Genetic associations between myasthenia gravis and the HL-A system

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SYNOPSIS HL-A antigens were determined in 159 myasthenic patients, 112 of whom were females and 47 males. Fifty-seven patients were thymectomized. The relationship between 23 different HL-A antigens and myasthenia gravis (MG) with reference to sex, age at onset, clinical course of MG, thymus histology, and auxiliary diseases of the patients, as well as the significance of these antigens to 245 relatives of myasthenics, were analysed. The frequencies of HL-A1 and HL-A8 were highly significantly higher in myasthenic patients than in normal Finnish controls. The increase of HL-A1 is secondary and due to a strong linkage disequilibrium between HL-A8 and HL-A1. HL-A8 appeared most often in females with onset of MG before the age of 35 years, and inpatients with thymus hyperplasia. No significant HL-A deviations were found in males or in females with later onset of disease. In seven thymoma patients the occurrence of W10 antigen was almost significantly increased.

In 1960 Simpson, Strauss *et al.*, and Nastuk *et al.* (1959), independently suggested that myasthenia gravis (MG) might be some kind of autoimmune disease. This is well accepted today, even though the basic mechanism of the immunological dysfunction remains unresolved.

Many kinds of humoral autoantibodies have been found in myasthenic patients but their pathogenetic significance is still obscure. Several findings support the concept of alterations in the cell-mediated immunity, the well-known thymus pathology in about 80% of patients, the frequent presence of muscle lymphorrhages, the increased cytotoxicity of myasthenic lymphocytes both *in vivo* (Namba *et al.*, 1969a, b) and *in vitro* (Armstrong *et al.*, 1973), and the migration inhibition of myasthenic lymphocytes in the presence of muscle antigen (Alpert *et al.*, 1972; Kott *et al.*, 1973) and thymus antigen (Vejjajiva *et al.*, 1974).

The increased occurrence of other autoimmune disorders in patients with MG has also been confirmed by many authors (Simpson, 1960; Oosterhuis *et al.*, 1964; Oosterhuis and de Haas, 1968; Whittingham *et al.*, 1970).

The familial incidence of MG is higher than would be expected by chance: calculated by Namba *et al.* (1971), 3.4% of 1875 patients are related as compared with approximately 0.01% in the population (Kurland and Alter, 1961). The pattern of inheritance is not clear and seems to be multifactorial.

We have sought evidence for the possible influence of inheritance by investigating one genetic polymorphism of tissue cells, the so-called human leucocyte antigens, or HL-A. The frequency of HL-A8 was found to be significantly increased in 38 unselected myasthenics, especially in women with onset of MG below the age of 30 years (Pirskanen *et al.*, 1972). This finding has been confirmed by many others (Behan *et al.*, 1973; Säfwenberg *et al.*, 1973; Dick *et al.*, 1974; Feltkamp *et al.*, 1974; Fritze *et al.*, 1974). Fritze *et al.* (1974) found in addition that HL-A3 was significantly increased in male patients and was also linked to thymoma. Feltkamp *et al.* (1974) did not confirm these findings but observed HL-A2 to be increased in patients with thymoma or with late onset of the disease. Both teams reported that HL-A8 was significantly increased in females with earlier

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TABLE 1
CLINICAL COURSE AND SEX OF 159 MYASTHENIC PATIENTS

	Group*							Total
	I	IIA	IIB	III-IV	IIB-IV →IIA	I, IIA →A	IIB-IV →A	
Female	3	40	23	6	30	3	7	112
Male	6	14	10	4	6	3	4	47
Total	9	54	33	10	36	6	11	159

* Classification according to Osserman modified by Oosterhuis (1964).

A: patients in full remission.

→: clinical type changed to

onset of MG than the age of 30 years or with hyperplastic thymus. In addition, they both showed that HL-A7 was more common in males.

The overall findings concerning HL-A antigens in myasthenia gravis seem to be somewhat controversial.

The purpose of this communication is to analyse further, with adequate statistical methods, the relationship between different HL-A antigens and myasthenia gravis with reference to the age at onset, sex, clinical form of MG, thymus histology, and associated diseases of patients. The haplotype and gene frequencies of different HL-A antigens are determined. The significance of the HL-A8 antigen to family members of myasthenics will be discussed.

