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Differential Immune Response to the Variable Surface Loop Antigen of P66 of Borrelia burgdorferi Sensu Lato Species in Geographically Diverse Populations of Lyme Borreliosis Patients

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We have studied the immune response to a variable surface-exposed loop region of the P66 outer membrane protein from Borrelia burgdorferi sensu stricto and B. garinii and B. afzelii, respectively.

Lyme borreliosis is the most common tick-borne infection in Europe and North America (7, 15). In two recent reviews, genospecies distribution was shown to be associated with geographic origin (11, 19). Borrelia burgdorferi sensu stricto is the only species found in North American Lyme disease, while three species, B. burgdorferi sensu stricto, B. afzelii, and B. garinii, are associated with Lyme borreliosis in Europe (11, 19). In earlier studies, a correlation between the serological response among Lyme borreliosis patients living in different geographic regions to antigens from various B. burgdorferi sensu lato genospecies has been shown (2, 10, 13).

The P66 membrane protein has a surface-exposed loop region including a hypervariable immunogenic determinant that is polymorphic among Borrelia species (3, 4, 5). That this antigen is species specific was suggested by its reactivity in immunoblot assays with P66 protein derived from a B. burgdorferi sensu stricto strain from North American patients with Lyme disease (5). The aim of this investigation was to further characterize this antigenic site by determining the effect of sequence polymorphism on the ability of anti-P66 antibodies to detect P66 in Lyme borreliosis patients from Sweden and the United States.

The strains used were B. burgdorferi sensu stricto B31 (ATCC 35210) and B. garinii Ip90 (12) and Swedish strains B. afzelii LU81 and B. garinii LU59, LU116, LU118, LU170, LU185, LU190, and LU222 (16).

The sequence diversity of the P66 loop of Lyme borreliosis strains recovered in Sweden was studied by performing partial p66 gene sequence analysis by PCR and cycle sequencing. A primer pair targeting the P66 loop was chosen (5′-GAAATT TCAAGCTATGAAGAC-3′ and 5′-CTACATATGCTTCTG TTGAATGG-3′). For subsequent recombinant enzyme immunoassays (EIAs), we generated peptides corresponding to the P66 loop region (rP66) of B. burgdorferi sensu stricto B31, B. afzelii LU81, and B. garinii Ip90 and LU59 as previously described (5). The following primer pairs were chosen in accordance with previously published sequences (4): 5′-TGGAT TAGGATCCATAACATCCTACGGTC-3′ and 5′-TTTCTATT GCGAATTCATAATGTGATTTAGG-3′ for B. burgdorferi sensu stricto, 5′-GGACTGGTAGTGGATCCACATCATATCG GTC-3′ and 5′-TCATTGCGAATTCAATGTTAATATAA TTTAGG-3′ for B. afzelii, and 5′-GGACTGGATGTGATCCACATC TATTAGGC-3′ and 5′-TCATTGCGAATTCAATGTTAG TATTAGG-3′ for B. garinii. The endonuclease restriction site (11) is underlined in each primer sequence.

Serum samples were collected from two geographically separated Lyme borreliosis populations, i.e., 100 patients from southern Sweden (50 with neuroborreliosis, 25 with arthritis, and 25 with acrodermatitis) and 38 patients from North America (23 with erythema migrans and 15 with disseminated Lyme disease [kindly provided by Martin Schäfer, Centers for Disease Control and Prevention, Fort Collins, Colo.,]). Diagnoses were based on established case definitions (6). Serum samples from healthy Swedish blood donors (n = 100) collected in a tick-free area in Sweden were used to define the cutoff value for seropositivity in the EIAs.

The rP66 peptides were used as antigens in the EIAs. Preparation of the microtiter plates and the EIA protocol were performed as previously described (13). A protein concentration of 5 μg/ml was used. Serum samples from patients were diluted 1:200. Each plate contained a positive control. Samples were run in duplicate. An EIA index was calculated for each sample by subtracting the background activity (estimated by using an antigen-free well) from the mean optical density value and thereafter divided by the positive control. The cutoff values were calculated as the 95th percentile of the blood donor EIA index. The seroreactivity in the Lyme borreliosis groups was compared by using the Mann-Whitney rank sum test. The proportions of seropositive samples were compared by using McNemar’s chi-square test (paired proportions) and the contingency tables chi-square test (independent proportions). A P value of <0.05 was considered statistically significant. The
The sequences determined in this study were deposited in the GenBank database and assigned accession numbers AY009472 (LU59), AY009473 (LU81), AY009474 (LU116), AY009475 (LU118), AY009476 (LU170), AY009477 (LU185), AY009478 (LU190), and AY009479 (LU222).
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