Human T Lymphocyte Response to *Borrelia burgdorferi* Infection: No Correlation between Human Leukocyte Function Antigen Type 1 Peptide Response and Clinical Status


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We tested the hypothesis that cross-reactivity between the outer surface protein A (OspA) of *Borrelia burgdorferi* and human leukocyte function antigen (LFA) type 1 mediates chronic autoimmune sequelae of Lyme disease. T cell response was studied in subjects with Lyme disease presenting with erythema migrans alone (*n* = 36), erythema migrans with neurological disease (*n* = 12), and chronic Lyme disease syndrome (*n* = 20), as well as healthy control subjects from Lyme-endemic (*n* = 50) and -nonendemic (*n* = 18) regions. Antigens included recombinant OspA and OspC (all strain B31) and human LFA-1 peptide (IYVIEGTSKQDLTSF). Proliferation to OspA was detected in 11 (28%) of 39 subjects presenting with erythema migrans, which increased to 50% at 4 weeks of follow-up. Reactivity to OspA and LFA-1 was significantly correlated (*P* < .001) and was observed in 18 (78%) of 23 OspA-responsive subjects. However, there was no correlation between T cell response to human LFA-1 peptide and clinical status.

The mechanisms of chronic sequelae of Lyme disease in humans, particularly neurological disease and arthritis, have not been well elucidated. It is hypothesized that chronic sequelae result from an autoimmune process triggered by the infection. Alternatively, chronic sequelae may result from the inability to rid the host of the *Borrelia burgdorferi* spirochete. Other possibilities include bacterial antigens resistant to enzymatic degradation and irreversible damage to joints.

The human immune response to *B. burgdorferi* infection depends at least in part on T cell responses. Evidence from passive transfer studies and active immunization with outer surface protein A (OspA) and OspC has indicated that protection from *B. burgdorferi* can be antibody mediated [1–4]. T cell help is essential for the generation of IgG, and a human T cell response to *B. burgdorferi* has been well documented. Furthermore, it is possible that the T cell response to *B. burgdorferi* contributes to chronic sequelae after infection. Peripheral blood T cells from patients with Lyme disease exhibit proliferation in vitro to *B. burgdorferi* antigens [5–7]. These T cell responses can be detected after proven infection in patients with negative antibody responses, as measured by ELISA [8]. T cells from...
Multiple B. burgdorferi antigens are recognized by T cells, including OspA, OspB, OspC, flagellin, and Hsp70 proteins [12–15]. T cells from affected patients respond to many of these antigens [16], and human T cell clones have been generated demonstrating a greater response than peripheral blood T cells. During a compartmentalized response, with synovial T cells demonstrating a greater response than peripheral blood T cells. During murine infection with B. burgdorferi, OspA expression is lost by day 30, whereas OspC expression is up regulated [17]. For this reason, it has been thought that the immune response is primarily directed toward OspC, in preference to OspA.

OspA from B. burgdorferi sensu stricto contains HLA-DR4–restricted epitopes that cross-react with human LFA-1 [18–20]. It has been proposed that this molecular mimicry explains the rare occurrence of antibiotic-resistant chronic arthritis in patients with HLA-DR4. The purpose of the present project was to test the clinical relevance of T cell recognition of the cross-reactive epitope of human LFA-1 peptide.

### SUBJECCTS, MATERIALS, AND METHODS

**Human subjects.** The study was confined to subjects aged between 18–70 years who were residents of New York, New Jersey, or Connecticut, and control subjects who were residents of New Mexico. This was part of a larger prospective study of the clinical outcome of neurological Lyme disease that is still ongoing and will be reported elsewhere.

**Early Lyme disease criteria.** Subjects presented with the erythema migrans rash of Lyme disease, as confirmed by an experienced physician. Work included documentation of the size and number of erythema migrans lesions, review of systems, and neurological examination. Serologic test results were obtained, but positive results were not necessary for inclusion in the presence of a documented rash of erythema migrans. Lumbar puncture was performed when indicated on patients with neurological findings. Subjects with multiple lesions of erythema migrans were included. Subjects with neurological findings (meningitis or cranial neuropathy) on presentation with erythema migrans were classified as having early neurological disease and were analyzed separately. Forty-eight patients with early infection presented with erythema migrans. Thirty-six had erythema migrans without neurological involvement, whereas 12 had concurrent neurological disease. Blood samples were obtained for studies on the day of presentation. Convalescent (4 weeks of follow-up) blood samples also were obtained from 16 of these patients.