METHODS

PATIENTS On 1 November 1974, there were in Finland 235 myasthenics per 4,560,000 inhabitants, 169 (72%) of whom were females and 66 (28%) males. The number of familial cases was 12 per 235, or 5.1%. During the clinical follow-up examination from January 1972 to August 1974, the HL-A antigens were determined in 159 (68%) myasthenics from various parts of Finland. Of these 112 (70.4%) were women and 47 (29.6%) men, the sex distribution thus being the same as in the entire group of patients. The sex distribution and the clinical forms of MG grouped according to Osserman modified by Oosterhuis (Oosterhuis, 1964) are shown in Table 1. Thymectomy was performed on 57 of the 159 myasthenics, 45 females and 12 males. Thymoma was found in five females and two males (12.5%), 38 patients (32 females and six males—or 66.1%) had lymphoid hyperplasia and two females and four males (10.7%) had a normal thymus histology. In six patients the histological picture of the removed thymus had not been recorded. The sex distribution

TABLE 2
THYMUS HISTOLOGY AND SEX OF 57 THYMECTOMIZED MYASTHENICS

	Hyper- plasia	Thymoma	Normal	Unknown	Total
Female	32	5	2	6	45
Male	6	2	4	0	12
Total	38	7	6	6	57

and the histological findings in the thymus gland of 57 thymectomized patients are shown in Table 2.

The HL-A antigens of 245 relatives of 106 myasthenic patients were determined. The haplotypes of 93 myasthenic patients were thus ascertained. The diseases of these relatives in relation to HL-A antigens were also analysed.

The blood samples were collected during the clinical follow-up examination. The lymphocytes were separated immediately and the tissue typing was performed by Dr Anja Tiilikainen at the Department of Serology and Bacteriology, University of Helsinki. The investigation included the following HL-A antigens: first (SD I) or LA locus: HL-A1, 2, 3, 9, 10, 11, 28, W19 (W29, 30, 31, 32 were all called W19 because they could not be separately identified throughout the work); the identified second (SD II) or FOUR locus antigens included HL-A5, 7, 8, 12, 13, 14, 17, 27, W5, W10, W15, W16, W18, W21, W22 throughout.

The statistical significance of deviations from the controls was based on calculations of χ^2 values. Because of a possibility of overestimation of the association between an antigen and the disease, the resultant P-values were multiplied by 23, the number of specificities being tested ('corrected-P-values'). P-value <0.001 will be described as 'highly significant', <0.01 as 'significant', and <0.05 as 'almost significant'. The corresponding gene frequencies were calculated in two ways (see

Table 7). P_1 states the observed gene frequency calculated directly from the haplotyped patients. P_2 is the gene frequency estimated from all the myasthenics by the formula: $P = 1 - \sqrt{1 - A}$; where A is the phenotype frequency of the antigen.

When evaluating the significance of deviations of HL-A haplotype frequencies from controls the so called delta (Δ) values are of importance because they provide the estimate of a genetic association between certain SDI and SDII antigens. The delta value, or linkage disequilibrium parameter, is calculated by the formula: $\Delta = f_{ij} - g_i \times g_j$; where f_{ij} is the frequency of joint occurrence of the antigens i and j on the same haplotype and g_i and g_j are the gene frequencies (Bodmer and Payne, 1965). In other words, f_{ij} is the observed frequency of a given HL-A haplotype and $g_i \times g_j$ gives the expected value of that haplotype frequency if all the antigens were linked together by chance.

RESULTS

In Table 3 the HL-A phenotype frequencies of 159 myasthenics are compared with those of 326 normal Finnish controls (Tiilikainen *et al.*, 1972). The corrected P-values show that the increases of HL-A1 (35.2%) in the SD I and of HL-A8 (47.8%) in the SD II segregation group are highly significant.

Among the SD II antigens HL-A7 (16.7%) is almost significantly and W15 (11.3%) highly significantly decreased.

