E and P Selectins Are Not Required for Resistance to Severe Murine Lyme Arthritis

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Borrelia burgdorferi-induced arthritis in mice is characterized by tendonitis, synovitis, and inflammatory-cell infiltrate, predominantly of neutrophils. Because genetic deficiency in E and P selectins results in delayed recruitment of neutrophils to sites of inflammation, mice with this deficiency were tested for their response to infection with B. burgdorferi. E and P selectins were not required for the control of B. burgdorferi numbers, nor did deficiency in E and P selectins result in alteration of arthritis severity.

Arthritis can develop in humans and in certain inbred mice upon infection with the spirochete Borrelia burgdorferi (1, 11). The murine model of Lyme disease has been extensively studied, but the mechanism of arthritis development remains unknown. In the murine model, C3H/He mice develop severe arthritis when infected with B. burgdorferi. This arthritis is reminiscent of human Lyme arthritis, with edema, infiltration of neutrophils, and the hyperproliferation of the synovial membranes within the joint (3). Ankles taken from infected mice exhibiting severe Lyme arthritis show the presence of high numbers of spirochetes in the joints by PCR analysis (9, 19).

In contrast to the severe arthritis seen in C3H/He mice, B. burgdorferi-infected BALB/cAN and C57BL/6N mice develop mild to moderate arthritis (1). The pathology in the joints of these animals shows mild edema and proliferation of synovial membranes, with fewer infiltrating neutrophils. Infected BALB/cAN mice show low numbers of spirochetes in tissues by PCR, whereas C57BL/6N mice show high numbers of spirochetes in the joint but low numbers in the heart (9). Interestingly, the mechanisms of resistance to severe arthritis are different in these two strains of mice; the resistance of BALB/cAN mice can be overcome by a high infectious dose of B. burgdorferi, whereas C57BL/6N mice are resistant to a very high infectious challenge (9). Severe arthritis in C3H/He mice infected with B. burgdorferi is characterized by tendonitis, synovitis, and neutrophil influx into joint tissues. The mild arthritis seen in resistant mice displays little synovial hyperproliferation and tendonitis, with little evidence of neutrophil infiltration. We have observed a consistent correlation between high neutrophil influx into joint tissues and histopathologically severe arthritis.

The induction of chemokines and adhesion molecule up-regulation on both endothelial and infiltrating cells is required for the infiltration of inflammatory cells, such as neutrophils, into tissues (15). Outer-surface components of B. burgdorferi have been documented to upregulate both chemokine and adhesion molecule expression on inflammatory cells (4, 5, 12–14, 18). Neutrophil degranulation is induced by stimulation with B. burgdorferi lipoproteins in vitro as well (10). This study was designed to determine the role of the adhesion molecules E selectin and P selectin in the development of murine Lyme arthritis and their role in the control of spirochete persistence in tissues. To this end, we utilized a strain of mouse in which the genes encoding the E and P selectin molecules were disrupted by homologous recombination (8). These mice previously were shown to have delayed neutrophil extravasation in thioglycollate-induced peritonitis and cytokine-induced meningitis (8, 17).

Male mice homozygous for the E and P selectin deficiency were bred in the Center for Cancer Research, Massachusetts Institute of Technology. Both wild-type controls and E and P selectin double-deficient mice were descendants of F2 intercrosses between 129sv and C57BL/6 strains. Intercross populations of 129sv × C57BL/6 and 129sv × Black Swiss mice have been found to resist severe Lyme arthritis in other studies (6, 7), suggesting that the 129sv mouse is arthritis resistant, like the C57BL/6 mouse. Male C3H/HeJ and C57BL/6N mice were obtained from the National Cancer Institute. Mice were infected with 2 × 10^3 B. burgdorferi organisms (N40 strain) by intradermal injection into the shaved back or were mock infected by injection of an equivalent volume of sterile BSK-H medium (9). Four to five mice from each experimental group were sacrificed at 2 weeks postinfection, and the remainder were sacrificed at 4 weeks postinfection. At the time of sacrifice, multiple tissues were taken for culture (blood, ear, and spleen), histological analysis (rear ankle joint), or isolation of DNA for determination of spirochete levels (rear ankle joint, heart, ear, urinary bladder, and brain), as previously described (9).

