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Multiple sclerosis in the Orkney and Shetland Islands

III: Histocompatibility determinants

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SUMMARY Histocompatibility testing was performed in 48 multiple sclerosis patients and two carefully matched control groups in the Orkney Islands, an area of high multiple sclerosis prevalence. The frequency of HLA-A3, HLA-B7, and DW2 was comparable in patients and controls. However, HLA-B7 was significantly more common in female patients compared with male patients. A B-cell alloantigen (B-cell 4) was also as frequent in patients as among controls. Strong linkage between HLA-B7, DW2, and B-cell 4 occurred in controls, but not patients; the linkage was particularly striking in female controls. These data are not consistent with theories that relate certain of the histocompatibility antigens to the aetiology of multiple sclerosis.

Epidemiologic studies of multiple sclerosis (MS) suggest that the disease is caused by an exogenous agent.[1 2] On the other hand, recent evidence tends to confirm the hypothesis that there is a genetic contribution to the aetiology. The histocompatibility antigen complex, located on chromosome 6 and containing specific A, B, C, and D locus antigens, may play a direct or indirect role in many diseases, MS among them. In most studies, HLA-A3 (locus A), HLA-B7 (locus B), and DW2 (locus D) have been shown to be associated with MS either singly or in combination. Table 1 illustrates the frequency of HLA-A3, HLA-B7, and, in a few instances, the lymphocyte-determined (LD) DW2 response reported for MS patients and controls in different populations.[3–11] In addition, a small number of studies have shown that a B-lymphocyte alloantigen, linked to the histocompatibility complex and presumed to be closely related to the D locus, is strongly associated with MS although its locus is still unknown.[5 12–13]

Some investigators theorise that an immune response (Ir) gene (s) exists, which is linked to the determinants of the D locus.[4 14] Although an Ir gene has not been demonstrated in man, it has been found in other vertebrates including mice, guinea pigs, and rhesus monkeys. It is also postulated that Ir genes confer dominant susceptibility to autoimmune disorders, possibly triggered by a virus carrying antigens that are structurally similar to host antigens.[14]

Terasaki and Mickey[15] propose an MS susceptibility gene located within the histocompatibility complex which is in linkage disequilibrium with certain antigens of the A, B, and D loci. This gene is suggested to have arisen by

Table 1 *Frequency of histocompatibility determinants in multiple sclerosis patients and controls from different populations*

Geographic area	*Latitude*	*No. of patients*	*No. of controls*	*%HLA-A3*		*%HLA-B7*		*%DW2*	
				P	*C*	*P*	*C*	*P*	*C*
Norway (Lapps) (3)	70° N.				47		41		
Orkney Islands	60° N.	51	96	25	30	35	36	44	40
Copenhagen (4)	56° N.	209	1967	36*	27	40*	27	60*	18
England (5)	52° N.	59	30	32*	20	56*	10		
Germany (6)	51° N.	1000	1000	36*	29	35*	26		
Minnesota (7)	45° N.	59	401	31	23	41*	24		
Massachusetts (8)	42° N.	56	100	43*	23	39*	21		
Japan (9)	39° N.	41	213	0	0·5	17	15		
Los Angeles (10)	34° N.	143	136	34	29	34	29	52*	18
Israel (European Jews) (11)	32° N.	152	292	14	18	10	9		

Multiple sclerosis is most prevalent in the temperate zone from latitude 40° to 60° N. Frequencies of HLA-A3, HLA-B7, and DW2 are arranged in descending order from latitude 60° N. The incidence of HLA-B7 in the Lapp population is given for reference. Numbers in brackets refer to studies listed in the references.
*Statistically significant.

mutation in an individual who had the HLA haplotype B-cell 4, DW2, B7, A3.

In nearly every study of MS in which antigens of the A, B, and D loci have been investigated, linkage of two or all three of the determinants has been associated with the disease. Opelz and his colleagues[10] ascribe the occasional lack of linkage between HLA-A3, HLA-B7 and DW2 in MS patients to different degrees of disturbance of the original hypothesised B-cell 4, DW2, B7, A3 haplotype. In their study of 330 patients with MS from the United States of America, no association was observed between MS and HLA-A3 or HLA-B7 in patients; however, there was an increased incidence of DW2, suggesting a dissociation of the B locus from the D locus in US patients.