In the random Finnish population HL-A7 is the most common and W15 the second most common antigen of the SD II group. The HL-A8 frequency in myasthenics is 2.73-fold as compared with controls. If non-HL-A8 antigens were

TABLE 3
HL-A PHENOTYPES IN 159 MYASTHENICS AND 326 NORMAL FINNISH CONTROLS

Antigen		159 myasthenic patients			326 normal controls phenotype frequency (%)
		Number of antigens		Phenotype frequency (%)	
		Observed	Expected		
<hr/>					
SD I: First segr. group (LA)					
HL-A	1	56	26.8	35.2*	16.9
	2	77	87.3	48.4	55.2
	3	53	66.3	33.3 ⁽³⁾	42.0
	9	30	28.8	18.9	18.4
	10	5	15.1	3.1 ⁽²⁾	9.8
	11	14	16.1	8.8	10.4
	28	20	19.0	12.6	12.0
W-	19	22	24.4	13.8	15.6
SD II: second segr. group (FOUR)					
HL-A	5	18	20.0	11.3	12.6
	7	26	45.9	16.4 [†] ⁽¹⁾	29.1
	8	76	27.8	47.8*	17.5
	12	22	22.9	13.8	14.7
	13	6	12.2	3.8	7.7
	14	2	0.0	1.3	—
	17	7	5.9	4.4	3.7
	27	10	22.0	6.3 ⁽²⁾	14.1
W-	5	35	29.3	22.1	18.7
-	10	35	24.0	22.1 ⁽³⁾	15.3
-	15	18	41.0	11.3*	25.8
-	16	18	19.0	11.3	12.0
-	18	13	7.3	8.2 ⁽³⁾	4.9
-	21	—	0.0	—	—
-	22	6	11.2	3.8	7.1

P-values:
P < 0.001 (1)
0.001 < P < 0.01 (2)
0.01 < P < 0.05 (3)
Uncorrected
Corrected multiplied by 23
* Highly significant
† Significant
‡ Almost significant

TABLE 4
HL-A PHENOTYPES (PER CENT) IN 159 MYASTHENICS ACCORDING TO AGE AT ONSET AND SEX

Antigen	112 female myasthenics		47 male myasthenics		326 normal controls
	Before age 35 yr (75 patients)	After age 35 yr (37 patients)	Before age 35 yr (22 patients)	After age 35 yr (25 patients)	
HL-A 1	48.0*	28.9	27.3	12.5	16.9
2	42.7	36.8	59.1	75.0	55.2
3	29.3	31.6	36.4	45.8	42.0
9	14.7	23.7	36.4	8.3	18.4
10	2.7	—	4.5	8.3	9.8
11	6.7	15.8	—	12.5	10.4
28	16.0	10.5	4.5	12.5	12.0
W-19	14.7	15.8	4.5	16.7	15.6
HL-A 5	8.0	7.9	22.7	16.7	12.6
7	16.0	18.4	13.6	16.7	29.1
8	72.0*	26.3	40.9	12.5	17.5
12	9.3	13.2	18.2	25.0	14.7
13	2.7	7.9	—	4.2	7.7
14	1.3	2.6	—	—	—
17	1.3	10.5	9.1	—	3.7
27	10.7	2.6	—	4.2	14.1
W- 5	24.0	15.8	13.6	33.3	18.7
10	16.0	31.6	27.3	20.8	15.3
15	9.3‡	18.4	13.6	8.3	25.8
16	9.3	10.5	18.2	12.5	12.0
18	8.0	7.9	9.1	8.3	4.9
21	—	—	—	16.7	—
22	—	2.6	4.5	—	7.1

*, ‡: see footnotes to Table 3.

present in the same proportions as in the random population, the expected frequencies of HL-A7 and W15 would be 18.4% and 16.4%, respectively. These figures differ from the observed only by 2 and 5% units. The differences are not statistically significant and the decreases of HL-A7 and W15 seem to be secondary and caused by the increase of HL-A8. Deviations in the uncorrected P-values are also given in the table.

Table 4 presents the phenotype frequencies in the different age and sex groups. We also determined the frequencies in the group contracting the disease before the age of 16 years, but these did not differ from those of the group with onset at below the age of 35 years. The age of 35 years was selected for the division of patients according to age because of all groupings at five year intervals this revealed the best discrimination for HL-A8 frequencies.