**Chronic Lyme syndrome criteria.** Subjects had a history of symptoms of >3 months’ duration. The history of Lyme disease was confirmed either by physician-documented erythema migrans rash or late clinical manifestations, as well as laboratory confirmation of infection. Late manifestations included recurrent briefly swollen joints, acute onset of secondary or tertiary atriocentral conduction defect, meningitis, cranial neuropathy, radiculoneuritis, and encephalomyelitis with intrathecal antibody production. Chronic antibiotic-resistant arthritis was not essential for inclusion. Acceptable laboratory confirmation included culture of B. burgdorferi, positive antibody titer in CSF, or acute and convalescent serum documentation of increasing titers. Serologic test results were confirmed by Western blot according to the Centers for Disease Control and Prevention criteria. Twenty patients with chronic Lyme disease syndrome were studied. LFA-1 peptide data were available from 13 of these patients.

**Endemic-area control subjects from Suffolk County, New York.** Fifty Lyme endemic–area healthy control subjects, aged 18–70 years, were recruited by random digit dialing and matched for area code with the patients with Lyme disease. Control subjects were excluded if they had a history of or syndromes consistent with Lyme disease. Evaluation included physical examination, including neurological examination, and serologic testing.

**Nonendemic-area control subjects from New Mexico.** Eighteen nonendemic-area (New Mexico) control subjects were recruited by Dr. Larry Davis, New Mexico Veterans Affairs Health Care system. New Mexico control subjects had always lived in New Mexico, never extensively vacationed in the northeastern United States, and never been diagnosed as having Lyme disease. Blood was obtained from 18 healthy subjects (2 women and 16 men), with a mean age of 50 years. Subjects had no history of immunosuppressive medication or acute illness.

**Recombinant B31 OspA and OspC.** Recombinant antigens from a B31 strain isolate of B. burgdorferi were prepared, as described elsewhere [21, 22].

**LFA-1 peptide.** The human LFA-1 peptide IYVIEGTSKQD-LTSTF recognized by OspA-reactive T cells [18–20] was synthesized commercially by Chiron Technologies. The peptide was 92% pure by high-performance liquid chromatography analysis, with free (unblocked) C- and N-terminal amino acids.

**Proliferation assays.** PBMC were isolated from heparinized blood by centrifugation on Ficoll-hypaque gradients (Pharmacia). They were then washed and resuspended in AIM V media (Gibco) at $1 \times 10^6$ cells/mL for proliferation assays. The PBMC (0.1 mL/well) were added to U-bottom 96-well trays (Linbro), with 0.1 mL of appropriate antigen. Antigens were added at the following final concentrations: OspA and OspB (0.1 µg/mL) and LFA-1 peptide (10 µg/mL). At the initiation
Table 1. Stimulation indices in response to Borrelia antigens.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Positive response, total no. (%)</th>
<th>P vs. reference</th>
<th>Positive serologic test result, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recombinant OspA response</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Mexico control subjects</td>
<td>3/18 (17)</td>
<td>Ref.</td>
<td>6</td>
</tr>
<tr>
<td>Endemic LI control subjects</td>
<td>22/50 (44)</td>
<td>.049</td>
<td>20</td>
</tr>
<tr>
<td>Erythema migrans, no neurological disease</td>
<td>11/39 (28)</td>
<td>.511</td>
<td>33</td>
</tr>
<tr>
<td>Erythema migrans, 4 wk of follow-up</td>
<td>8/16 (50)</td>
<td>.066</td>
<td></td>
</tr>
<tr>
<td>Erythema migrans, neurological disease</td>
<td>3/11 (27)</td>
<td>.646</td>
<td>83</td>
</tr>
<tr>
<td>Chronic Lyme syndrome</td>
<td>8/20 (40)</td>
<td>.160</td>
<td>100</td>
</tr>
<tr>
<td>Recombinant OspC response</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Mexico control subjects</td>
<td>3/18 (17)</td>
<td>Ref.</td>
<td>6</td>
</tr>
<tr>
<td>Endemic LI control subjects</td>
<td>15/50 (30)</td>
<td>.359</td>
<td></td>
</tr>
<tr>
<td>Erythema migrans, no neurological disease</td>
<td>5/40 (13)</td>
<td>.649</td>
<td></td>
</tr>
<tr>
<td>Erythema migrans, 4 wk of follow-up</td>
<td>6/16 (38)</td>
<td>.250</td>
<td></td>
</tr>
<tr>
<td>Erythema migrans, neurological disease</td>
<td>4/11 (36)</td>
<td>.375</td>
<td></td>
</tr>
<tr>
<td>Chronic Lyme disease syndrome</td>
<td>6/19 (32)</td>
<td>.447</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** LI, Long Island; Osp, outer surface protein; Ref., reference; wk, weeks. Positive stimulation index ≥2.
who showed a 35% response rate (7/20). This was significantly different ($P = .005$) from the response of other Lyme disease groups, as well as endemic-area control subjects.