All tissues taken for culture were incubated in BSK-H medium containing 6% rabbit serum, 100 mg of phosphomycin/mL, and 50 mg of rifampin (Sigma, St. Louis, Mo)/mL for up to 14 days at 32°C. Tissues taken from mock-infected animals had no spirochetes present in any tissue cultured. All ear cultures from infected mice contained spirochetes, confirming that 100% of the mice injected with B. burgdorferi were infected. Although culture positivity among blood or spleen cultures

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from infected mice was variable, differences were not noted between the different mouse strains.

At the time of sacrifice, both rear ankle joints from each mouse were measured for swelling with metric calipers as previously described (9). Figure 1 shows the average rear ankle swelling for each group of animals. C3H/HeJNCr mice had severe ankle swelling at both 2 and 4 weeks postinfection, while C57BL/6NCr mice showed little to no swelling at both time points. Infected E and P selectin-deficient mice and their wild-type littermates showed very little swelling at either 2 or 4 weeks postinfection. Based on these data, the E and P selectin-deficient mice and the wild-type controls showed an arthritis-resistant phenotype. Others have also observed that 129SvC57BL/6 mice are resistant to severe arthritis development during infection with *B. burgdorferi* (7). In this case, the lack of E and P selectin molecules did not result in increased ankle swelling upon infection with *B. burgdorferi*.

The rear ankle joint displaying the greatest degree of swelling was taken for histological analysis. Hematoxylin and eosin-stained sections of the joint were examined microscopically and assigned scores from 0 to 4+, as previously described (9). The examiner was not aware of the identity of the experimental group from which the sections were taken. Histological analysis revealed severe pathology in infected C3H/HeJ mice (3+ to 4+) and mild to moderate pathology in C57BL/6NCr mice (1+ to 2+), as previously reported (9).

A range of pathology was noted in ankle sections from infected E and P selectin-deficient mice and wild-type littermates (0 to 2+). Significant alteration in joints was not noted until 4 weeks postinfection, at which point pathology ranged from zero to moderate inflammation, as assessed by degree of proliferation of synovial membranes, edema, and neutrophil influx. Interestingly, synovial thickening and edema were always associated with the presence of neutrophils in joint tissues. Thus, the defect in neutrophil extravasation into tissues in the E and P selectin-deficient mice appeared to be overcome by 2 to 4 weeks following infection with *B. burgdorferi*. This is consistent with recent studies by others in a model of wound healing, in which the defect in neutrophil extravasation in E and P selectin-deficient mice was most apparent at early time points. By 3 days following the trauma, neutrophils were present at one-third the numbers found in wild-type mice, suggesting that alternative adhesion mechanisms were allowing neutrophil influx into tissues (16). The similar ranges of pathology seen in E and P selectin-deficient mice and wild-type mice indicate that the absence of the E and P selectin molecules in vivo does not significantly affect the outcome of arthritis during infection with *B. burgdorferi*. Additionally, the histological sections supported the ankle swelling measurements, indicating that E and P selectin-deficient mice and the wild-type controls show an arthritis-resistant phenotype when infected with *B. burgdorferi*.

To assess the role of E and P selectins in clearance of tissue spirochetes during infection with *B. burgdorferi*, DNA was isolated from various tissues of mice for PCR determination of spirochete levels. Five tissues were harvested for PCR analysis: one ear, one rear ankle joint, the heart, the urinary bladder, and the brain. Primers and conditions used in PCR analysis were as previously published (9). A standard curve containing known numbers of *B. burgdorferi* organisms was run with each set of samples, allowing an estimation of the number of *B. burgdorferi* organisms in infected tissues for comparison purposes. Sample

![FIG. 1. Effect of E and P selectin deficiency on ankle swelling in mice infected with *B. burgdorferi*. Rear ankle joints were measured in mice at 2 (A) and 4 (B) weeks following infection, and the measurement of the most severely swollen rear ankle joint from each mouse is indicated on the graph. Mock-infected mice include C3H/HeJ mice, E and P selectin-deficient mice, and wild-type controls.](http://iai.asm.org/)

![FIG. 2. Effect of E and P selectin deficiency on spirochete levels in ankle joints and hearts. Ankle joints (A) and hearts (B) were collected from mice 2 weeks (open circles) or 4 weeks (closed circles) following infection, as indicated. Spirochete levels were determined by linear range PCR, with standard curves of known *B. burgdorferi* numbers included on each gel. Circles represent results from individual mice, and bars represent the average value for each group. Values for E and P selectin-deficient mice were not significantly different from values for wild-type controls at either time point, in either tissue (*P > 0.05*).](http://iai.asm.org/)
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