

No Association with HLA Class II Alleles in Swedish Patients with Cutaneous Manifestations of Lyme Borreliosis

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The possibility of an association between manifestations of Lyme borreliosis and HLA class II alleles has been investigated with varying results. In the present study, we used genomic typing techniques to determine the DR, DQ and DP allele frequencies in 29 patients with erythema migrans and 36 patients with acrodermatitis chronica atrophicans. We did not find a significant deviation from controls in the distribution of the HLA class II alleles in any of these disease manifestations, nor in the subgroup of 8 patients with acrodermatitis chronica atrophicans and long-standing arthritis. With the additional information obtained by the typing techniques used, our results are thus in accord with those studies where no association between the development of the late disease manifestation acrodermatitis chronica atrophicans and HLA class II alleles has been found. **Key words:** genetic susceptibility; HLA antigens; borrelia.

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The natural course of Lyme borreliosis is very variable (1). Schematically, the disease can be divided into localized infection, early disseminated infection and late or chronic infection (2). Uncomplicated erythema migrans and borrelial lymphocytoma represent localized infection. Early disseminated infection can result in multiple skin lesions, arthritis, carditis and/or meningopolyneuritis. In late or chronic disease, manifestations such as chronic arthritis, chronic neurological involvement, and/or acrodermatitis chronica atrophicans (ACA) may develop. ACA is a progressive disorder with inflammatory and in time atrophic skin changes (3). However, most patients do not develop all three stages, and any one of them may be the sole manifestation of the infection. Thus, in only some infected persons does chronic disease evolve.

The factors that determine the outcome of the infection are not known. The possibility of an association between manifestations of the infection and HLA specificities has been investigated with the main interest focused on HLA class II antigens (4–13). The studies have yielded divergent results. Steere et al. reported an overrepresentation of HLA-DR4 and -DR2 alleles in chronic Lyme arthritis (5). As regards ACA, an HLA-DR2 association was initially found (13) but not confirmed in subsequent studies (6, 9, 11). Conventional serological methods for HLA-typing have been used in these studies. In recent years, additional possibilities for identifying HLA class II variability have been provided by genomic typing techniques, such as restriction fragment length polymorphism (RFLP)-analysis and polymerase chain reaction (PCR)-based methods. A PCR analy-

sis of HLA class II alleles in patients with Lyme arthritis was presented in 1991 and, compared to controls, a unique distribution of alleles was reported (14).

In the present study, genomic typing techniques have been used to analyse the frequencies of HLA class II alleles in patients with ACA and, for comparison, in patients with erythema migrans as well.

MATERIAL AND METHODS

Patients

Blood samples for the HLA-analyses were obtained from 29 patients with erythema migrans and 36 patients with ACA. The patients had all been examined by the authors. The erythema migrans diagnosis was based on the clinical picture which was typical in all the cases. The ACA diagnosis was based on the clinical and histopathological pictures and on elevated IgG antibodies to *Borrelia* in serum determined by enzyme-linked immunosorbent assay. Ten of the 36 patients with ACA had arthritis, defined by pain and swelling in one or more joints, which was considered to have a reasonable connection with the borreliosis and of which other causes could not be established. Eight of these 10 patients had had continuous or recurrent arthritis for more than one year.

Controls

Two-hundred and fifty randomly selected, healthy Swedes were used as controls.

Southern blot analysis

DNA was isolated from peripheral blood leukocytes by phenol/chloroform extraction of proteinase-K treated nuclei in microscale. Aliquots of 8–10 µg DNA were digested with *MspI* and *TaqI* (Boehringer-Mannheim) according to the manufacturer's recommendations. Agarose gel electrophoresis, capillary blotting of size-separated DNA fragments onto nylon membranes, labelling of purified probe inserts, prehybridization, hybridization, stringency washes and autoradiography were performed according to standard techniques.

TaqI DRB, DQA and DQB RFLP typing

Allelic *TaqI* DRB, DQA and DQB restriction fragment patterns were analyzed as previously described (15, 16). To avoid local RFLP nomenclature, the serologically defined DR and DQ specificities associated with different allelic *TaqI* DRB-DQA-DQB haplotypes are given in the text and Tables I and II.

MspI DPA and DPB RFLP typing

Allelic *MspI* DPA and DPB restriction fragment patterns were analyzed as previously described (17). The cellularly defined DP specificities associated with the different allelic *MspI* patterns are given in the text and Tables.