**T cell response to human LFA-1 peptide.** It has been proposed that chronic arthritis can be induced by *B. burgdorferi* infection as a result of cross-reactivity between OspA and a human LFA-1 peptide, as described elsewhere [18–20]. T cell proliferation to this LFA-1 peptide was tested in a subset of 85 human LFA-1 peptide, as described elsewhere [18–20]. T cell infection as a result of cross-reactivity between OspA and a posed that chronic arthritis can be induced by *B. burgdorferi* groups, as well as endemic-area control subjects.

There was a significant ($P < .001$; Spearman’s correlation coefficient) association between the stimulation index of the response to OspA and LFA-1 peptide reactivity for all patient groups, including those without systemic symptoms and endemic control subjects with no history of disease.

It should be noted that patients with chronic Lyme syndrome were not selected for antibiotic-resistant arthritis, and no conclusion can be made about the level of LFA-1 reactivity in the arthritis subset. However, among those patients reacting to OspA, almost all of them also reacted to human LFA-1 peptide, and LFA-1 peptide reactivity did not correlate with disease status.

The human T cell response to infection with *B. burgdorferi* was marked by an early proliferative response to recombinant OspA and OspC, which approximately doubled in frequency at 4 weeks after presentation. Patients with neurological disease at presentation exhibited higher levels of T cell reactivity, close to that of the 4-week follow-up patients. This may simply reflect a time delay in diagnosis. The cytokine response to OspA and OspC was characterized by IFN-$\gamma$ production, with minimal IL-4 or IL-5, and exhibited a similar boost at 4 weeks. There was no difference in the IFN-$\gamma$ bias of the cytokine response between patient groups. The only significant difference between
Table 3. T cell response to human leukocyte function antigen (LFA)-1 peptide.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>LFA-1⁺</th>
<th>OspA⁺</th>
<th>LFA-1⁺, OspA⁻</th>
<th>LFA-1⁻, OspA⁺</th>
<th>LFA-1⁻, OspA⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythema migrans, no neurological disease</td>
<td>1/7 (14)</td>
<td>2/7 (29)</td>
<td>0/7</td>
<td>1/7</td>
<td>1/7</td>
</tr>
<tr>
<td>4 wk of follow-up, no neurological disease</td>
<td>3/10 (30)</td>
<td>4/10 (40)</td>
<td>0/10</td>
<td>1/10</td>
<td>3/10</td>
</tr>
<tr>
<td>Neurological disease on presentation</td>
<td>2/10 (20)</td>
<td>2/10 (20)</td>
<td>0/10</td>
<td>0/10</td>
<td>2/10</td>
</tr>
<tr>
<td>Chronic Lyme syndrome</td>
<td>3/13 (23)</td>
<td>2/13 (5)</td>
<td>1/13</td>
<td>0/13</td>
<td>2/13</td>
</tr>
<tr>
<td>Endemic control subjects, Suffolk County, NY</td>
<td>8/27 (30)</td>
<td>10/27 (37)</td>
<td>0/27</td>
<td>2/27</td>
<td>8/27</td>
</tr>
<tr>
<td>Nonendemic control subjects, New Mexico</td>
<td>3/18 (17)</td>
<td>3/18 (17)</td>
<td>1/18</td>
<td>1/18</td>
<td>2/18</td>
</tr>
<tr>
<td>Total</td>
<td>20/85 (24)</td>
<td>23/85 (27)</td>
<td>2/85 (2)</td>
<td>5/85 (5)</td>
<td>18/85 (21)</td>
</tr>
<tr>
<td>Proportion of positive results</td>
<td>20/25 (80)</td>
<td>23/25 (92)</td>
<td>2/25 (8)</td>
<td>5/25 (20)</td>
<td>18/25 (72)</td>
</tr>
</tbody>
</table>

NOTE. Data are no. of patients positive (%)/no. of patients. Osp, Outer surface protein. OspA and LFA-1 responses were significantly correlated (P<.001, k-statistic analysis).

patients with chronic Lyme disease syndrome and patients with early disease was the production of IFN-γ in response to OspC by patients with chronic Lyme syndrome.