The only significantly increased frequencies are those of HL-A1 and 8 in the younger female group. The decrease of W15 in the same group is secondary and due to the increase of HL-A8.

In males and in the older age group of females no significant deviations from normal controls

were found when only the corrected P-values are considered.

Table 5 compares the occurrence of HL-A antigens in the different clinical groups. The figures are given in numbers of cases because of the small size of some patient groups.

In the ocular group (I) there are no significant deviations from the controls. In the milder MG group (IIA) HL-A8 is found in 41.4%; in the more severe form of the disease (IIB-IV) in 37.2%; and in patients who improved with time (IIB-IV changes to IIA or to A) (the majority after thymectomy) in 72.3%. All these increases are significant. In the IIB-IV group the decrease of HL-A7 is almost significant.

Table 6 presents the occurrence of HL-A antigens of the thymectomized patients, grouped according to the microscopic picture of the thymus gland. HL-A1 (55.3%) and HL-A8 (73.7%) were highly significantly increased in the hyperplasia group and W10 (57.1%) almost significantly increased in the thymoma group.

The haplotype and exact gene frequencies (p_1) of the randomly haplotyped 93 patients are given in Table 7a. The estimated gene frequencies

TABLE 5

HL-A PHENOTYPES (NUMBER OF CASES) IN 159 MYASTHENICS WITH DIFFERENT CLINICAL COURSES

Antigen	Groups			
	I, I→A (11 patients)	IIA, IIA→A (58 patients)	IIB, III, IV (43 patients)	IIB-IV→IIA or A (47 patients)
HL-A 1	5	19 (32.7%)‡	13	22 (46.8%)*
2	6	28	21	22
3	5	19	13	16
9	3	10	10	7
10	1	1	2	1
11	—	9	4	1
28	1	7	6	6
W-19	—	7	6	9
HL-A 5	2	6	9	1
7	2	12	3 (7.0%)‡	9
8	2	24 (41.4%)*	16 (37.2%)†	34 (72.3%)*
12	1	14	4	4
13	1	2	2	1
14	—	1	1	—
17	—	3	2	2
27	—	3	2	5
W- 5	4	10	8	13
-10	3	12	9	11
-15	1	7	5	15
-16	2	6	8	2
-18	1	4	4	4
-21	—	—	—	—
-22	1	2	3	—

*, †, ‡: see footnotes to Table 3.

→: clinical type changed to.

TABLE 6

HL-A PHENOTYPES (NUMBER OF CASES) AND THYMUS HISTOLOGY IN 57 THYMECTOMIZED PATIENTS

Antigen	Normal histology	Hyperplastic thymus	Thymoma	Unknown histology
HL-A 1	2	21 (55.3%)*	1	3
2	5	12	5	4
3	2	10	4	—
9	—	5	2	1
10	—	1	—	—
11	1	2	—	—
28	—	6	—	1
W-19	—	9	1	—
HL-A 5	2	2	1	—
7	1	5	2	—
8	3	28 (73.7%)*	1	6
12	3	4	—	1
13	—	1	—	—
14	—	1	—	—
17	—	1	—	—
27	—	2	1	—
W- 5	3	6	2	—
10	1	10	4 (57.1%)‡	—
15	—	2	2	2
16	—	3	—	1
18	—	4	—	—
21	—	—	—	—
22	—	—	—	—
Total number of patients	6	38	7	6

*, †, ‡: see footnotes to Table 3.