*DRB1*04* subtyping by PCR amplification with sequence-specific primers

The *DRB1*0401*-*DRB1*0412* alleles were distinguished by PCR amplification with sequence-specific primers (18). In brief, genomic DNA from DR4-positive individuals was amplified in eight separate PCR

Table I. Frequencies of DR-DQ phenotypes in patients with erythema migrans (EM) and acrodermatitis chronica atrophicans (ACA) compared to healthy controls

HLA class II		EM n = 29	ACA n = 36	EM + ACA n = 65	Controls n = 250
DR	DQ	%	%	%	%
1, 'Br' ^{a)}	5	31	17	23	18
2 (15)	6	28	17	22	30
2 (16)	5	0	3	2	1
3 (17)	2	31 ^{b)}	28 ^{b)}	28 ^{b)}	17
4	7	3	8	6	12
4	8	31	31	31	26
5 (11)	7	0 ^{b)}	11	6	14
5 (12)	7	7	6	3	6
6 (13)	6	48 ^{b)c)}	25	35	29
6 (13)	7	0	0	0	2
6 (14)	5	0	0	0	6
7	2	0 ^{c)}	19 ^{b)}	11	7
7	9	7	0	3	5
8	4	0 ^{c)}	14	8	9
8	7	0	0	0	1
9	9	0	0	0	4
10	5	3	0	2	2

^{a)} DR1 and DR'Br' cannot be separated by TaqI RFLP analysis.

^{b)} EM, ACA, or EM + ACA versus controls, $p < 0.05$, p_{corr} n.s.

^{c)} EM versus ACA, $p < 0.05$, p_{corr} n.s.

reactions, each containing the same DR4-specific 5'-primer but different 3'-primers. The presence or absence of PCR product was detected by means of agarose gel electrophoresis, and amplification patterns were interpreted (18).

Statistical analysis

Data were analyzed by the chi-square test or, in the case of small numbers, by Fisher's exact test. Probability (p) values were corrected (p_{corr}) for multiple comparisons with a factor of 24, the number of TaqI DRB-DQA-DQB haplotypes observed in North Europeans, for DR-DQ haplotypes, 12 for DRB1*04 alleles, and 6 for DP alleles.

RESULTS

DR-DQ haplotypes

No significant differences in the distribution of DR-DQ haplotypes were found in patients with erythema migrans or ACA compared to controls. The haplotypes observed in either patients or controls are shown in Table I. The frequency of the DR17, DQ2 haplotype was insignificantly increased in both groups of patients. There was no increased frequency of rare DR or DQ alleles. No aberrant DR-DQ haplotypes were observed among the patients.

In erythema migrans patients, the frequency of the DR13, DQ6 haplotype was increased and the DR11, DQ7, DR7, DQ2, DR8, DQ4 haplotypes were less frequent compared to patients with ACA. However, none of these changes was significant after correcting for multiple comparisons.

The DQB1*0201 allele, found on DR17, DQ2 and DR7, DQ2 haplotypes, was more frequent in patients with ACA than among the controls ($p < 0.01$, p_{corr} n.s.). The DQ7 specificity, present on, for example, DR4, DQ7, DR11, DQ7 and DR12, DQ7 haplotypes, was reduced in frequency among both groups of

Table II. Distribution of DRB1*04 alleles in DR4-positive haplotypes in patients with erythema migrans (EM) and acrodermatitis chronica atrophicans (ACA) compared to healthy controls

DRB1*04 allele	EM n = 9	ACA n = 16	EM + ACA n = 25	Controls n = 92
	%	%	%	%
*0401	56	38	44 ^{a)}	65
*0402	0	0	0	1
*0403	0	6	4	3
*0404	44	31	36	20
*0405	0	0	0	2
*0406	0	0	0	2
*0407	0	6	4	2
*0408	0	19 ^{a)}	12	4

^{a)} ACA or EM + ACA versus controls, $p < 0.05$, p_{corr} n.s.

patients: erythema migrans $p < 0.05$, p_{corr} n.s.; ACA p n.s. In the 8 ACA patients with long-standing arthritis, no statistically significant deviations in the distribution of DR-DQ haplotypes were found (data not shown). The only deviation was that 50% of these 8 patients with arthritis were DR8-positive compared to 10% of the controls ($p < 0.005$, p_{corr} n.s.).

DRB1*04 alleles

In the DR4-positive erythema migrans and ACA patients, as among the DR4-positive controls, the most frequent DR4 alleles were DRB1*0401 and DRB1*0404 (Table II).

Only 3 of the 8 ACA patients with arthritis for more than one year were DR4-positive. Two carried the most frequent DR4 allele, DRB1*0401; the third patient was DRB1*0408-positive.

DP alleles

The distribution of DP alleles was similar in patients with erythema migrans and ACA, and not different from the distribution observed in healthy controls (Table III). The only deviation was that the frequency of DPw3 was insignificantly decreased among the erythema migrans patients.