The high rates of T cell response of endemic-area control subjects to recombinant OspA and OspC raises the question of cross-reactivity of the T cell response with other Borrelia species. Evidence that the observed T cell response to OspA was related to Borrelia species exposure includes the low level of reactivity found in nonendemic New Mexico control subjects, as well as the significant boost in reactivity detected between presentation and at 4 weeks’ follow-up (acute vs. convalescent).

We have reported elsewhere that, of >20 genotypes of OspC expressed in ticks, only a minority are associated with human skin infection, and only 4 genotypes are associated with invasive disease [24]. This may explain both the low rate of T cell reactivity to the B31 strain OspC used for the present study and the high incidence of T cell reactivity to Borrelia antigens in endemic-area control subjects with no history of Lyme disease. Cross-reactivity between the OspC genotype proteins is minimal [25], whereas Lyme serologies in North America recognize the B31 strain of OspA [26]. The high level of T cell reactivity to Borrelia species in endemic-area subjects with no history of Lyme disease may result from infection with Borrelia OspC genotypes not associated with clinical disease.

OspA was demonstrated to be an important target of the early T cell immune response to B. burgdorferi, and OspA T cell responses increased over the first 4 weeks after infection. Human T cell responses to OspA have been reported elsewhere and do not correlate with serum antibody responses to OspA [12, 14]. OspA expression is down regulated by Borrelia in the mammalian host [17]. For this reason, the maintenance and expansion of T cell responses to OspA in humans is not well

Figure 1. Correlation of stimulation indices for outer surface protein A (OspA) and leukocyte function antigen (LFA) type 1 peptide. Spearman’s correlation coefficient, 0.737 (P<.001).
understood. Many patients with a T cell response exhibited proliferation to both OspA and OspC. However, there was no correlation in the stimulation index to these 2 antigens.

The association of OspA T cell response and chronic sequelae has been theorized to result from cross-reaction between a DRB1*0401-restricted OspA T cell epitope and human LFA-1 [18, 19]. This cross-reactivity to LFA-1 has been demonstrated for T cells from patients with Lyme disease who have chronic arthritis. Inflammatory cells within the synovium express LFA-1 [27]. Only a subset of human OspA–reactive T cell clones recognize this human LFA-1 peptide, and the response to LFA-1 peptide tends to be suboptimal, which suggests that it is a partial agonist [20]. T cell clones proliferate less well to LFA-1 peptide than OspA, and the cytokine response favors IL-13 rather than IFN-γ. It has been proposed that human LFA-1 peptide is a partial agonist [20]. It is likely that the human LFA-1 peptide is not processed and presented in vivo. When autologous Epstein-Barr virus (EBV)–transformed B cells were used as antigen-presenting cells, it was necessary to add exogenous LFA-1 peptide to induce a T cell response, despite the expression of LFA-1 on EBV lymphoblasts [20].

Our data demonstrate that T cell recognition of human LFA-1 peptide correlated significantly with T cell response to OspA. T cell response to both LFA-1 peptide and OspA was detected in all groups, including those without neurological disease or arthritis and healthy endemic control subjects. The antigen specificity of the LFA-1 response of endemic control subjects was supported by a response to recombinant OspA in all 18 of these subjects, as well as the low level of LFA-1 response among nonendemic control subjects. The principle limitation of our study was that the subjects were not typed for HLA-DR, precluding conclusions specific for HLA-DR4 patients. The strength of our study was that it was a prospective epidemiological study with matched endemic-area control subjects. None of the previous studies on LFA-1 reactivity have had similar numbers of patients or control subjects. There was no correlation between LFA-1 response and clinical status, arguing that the T cell response to human LFA-1 peptide is not clinically relevant. This is supported by the evidence, discussed above, that the human LFA-1 peptide epitope is not processed by LFA-1–positive human antigen-presenting cells and must be added as exogenous peptide [20].

Acknowledgment

We thank Julia Coleman for her assistance in collecting blood from the New Mexico subjects.

References

21. Schutzer SE, Coyle PK, Krupp LB, et al. Simultaneous expression of