TABLE 7A
HL-A HAPLOTYPE AND GENE FREQUENCIES IN 93 MYASTHENICS (186 HAPLOTYPES)

HL-A	5	7	8	12	13	14	17	27	W5	W10	W15	W16	W18	W21	W22	X	P ₁	P ₂
1			0.194*				0.005				0.005					0.005	0.209‡	0.195‡
2	0.016	0.032	0.032	0.048	0.005		0.011	0.022	0.016	0.005‡	0.043	0.022	0.016		0.011	0.005	0.285	0.282
3		0.038	0.011	0.011					0.070	0.022	0.032						0.183	0.183
9	0.005	0.005	0.011							0.032 ‡	0.005	0.032					0.091	0.099
10		0.005								0.011			0.005				0.022	0.016
11	0.011	0.005		0.011					0.011				0.005		0.005		0.048	0.045
28			0.027 †	0.011			0.005		0.011	0.027							0.054	0.065
W19	0.005		0.016						0.011	0.027		0.011	0.011				0.081	0.072
X	0.011				0.005	0.005				0.005							0.026	
P ₁	0.048	0.086	0.290*	0.081	0.011	0.005	0.016	0.027	0.108	0.113	0.085	0.065	0.038		0.016	0.010	0.999	
P ₂	0.058	0.091	0.278*	0.072	0.019	0.007	0.022	0.032	0.117	0.117	0.058	0.058	0.042		0.019			

P₁: gene frequency determined from 186 haplotypes of 93 myasthenics. P₂: gene frequency calculated from the phenotypes of 159 myasthenics. Bold type: positive delta value (see text).
Italic type: negative delta value (see text). *, †, ‡, see footnotes to Table 3.

TABLE 7B
HL-A HAPLOTYPE AND GENE FREQUENCIES IN CONTROLS: 232 NORMAL FINNS (464 HAPLOTYPES)

HL-A	5	7	8	12	13	14	17	27	W5	W10	W15	W16	W18	W21	W22	X	P ₀
1			0.050	0.004			0.006		0.006	0.002	0.009	0.009	0.002			0.002	0.093
2	0.019	0.043	0.011	0.030	0.013		0.004	0.034	0.024	0.034	0.069	0.015	0.006		0.002	0.002	0.310
3		0.078	0.004	0.015	0.004			0.026	0.060	0.011	0.026	0.004	0.006		0.002	0.009	0.246
9		0.006	0.019	0.002	0.006	0.002			0.004	0.009	0.009	0.022	0.004			0.006	0.091
10			0.004	0.002	0.004					0.002		0.009	0.006		0.004	0.002	0.034
11	0.002	0.009	0.004	0.004					0.006	0.002	0.004		0.004		0.002	0.002	0.041
28	0.006	0.004	0.002	0.009				0.002	0.006	0.006	0.004		0.002		0.002		0.045
W19	0.002	0.004	0.011	0.004			0.002	0.004	0.011	0.017	0.011	0.011	0.011			0.002	0.097
X		0.006					0.002	0.002	0.009	0.002	0.009	0.002	0.002	0.002			0.039
P ₀	0.038	0.168	0.086	0.093	0.019		0.015	0.067	0.127	0.086	0.140	0.071	0.045	0.002	0.013	0.026	0.996

The control population is part of an anthropological material being collected by Dr Anja Tiilikainen, and is published with her permission.
P₀ = Gene frequency in the control population. Bold type: positive delta value (see text). Italic type: negative delta value (see text).

TABLE 8
INHERITANCE OF HL-A8 BY FEMALE AND MALE
MYASTHENICS

<i>Patients</i>	<i>From father</i>	<i>From mother</i>	<i>Total</i>
Female	16	11	27
Male	3	0	3
Total	19	11	30
	63.3%	36.6%	

A comparison of the frequencies in myasthenics and controls (Table 7b) shows an increase of haplotype 1.8 (highly significant), 28.8 (significant), and 9.W10 (almost significant), and an almost significant decrease of haplotype 2.W10 in MG patients. When only corrected P values are taken into account, the increase of the gene determining HL-A1 in myasthenics is almost significant and that one determining HL-A8 highly significant. If uncorrected P-values are

TABLE 9
FAMILIAL CASES OF MYASTHENIA AND THEIR HL-A TYPES

<i>Relationship</i>	<i>HL-A type</i>	<i>Comments</i>	<i>Age at onset (yr)</i>
Mother	1,8/3 W5	Thymus radiated	21
Son	1,8/2,W16	Mild myasthenia	7
First cousin A	1,8/11,W5	Systemic lupus erythematosus	23
First cousin B	1,8/9,W10	Normal thymus histology	12
First cousin C	2,27/28,5	Very mild myasthenia	15
Second cousin D	1,8/3,W5	Hyperplastic thymus	12
Sister A	28,8/W19,W5	Hyperplastic thymus	19
Sister B	9,7/W19,W5	Very mild myasthenia	16
Sister	W19,8/W19,W16	Hyperplastic thymus	19
Brother	W19,8/W19,W16	Very mild myasthenia	22