A unique opportunity to investigate MS in an area with the highest reported prevalence rates resulted in the present study of the disease in the Orkney and Shetland Islands of northern Scotland. The prevalence rates of MS in Orkney and Shetland were 309 and 184/100 000, respectively, on prevalence day (1 December 1974).[16] The two archipelagoes are well-defined geographic isolates. Their populations are homogeneous and have remained so for a long period of time. Although there has been considerable out-migration from the islands since 1900, there has been little permanent in-migration.

Material and methods

Because of the poor cell viability of blood specimens from the Shetland Islands, this report is limited to results from Orkney. Patients with MS were located through each of the general practitioners (GPs) in the Orkney Islands. Contact with the local GPs was sustained throughout the period of study so that cases of quiescent disease or those patients who had not consulted their doctors for long periods of time were ultimately brought to attention. It is believed that virtually all cases of MS in the islands were identified.

For each patient, an unrelated individual of the same age and sex was randomly selected from birth registers of the same parish as the patient. Because of the possibility that patients and parish controls were overmatched for environmental factors, a second, discontiguous, control was selected in the same manner. The discontiguous control was of the same age and sex but born in a parish not contiguous with that of the patient and was selected randomly from a list of parishes weighted for the population distribution in each 10-year period.

Table 2 *Frequencies of HLA antigens among multiple sclerosis patients and controls in Orkney*

	PATIENTS			CONTROLS		
Locus A	*Male* %	*Female* %	*Total* %	*Male* %	*Female* %	*Total* %
HLA—A1	48	37	41	49	33	40
A2	62	33	45	51	53	52
A3	14	33	25	41	23	30
A11	14	17	16	10	19	16
A23	—	3	2	—	2	1
A24	24	13	18	5	12	9
A25	10	13	12	2	4	3
A26	—	—	—	8	7	7
A28	—	7	4	5	5	5
A29	5	3	4	8	4	5
AW30	5	7	6	—	2	1
AW31	—	—	—	—	5	3
AW32	—	13	8	13	12	12
TOTAL No. OF CASES	21	30	51	39	57	96
Locus B						
HLA—B5	5	—	2	2	9	6
B7	10	53	35	36	37	36
B8	38	20	27	31	26	28
B12	24	20	22	31	30	30
B13	10	3	6	5	2	3
B14	24	13	18	15	14	14
BW15	14	17	16	13	12	12
BW17	5	10	8	5	7	6
BW18	14	23	20	5	4	4
BW21	—	7	4	—	5	3
BW22	—	—	—	2	7	5
BW27	14	13	14	18	12	14
BW35	10	3	6	10	9	9
BW37	—	7	4	—	4	2
BW38	—	—	—	5	2	3
BW39	—	3	2	5	4	4
BW40	24	3	12	10	7	8
TOTAL No. OF CASES	21	30	51	39	57	96

Blood samples were collected in March 1976 from 51 MS patients, 48 parish controls, and 48 discontiguous controls. The samples were tested for histocompatibility antigens of the A, B, and D loci as well as B-cell alloantigens. For three patients who were not born in Orkney, no controls were selected. Forty-three of the MS patients were classified as 'probable' cases and eight as 'possible' cases. Two patients not personally examined were included in the 'possible' group. Thirty (59%) of the patients were female and 21 (41%) were male.

Heparinised blood taken from patients and controls in the Orkney Islands was processed at the field laboratory within six hours. Lymphocytes were isolated by using Ficoll-Hypaque, and care was taken to remove contaminating granulocytes, platelets, and erythrocytes. The purified lymphocyte suspension was frozen in 0·4 ml plastic tubes and stored in liquid nitrogen thereafter. Histocompatibility typing was done by the standard microcytotoxicity test.[17] The LD typing was performed with homozygous typing cells, using the microlymphocyte culture method described previously.[18] B-cell testing was done on B lymphocytes that were isolated from the frozen lymphocytes, rosetted with neuraminadase-treated sheep red cells and centrifugation over Ficoll-Hypaque.[19] All specimens were tested blindly. Only two patients were receiving corticosteroid therapy at the time the blood specimens were taken.

The two control groups were combined for statistical analysis. Student's t test, the χ^2 test, and the Fisher exact probability test were used in the analyses.

