurements of infected BALB/c (open circles) and C3H/HeJ mice (closed circles) are compared with anti-IL-4-treated BALB/c (open squares) and anti-IFN- γ -treated C3H/HeJ mice (closed squares). The results are expressed as individual animals from four separate experiments at the time of peak severity of the early arthritis.

the right popliteal nodes were harvested as early as 2 wk or as late as 6 wk after infection (data not shown). Examination of the left popliteal lymph nodes and spleens was not indicative of a difference in T helper subset maturation between strains (Fig. 2 B). In most instances there was little, if any, production of antigen-specific IL-4 or IFN- γ . When production did exceed background levels, it was usually manifest as slightly higher IFN- γ production by C3H/HeJ mice with no difference in IL-4 production between strains.

Cytokine Neutralization Alters Disease Phenotype. To establish whether the observed differences in cytokine production influence the course of infection, we treated C3H/HeJ mice with anti-IFN- γ and BALB/c mice with anti-IL-4 mAbs once or repeatedly at the initiation of infection (Fig. 3). There was marked reversal in disease phenotype in the course of early arthritis when these two interventions were made regardless of the number of doses given (data not shown).

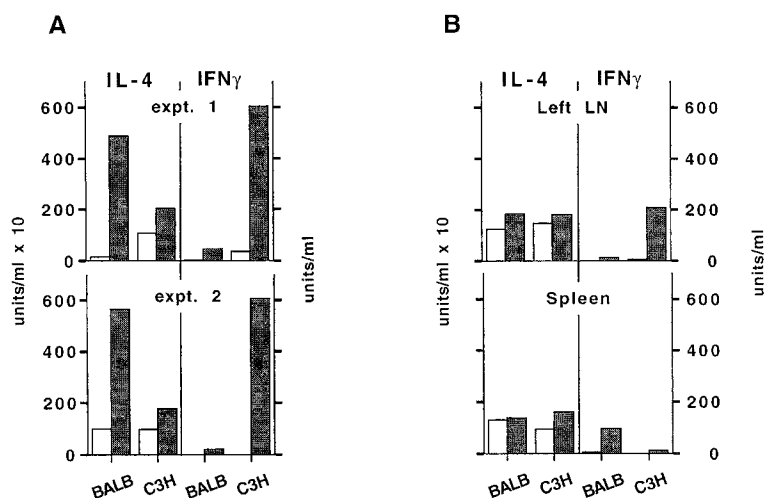


Figure 2. Antigen-specific cytokine production by proximal (A) and distant (B) lymphoid organs in *Borrelia burgdorferi*-infected BALB/c (BALB) and C3H/HeJ (C3H) mice. Spontaneous production of IL-4 and IFN- γ without borrelial antigen (open bars) is compared with antigen-stimulated cells (shaded bars). Results for draining right popliteal lymph nodes (A) are from two (of four) representative experiments (top and bottom). Results for the distant organs (B) are from a representative experiment (of four) examining left lymph node (LN) (top) and spleen (bottom). Values are the mean of duplicate wells with all SEM <15%.

Treated C3H/HeJ mice developed much milder arthritis at a time when severity had peaked in untreated animals and treated BALB/c mice developed peak severity far greater than untreated animals, often approximating susceptible C3H/HeJ mice.

Discussion

Infection of BALB/c and C3H/HeJ mice with *B. burgdorferi* has proven a useful model in illustrating a genetic basis for susceptibility to Lyme disease (2–4) although immunologic mechanisms for the observed differences have been obscure. In several infectious disease models that exhibit differential susceptibility, the immunologic mechanism underlying disease phenotype has been the type of T helper cell response (Th1 versus Th2) mounted against the invading pathogen (5–7). In a study of four humans with chronic Lyme disease, T cell clones exhibited an exclusively Th1 phenotype (8). Susceptible C3H/HeJ have higher levels of IgG2a than resistant mice during infection (3) and their lymphocytes are capable of IFN- γ production during restimulation with borrelial antigens (9). Comparable data regarding T helper cytokines produced by resistant mice (or asymptomatic infected humans) had not been available.