TABLE 10
HL-A8 IN 245 RELATIVES OF 106 MYASTHENICS

	<i>245 relatives</i>		<i>Total</i>
	<i>Healthy</i>	<i>Unhealthy</i>	
HL-A8 +	50 49.0%	52 51.0%	102 41.6%
HL-A8 -	78 54.6%	65 45.4%	143 58.4%
Total	128 52.2%	117 47.8%	245

also taken into account the frequencies of HL-A7 and HL-A27 are significantly and almost significantly decreased. Parental HL-A determination resolves whether HL-A8 is inherited from mother or father in 30 myasthenic patients (Table 8). It is received from the mother in 11 and from the father in 19 patients. The difference is statistically almost significant (χ^2 4.27 or $P < 0.05$).

HL-A antigens were examined in 10 familial myasthenic patients and are presented in Table 9. One mother and son, three first cousins and one second cousin, two sisters, and a sister and a brother belonging to four different families are included. Not even in them is myasthenia absolutely associated with HL-A8. The two patients lacking HL-A8 have very mild myasthenia symptoms. It is worth mentioning that in these cousins the LD¹ antigen commonly associated with HL-A8 and with myasthenia is not detected

¹ 'Lymphocyte defined' antigens are controlled by a further locus in the MHC region.

(p_2) of the whole myasthenic series are also presented. The exact and estimated gene frequencies (p_1 and p_2) do not differ significantly from each other, which confirms that the haplotyping is performed randomly.

In this Table the bold type indicates positive delta values exceeding 0.015—for example, the observed haplotype frequency is higher than the expected frequency calculated from corresponding gene frequencies (Bodmer, 1972). The italic type indicates negative delta values (correspondingly calculated).

(Kaakinen *et al.*, in press). The increased frequency of HL-A8 both in myasthenia gravis and in certain other autoimmune/endocrine disorders, confirmed by many authors, raises the question whether this antigen or the gene coding for it might be associated with an autoimmune or endocrine disorder present, either in myasthenics or in their relatives.

The HL-A antigens of 202 first degree relatives (parents, children, siblings) and of 43 second degree relatives of 106 myasthenic patients in 102 different families were determined. The average number of relatives in one family was 2.4 (range one to nine) in addition to the patient.

Of these 245 relatives, 128 (52.2%) are totally healthy, and 50 (49.0%) of them have HL-A8 determinant. Concerning various disease groups, it can be mentioned that seven of 245 relatives (2.9%) have some kind of autoimmune disease—for example, rheumatoid arthritis, systemic lupus erythematosus, glomerulonephritis—and two of them have HL-A8; 16 (6.5%) have thyroid gland disorders (including non-toxic goitre), of whom six (37.5%) are HL-A8+; 12 (4.9%) have some other endocrinopathy—for example, diabetes mellitus—of whom six (50%) are HL-A8+; and 18 (7.4%) of these 245 relatives have some kind of atopic tendency—for example, bronchial asthma, hay fever, or allergic eczema—of whom six (33.3%) have HL-A8.

The incidence of autoimmune, allergic, or endocrine disorders is even lower in HL-A8 positive than HL-A8 negative relatives. Table 10 summarizes the frequency of HL-A8 in these 245 healthy and unhealthy relatives of myasthenics.

With regard to segregation of any of these diseases with HL-A antigens, these family pedigree studies turned out to be unproductive, even though in some families some atopic tendency seems to segregate with HL-A8.