Since completion of the HLA testing, the nomenclature for factors of the HLA system has been revised.[20] To avoid confusion, we report results using the nomenclature extant in 1976.

Results

HLA (A and B locus antigens)

The frequencies of the locus A HLA antigens (Table 2) were comparable in MS patients and both control groups. The most prevalent specificities in decreasing frequency were HLA-A2, HLA-A1, and HLA-A3. The HLA-A3 antigen was found in 25% of MS patients and 30% of controls.

Of the B-locus antigens, HLA-B7 was present in 35% of patients and in 36% of the controls (Table 2). HLA-B18 was found in 10 (20%) of the MS patients and only 4 (4%) of the controls ($\chi^2 = 7{\cdot}51$, 1 df, $P < 0{\cdot}01$). However, when the total number of comparisons made were considered, the P value was increased to greater than 0·05. Among patients, the most common of the B-locus specificities in decreasing frequency were HLA-B7, HLA-B8, and HLA-B12.

Table 3 *Frequency of locus A and locus B combinations in multiple sclerosis patients and controls in Orkney*

	Patients		*Controls*	
	No.	*%*	*No.*	*%*
HLA—A1, B8	13	25	24	25
HLA—A2, B12	8	16	18	19
HLA—A3, B7	7	14	13	14

When the frequencies of the specifications were controlled by sex, there was an excess of HLA-B7 among female patients compared with male patients, male controls, and female controls, as seen in Table 2. Fifty-three per cent of female patients had HLA-B7 compared with 10% of male patients; among female and male controls, the frequencies of HLA-B7 were 37% and 36% respectively. The presence of HLA-A3 and/or HLA-B7 in female patients was 63% compared with 14% in male patients.

The most prevalent locus A and B antigen haplotypes commonly thought to be in linkage disequilibrium, HLA-(A1-B8), HLA-(A2-B12), and HLA-(A3-B7), occurred with similar frequency in patients and controls (Table 3). However, the frequencies of these combinations in *both* patients and controls were considerably higher than those reported in other studies.[4 21]

The temporal course of MS was analysed for associations with antigens of the A, B, and D loci; no association between HLA type and temporal course was found.

No case of optic neuritis without MS was identified in the Orkney Islands. Fifteen patients in the Orkney Islands had optic neuritis as a presenting or subsequent symptom of their illness. No particular histocompatibility antigens were associated with the presence of optic neuritis in patients.

HLA-DW2 (D locus antigens)

Blood samples from 119 of the 147 Orcadians were tested for the mixed lymphocyte culture (DW2) determinant. The remaining samples were not tested because of poor cell viability. Individuals whose samples were not tested did not differ from those tested when compared for demographic, epidemiologic, or clinical factors. A response of 50 or less was considered a positive response. Eighteen of the 41 tested MS patients (44%) and 31 of the 78 (40%) tested controls were DW2 positive. From the Figure it is evident that the response of the controls were *either* strongly negative or strongly positive for DW2.

When the mixed lymphocyte culture (DW2) results were analysed by HLA type, there was a strong correlation between DW2 and HLA-B7 among controls but *not* among MS patients.

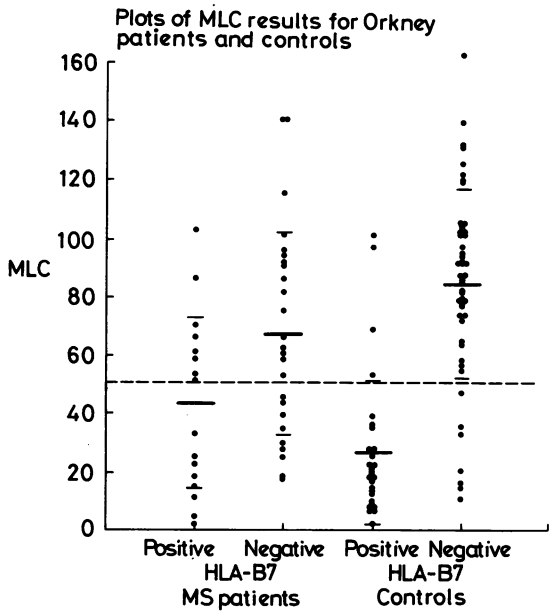

Figure *Responses of MS patients and controls to the mixed lymphocyte culture test for the DW2 antigen, controlled by HLA—B7. A response of 50 or less is considered a positive reponse, although those that fall between 40 and 60 are equivocal. Note the strong correlation between DW2 and HLA—B7 among controls but not among patients.*

Eighty-six per cent of controls either had both determinants or had neither ($\chi^2 = 35{\cdot}59$, 1 df, $P < 0{\cdot}0001$)*; only 50% of HLA-B7 patients had DW2.