Table III. Distribution of DP alleles in patients with erythema migrans (EM) and acrodermatitis chronica atrophicans (ACA) compared to healthy controls

DP	EM n = 29	ACA n = 36	EM + ACA n = 65	Controls n = 250
	%	%	%	%
w1	14	11	12	8
w2	28	17	22	21
w3/w6 ^{a)}	17 ^{b)}	36	28	36
w4	93	86	89	86
w5	3	3	3	6
'CDP HEI'	0	3	2	2

^{a)} DPw3 and the rare DPw6 allele cannot be distinguished by RFLP typing (17).

^{b)} EM versus controls, $p < 0.05$, p_{corr} n.s.

DISCUSSION

One of the most puzzling aspects of Lyme borreliosis is its varying course, with persistence of the infection for years and even decades in some individuals. It is today not known if this variability can be explained by the established differences in the spirochetes involved (19), or if host defence mechanisms are involved as well. Among host factors that might influence the outcome of the borreliosis, the possibility of a genetic disposition linked to the HLA-system has been considered. The HLA molecules have an important role in regulating the immune response of an individual. Moreover, a number of different diseases have been shown to be associated with HLA alleles, although the exact mechanisms behind these associations are not fully understood.

The finding of an increased frequency of the HLA-DR2 specificity in 10 patients with chronic Lyme arthritis raised the first suggestion of a correlation between manifestations of Lyme borreliosis and HLA genes (4). In a later and larger study, Steere et al. found increased frequencies of DR4 and DR2 in patients with chronic Lyme arthritis when compared to patients with arthritis of shorter duration but not when compared to healthy controls (5). However, a European investigation did not yield evidence of a DR2 or DR4 association either in patients with Lyme arthritis as such or in the subgroup with chronic Lyme arthritis (6).

In patients with neurological disease manifestations, a DR2 and DR4 association was mentioned by Májsky et al. (7), but the clinical details of that study are not clearly given in the report. Kristoferitsch et al. studied patients with early neuroborreliosis and did not find an association with the alleles investigated, DR1 to DR5 and DR7 (8). Later studies are in agreement with this (9–11). In a study of patients with early as well as chronic neuroborreliosis, there was a suggestion of an increased frequency of DR7 in patients with CNS involvement but not of DR2 or DR4 (12). An indication that HLA-Cw3 may be associated with *Borrelia burgdorferi* infection as such (9) was not confirmed by a later, extended study (11).

ACA, like chronic Lyme arthritis, represents a late manifestation of the infection. In an initial report, Kristoferitsch et al. did find a significant increase of DR2 in 23 patients with ACA (13). Two later studies including 15 and 34 patients with ACA, however, did not support this finding (6, 9). A recent large European report on HLA-A, B, Cw and DR antigens in 283 patients with various manifestations of Lyme borreliosis, of which 215 had been investigated in previous HLA studies (8, 9, 13), failed to confirm particular HLA antigens as risk factors for *Borrelia burgdorferi* infection or for a chronic course of the disease (11). Eighty-nine of these patients had late disease manifestations, and, of them, the vast majority had ACA. But it should be noted that only 5 patients with chronic arthritis were included.

As compared to conventional serological methods for HLA typing, the genomic tissue typing techniques have considerable advantages, primarily higher resolution and improved accuracy. As mentioned, a molecular analysis of the polymorphic class II genes in 25 patients with Lyme arthritis of varying duration has

been performed and, compared to controls, a unique distribution of alleles was reported (14).

In this study we used RFLP analysis to determine the DR, DQ, and DP allele frequencies in patients with erythema migrans and patients with ACA. The DR4 subtypes were further specified by the use of PCR amplification with sequence-specific primers. In previous studies of HLA class II antigens in ACA, by the use of serological methods, the DP and DQ alleles have not been analysed and the resolution of the DR locus has been less detailed. In the present study, additional polymorphism was thus identified and alleles were more reliably assigned since a genomic typing technique was used. We did not find a significant deviation from controls in the distribution of the HLA class II alleles in any of these cutaneous manifestations of Lyme borreliosis. The 8 patients with ACA and arthritis for more than one year were analysed separately, and no significant deviations in DR-DQ frequencies were found in this subgroup either. The number of patients in the subgroup with long-standing arthritis is, however, small and thus does not permit conclusions as to the possibility of an immunogenetic basis for chronic Lyme arthritis.

With the additional information obtained by the genomic typing techniques used, our material can be added to those previous studies that have not found the development of acrodermatitis chronica atrophicans to be associated with HLA class II alleles.

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