Table 11 presents certain associated diseases in 159 myasthenics and their relation to HL-A8. The significances are calculated by comparing the observed frequency of HL-A8 in a group of *n* myasthenics having a given additional disorder with the frequency of HL-A8 in the (159-*n*) myasthenics. The frequency of rheumatoid arthritis (criteria according to American Rheumatoid Association) was 3.8%, systemic lupus erythematosus 1.9%, thyroid hormone disorders 9.4%, and 'neurovegetative' symptoms—for example, general asthenia, acrocyanosis, cardiac arrhythmias, abnormal sweating, and chill, etc.—23.9%. These are all increased compared with Finnish control population. The frequencies of HL-A8 in these patients do not differ from the frequencies in the myasthenics without the said disorders with the exception of patients with 'neurovegetative' symptoms in whom HL-A8 is 'almost significantly' ($P < 0.05$) increased. It is also interesting to find that HL-A8 is almost

TABLE 11
SOME ASSOCIATED DISORDERS OF 159 MYASTHENICS AND THE INCIDENCE OF HL-A8

Disorder	Additional disorders in 159 myasthenics		With HL-A8	
	(no.)	(%)	(no.)	(%)
Rheumatoid arthritis (ARA criteria)	6	3.8	2	33.3
Systemic lupus erythematosus	3	1.9	1	33.3
Arthritis NUD	6	3.8	3	50.0
Scleroderma	1	0.6	1	100.0
Glomerulonephritis chronic	1	0.6	0	—
Sjögren's syndrome	1	0.6	0	—
Pemphigus	1	0.6	1	100.0
Atopia	14	8.8	10	71.4
Thyroid hormone disorder	15	9.4	7	46.7
Non-toxic goitre	18	11.3	8	44.4
'Vegetative' symptoms (acrocyanosis, etc.)	38	23.9	25	65.8‡
Neurological symptoms	35	22.0	12	34.3
Some effect of menstrual cycle on MG (102 females)	58	56.8	34	58.6
No additional disease	32	20.1	21	65.6‡

* ‡: see footnotes to Table 3.

significantly increased in myasthenics with no other illness. However, many of these associated disorders are too infrequent to justify conclusions concerning their association with HL-A8.

DISCUSSION

It is now well accepted that both immunogenicity of certain biological compounds and immune responsiveness are genetically controlled. Many other factors, of course, also have effects on the immune response.

Transplantation antigens, including HL-A antigens, are one group of genetically controlled immunogens. HL-A antigens are polypeptide structures mimicking immunoglobulins in the plasma membranes of all cells. They can be detected by serological methods and are thus also called SD (serologically defined) antigens. The genes coding for HL-A antigens are situated in autosome no. 6 in a region called Major Histocompatibility Complex (MHC) (Figure) and are in several loci, the best known of which

mentioned as candidates for a MHC-linked genetic control (Ceppellini and van Rood, 1974). The possible role of HL-A antigens in certain diseases (McDevitt and Bodmer, 1974) could be explained by molecular mimicry between these and microbial antigen, or HL-A might act as or interact with receptor sites on the cell surface for the attachment of microbes, or HL-A products might simply act as markers for closely linked genes in MHC that may be involved in the synthesis or control of cell surface structures, or otherwise influence the penetrance of a multi-genic disease.

In this study, the only really significant deviation from normal controls is the increased frequency of the FOUR locus antigen, HL-A8. All other deviations seem to be secondary and due to the very marked association of HL-A8 with myasthenia gravis. Thus there is an increase of HL-A1 only in the haplotype 1.8; most other haplotypes with HL-A8 are also increased. Surprisingly, the haplotype 2.W10 is almost significantly decreased, while haplotype 9.W10 is almost significantly increased. HL-A8 is increased especially in female myasthenics with neurovegetative lability, or with onset of myasthenia below the age of 35 years, with thymus hyperplasia, or with the typical generalized clinical course.

In our patients, HL-A8 is inherited almost significantly ($P < 0.05$) more often from the father (19 subjects) than from the mother (11 subjects). This could be connected with the combined evidence by various authors showing that, in familial cases, myasthenia appears more often in father and child than in mother and child. The same inheritance was not found by Behan *et al.* (1973). In their study of eight cases HL-A8 was inherited four times each from father and from mother.