Neither temporal course of disease nor the presence of various symptoms differed significantly between DW2 positive and DW2 negative patients. Unlike HLA-B7, the frequency of DW2 was not elevated in female patients.

B-lymphocyte alloantigen (B-cell 4)

Comparable proportions of MS patients and controls were B-cell 4 positive, 55% and 50% respectively. Sixty-three per cent of female patients compared with 41% of male patients had this determinant; among controls, 47% cent of females and 54% of males had B-cell 4.

* Fisher exact probability test.

Linkage between HLA-B7, DW2, and B-cell 4

When the linkage between HLA-B7, DW2, and B-cell 4 was examined, the presence or absence of all three determinants was significantly more common among *controls* than among MS patients ($\chi^2 = 11{\cdot}3$; 2 df, $P < 0{\cdot}01$), as seen in Table 4. This was especially evident among female controls, 84% of whom had all three determinants or none of them.

Discussion

In the Orkney Islands, MS patients and two carefully matched control groups were tested for locus A and locus B histocompatibility antigens, locus D antigens, and B-cell alloantigens. This is the first study of a well-defined population of MS patients in whom all four specificities have been tested.

These islands contain unique and homogeneous populations. For example, the frequencies of the haplotypes HLA-A1-B8, HLA-A3-B7, and HLA-A2-B12 are unusually high, although they are comparable in MS patients and controls.

The results of this study do not confirm the observations by some investigators that certain HLA antigens are more frequent in MS patients than controls.[4–8 10] No significant increase in HLA-A3, HLA-B7, DW2 or B-cell 4 was found in the entire MS patient group when compared with controls. In addition, MS patients did not exhibit the strong linkage between HLA-B7, DW2 and B-cell 4 that was evident among controls.

Few studies of HLA have attempted complete ascertainment of MS patients in a defined population. Frequently, patients are selected from a registry, neurological clinic, or hospital service. There is reason to suspect, therefore, that these patient groups may include a disproportionate number of MS cases with the classical, relapsing-remitting course and early onset. Cases characterised by non-classical course and later onset may be selectively excluded from studies of HLA. In Orkney, complete ascertainment of MS cases was probably attained. All reported cases of neurological disease were personally seen by one of the authors (DCP). Twenty-two per cent of the cases had non-classical disease and a generally later onset. If certain histocompatibility determinants are associated with only a subgroup of patients, then

Table 4 *Frequency of linkage between HLA—B7, DW2 and B-cell 4 in multiple sclerosis patients and controls in Orkney*

	PATIENTS						CONTROLS					
	Male No.	(%)	Female No.	(%)	Total No.	(%)	Male No.	(%)	Female No.	(%)	Total No.	(%)
Positive for HLA—B7, DW2 and B-cell 4	0	—	6	(25)	6	(16)	9	(31)	14	(32)	23	(32)
Negative for HLA—B7, DW2 and B-cell 4	6	(43)	4	(17)	10	(26)	8	(28)	23	(52)	31	(44)

patient selection may explain the different results in this and other studies.

In contrast to other studies, we found no increase of HLA-B7 among patients. However, when controlled by sex, a significantly greater proportion of HLA-B7 patients were female (Table 2). Because it is believed that sex is unrelated to HLA type, few studies have attempted to control for this variable. If the presence of histocompatibility antigens is sex-related or their detection affected by sex factors, the association between HLA-B7 and MS may be artificial because females have a higher rate of MS.[1] An association between myasthenia gravis, the female sex, and HLA-A8 has been reported.[22] However, no adequate explanations about the influence of sex and HLA on disease have been offered.

An unexpected finding in this investigation was the strong linkage observed between HLA-B7, DW2 and B-cell 4 in *controls* and not in patients; this was especially evident in female controls. On the basis of these data, variations in the HLA system appear to be neither necessary nor sufficient factors in the aetiology of MS.

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