We demonstrate that *Borrelia*-specific lymphokine patterns underlie the host genetic control of this infectious disease: lymphocytes from susceptible C3H/HeJ mice produce high levels of IFN- γ and low levels of IL-4 when restimulated with borrelial sonicates, and neutralization of IFN- γ during infection attenuated the initial severity of proximal arthritis. Antigen-stimulated lymphocytes from resistant BALB/c mice produced high levels of IL-4 with much lower levels of IFN- γ and neutralization of IL-4 in vivo significantly enhanced early disease severity.

The current study employed bulk lymphoid populations but we infer that the cellular sources of these lymphokines are T helper cells. During restimulation with antigen, only culture wells which exhibited vigorous clonal expansion characteristic of lymphocytes had significant production of lymphokines (Reiner, S., unpublished results). Since B cells do not produce IL-4 and IFN- γ , we believe their sources are CD4⁺ T cells, a conclusion supported by other murine infectious disease models with demonstrable polarization in IL-4 and IFN- γ production derived from CD4⁺ T cells (5–7). Whereas NK (or CD8⁺ T) cells may produce IFN- γ and basophils (or mast cells) may produce IL-4, their contribution to the polarized lymphokine phenotypes in this model is still being investigated.

To focus and amplify the immune response, we modified experimental Lyme disease by inoculating the spirochete distal to an easily visualized joint and a draining lymph node. Our model of localized Lyme arthritis faithfully reproduces the genetic and pathological differences found using intraperitoneal

or intradermal (nonfootpad) infection (2, 3, 10) although inoculation at other sites has yet to reveal a difference in the quality of the T cell response between susceptible and resistant hosts (3, 9, 11). The dramatic lymphokine polarization we observe would not have been detected without analyzing a proximal lymph node since the responses were essentially absent in the distant lymph node and spleen. Although disease is often attenuated in the left ankle and we are unsure whether carditis is present at all, the proximal arthritis provides an extremely useful model for the immunologic basis of susceptibility since the inability to control centripetal dissemination is under the same genetic regulation as the severity of distal arthritis and carditis (12).

The immunoprotective or immunopathologic mechanisms that the T helper subsets might mediate during this infection will require further analysis. Pathologic severity in mice has been linked with persistence of spirochete DNA in tissues (4) and spirochete DNA has been detected in the joints of humans with Lyme disease (13), indicating that control of the microbial burden is necessary (if not sufficient) to prevent the severe pathological changes associated with this infection. The ability to passively transfer protection to *scid* mice with an anti-OspA (a major surface protein of the spirochete) mAb (14) and protect C3H mice with recombinant OspA immunization (15) or passive transfer of human immune sera (16) suggests that effective humoral responses could be sufficient for protective immunity. The finding of higher IL-4 production, a B cell stimulatory factor, in resistant mice, is consistent with a protective role for humoral immunity. Although infected C3H/HeJ mice exhibit higher serum IgG levels than BALB/c mice (2, 3), our results suggest that secondary sampling sites may be misleading in detecting primary immunoregulatory events and that higher serum IgG may merely be an index of progressive disease. We, in contrast, observe increased B-to-T cell ratios in the right popliteal nodes of resistant BALB/c versus C3H/HeJ mice which were not evident in the left nodes and spleens (Reiner, S., unpublished results).

The production of Th1-type cytokines by susceptible mice may represent a deficiency in protective immunity but could also be immunopathologic. Consistent with our finding of higher IFN- γ production, infected C3H/HeJ mice exhibit marked increase in serum IgG2a (3). This IFN- γ -induced isotype fixes complement and binds Fc γ RI on monocytes with high affinity, perhaps inducing complement- or monokine-mediated tissue damage, as has been proposed by others (3, 9). Further clarification of the protective nature of a Th2 response or the exacerbative nature of a Th1 response in Lyme disease should help explain many of the unresolved features of its pathogenesis. Subsequent analysis should be greatly facilitated using the current modification of experimental Lyme borreliosis which illuminates critical genetic differences in immune regulation.

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