We did not find any significant increase of HL-A2 or 3 in thymoma patients, contrary to Feltkamp *et al.* (1974) and Fritze *et al.* (1974). Instead, we found W10 to be almost significantly increased in thymoma patients, but our thymoma material is too small for definite conclusions. In our study HL-A8 seems to be of no importance to 245 relatives of 106 myasthenics in respect of an increased risk of an autoimmune or endocrine disorder. Even in familial cases, HL-A8 is not always found, indicating that it may be only one

FIGURE Genetic map of the major histocompatibility complex (MHC) in man. SD-1 and SD-2—Genes for best-known HL-A antigens. LD-1—Genes for best-known LD-antigens. IR—Immune response-genes. DS—Disease susceptibility genes.

are called LA (SD I) and FOUR (SD II). LA includes at least 14 and FOUR 16 alleles. An individual inherits a haplotype with one LA and one FOUR gene from each parent and may thus produce principally four different HL-A antigens in his cells. The inheritance is codominant. There is increasing evidence that the MHC region, at least near the FOUR locus, includes genes that affect disease susceptibility or resistance. Susceptibility to certain viruses and some diseases with alterations in the immunomechanism may be

of the many factors that are required for the outbreak of myasthenia gravis or of another immunological disorder.

Myasthenics with no other additional symptoms have a high incidence of HL-A8 (65.6% HL-A8+). We can assume that this is the 'basic figure' indicating the effect of HL-A8 on the penetrance of myasthenia. Both the so-called collagen diseases and thyroid disorders occur in myasthenic patients more often than in the whole population. In this study the incidence of HL-A8 in these patients is a little lower than the 'basic figure'. Perhaps these disorders facilitate penetrance of myasthenia gravis. The joint influence of all these disease susceptibility factors may render the presence of the HL-A8-associated susceptibility factors superfluous. In any case, the number of examples of each disorder in the present study is too small to justify conclusions of their effect on the frequency of HL-A8. It is interesting to note that myasthenics with different neurovegetative symptoms (most often acrocyanosis and general asthenia) have HL-A8 more often than myasthenics without those symptoms.

HL-A8 has been demonstrated in other studies to be associated with several other diseases featuring lymphocytic infiltration of a target organ and/or the thymus: this is the case with chronic active autoimmune hepatitis (MacKay and Morris, 1972), dermatitis herpetiformis (Gebhard *et al.*, 1973), Graves' disease (Grumet *et al.*, 1973), idiopathic Addison's disease (Platz *et al.*, 1974), some forms of systemic lupus erythematosus (Grumet *et al.*, 1971), juvenile diabetes (Nerup *et al.*, 1974), coeliac disease (Stokes *et al.*, 1972), and perhaps with childhood asthma (Thorsby *et al.*, 1971). HL-A8 or an associated immune response (IR) gene might thus be one of the factors affecting endocrine autoimmunity. We have also looked for some specific LD (IR?) gene with a strong positive disequilibrium linkage with HL-A8 that would better explain the very marked association between myasthenia and HL-A8.

One specific LD gene (LD-8a) was found to be linked strongly to HL-A8 but only a little more often in myasthenics than in control persons. A specifically myasthenia-associated LD gene as determined by MLC technique was not found (Kaakinen *et al.*, in press). Thus a possible

myasthenia-susceptibility gene(s) may be situated closer to the FOUR locus than to the LD-1 locus. The clinical, serological, and familial overlap of certain autoimmune diseases suggests that they may have a common genetic base and that some extrinsic factor (possibly one causing somatic mutation in lymphoid stem cells) is needed to bring forth a particular autoimmune disease. In myasthenia gravis these mutated immunocytes or some product of theirs might then be directed against substances (for example, cholinacetylase?) in the synaptic area or against the neurotransmitter receptor site. This need not be a true antigen-antibody reaction. It is worth mentioning that HL-A8 is found in the plasma membrane of all cells, also of nerve and muscle cells, and that in MG morphological changes have been shown in the membranes of both these cells. HL-A8 may also represent a genetically determined weak point in either of these membranes.

Histocompatibility antigens have given neither an aetiological nor a pathogenetic explanation but have added to the evidence for inherited susceptibility to myasthenia gravis. The exact relationship between them remains to be clarified.

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