The Lambert-Eaton myasthenic syndrome

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Chapter 8

HLA class I and II in Lambert-Eaton myasthenic syndrome without associated tumour

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Hum Immunol 2001;62:809-813

Abstract

Lambert-Eaton myasthenic syndrome (LEMS) is an autoimmune disorder, in which antibodies against voltage-gated calcium channels located at nerve terminals cause muscle weakness and autonomic dysfunction. In approximately half of the patients the autoimmune process is initiated by a tumour. In the other half of patients no tumour is found and the etiology is unknown. The aims of this study were to investigate the strength of HLA-associations with nontumour LEMS (NT-LEMS) and to study the relation of HLA-haplotypes with age at onset of LEMS and other clinical features. Therefore, typing of HLA class I and II was performed in 19 patients with NT-LEMS, who were clinically evaluated. NT-LEMS was significantly associated with alleles of both HLA-class I (i.e. HLA-B8) as well as -class II (i.e. HLA-DR3 and –DQ2). HLA-B8+ patients had significantly younger age at onset of LEMS and tended to be female. This study shows that HLA-class I haplotype is associated with a distinct phenotype in NT-LEMS.

Introduction

Lambert-Eaton myasthenic syndrome (LEMS) is characterized by proximal muscle weakness and autonomic dysfunction. These clinical features are caused by antibodies directed against voltage-gated calcium channels (VGCC's) located at presynaptic nerve terminals. In approximately half of the patients a tumour, mostly small cell lung carcinoma (SCLC), is found, which probably initiates the production of crossreactive autoantibodies. In patients in whom no tumour is found, the etiology of LEMS is unknown.

The HLA genotype is considered to be the most important genetic marker of susceptibility to many autoimmune diseases. An increased frequency of HLA-B8 was found in both tumour and nontumour cases, but the association in nontumour LEMS (NT-LEMS) was much stronger.[1] Recently, an association of NT-LEMS was found with HLA-DR3 and -DQ2 in a study of HLA class II alleles in 23 cases.[2] Although HLA-B8, -DR3 and –DQ2 are known to be in strong linkage disequilibrium, it is unclear whether these associations of class II alleles with NT-LEMS are secondary to the class I association, and whether the HLA-associations are related to other clinical parameters of NT-LEMS.

The aim of this study was to confirm the previously found HLA-associations in one group of NT-LEMS and to investigate the relation of HLA-haplotypes with age and other clinical features.

Materials and methods

Subjects

Nineteen Dutch Caucasian patients with LEMS were studied. Diagnosis of LEMS was based on clinical features and electromyographic findings, including a low compound muscle action potential (CMAP) amplitude as well as an increase of this amplitude of more than 100% following high frequency repetitive nerve stimulation or following maximal voluntary contraction. In all patients, repeated extensive search for an underlying malignancy, especially SCLC, was negative. Follow-up was more than two years in all patients. The control group for comparison of phenotypes consisted of 1069 healthy donors. Informed consent was obtained from all subjects.

All patients were clinically evaluated in our hospital by taking a history of weakness and autonomic signs during the course of LEMS, use of medication for LEMS during the disease course and other immunological disorders. At the time of clinical evaluation, the levels of anti-P/Q-type VGCC were investigated in all patients using a commercial kit (RSR limited, Cardiff, UK). Titres of antibodies were considered positive if values were more than two standard deviations above the mean titre of 20

normal healthy controls. A patient was considered positive for having anti-VGCC antibodies if this test was positive or if testing of anti-VGCC antibodies had been positive in the past.

HLA typing

HLA-A, -B and –C typing was performed using the standard microcytotoxicity technique and for DR and DQ using the propidium iodide staining and automated reading method.[3]

Statistical analysis

HLA phenotype frequencies of patients and controls were compared using Haldane's modification of Woolf's method. P-values were corrected for 42 informative comparisons. For clinical comparison, a Mann Whitney test or Fisher's exact test was used when appropriate.

Results

The group consisted of 9 male and 10 female patients. Median age at onset of LEMS was 53 years (range 11-69 years). Six patients (32%) had an additional autoimmune disorder. Fifteen patients (79%) had signs of autonomic dysfunction. Ten patients (53%) used or had been using immunosuppressive therapy. Six patients (32%) had been wheelchair-dependent during the course of the disease. In 15 patients (79%) anti-P/Q-type VGCC antibodies were ≥18 pmol/l (2 SD above the mean for healthy controls) or had been present in the past.

HLA typing

The results for HLA-typing of the whole group for class I and class II are shown in table 1. With regard to class I alleles, a significant association was found for HLA-B8, which was present in 74% of the patients with LEMS and 27% of the controls ($p = 0.00006$). In HLA-class II, significantly higher frequencies of HLA-DR3 ($p = 0.0006$) and HLA-DQ2 ($p = 0.0003$) were found in LEMS patients. No other alleles showed a significant association with NT-LEMS after correction. Twelve LEMS patients (63%) were positive for HLA-B8, -DR3 and -DQ2. Results for each individual patient with regard to HLA-B8, -DR3, -DQ2 and clinical parameters are shown in table 2. All patients with age at onset less than 50 years were HLA-B8(+), against only 50% of the patients with age onset above 50 years (Fisher's exact test, $p = 0.03$).

Clinical comparison between B8(+) and B8(-) LEMS patients

A clinical comparison was made between B8(+) and B8(-) LEMS patients (table 3). Age at onset of LEMS was significantly lower in HLA-B8(+) patients. HLA-B8(+) patients tended to be female. Nine of the 10 females (90%) were HLA-B8(+), against

Table 1. HLA class I and II in patients with Lambert-Eaton myasthenic syndrome without associated tumour

HLA-loci	LEMS n (%)	Controls n (%)	OR	95% CI	*p* *	*Pc*†
A1	9 (47)	353 (33)	1.834	0.755 - 4.452	0.2204	1.0000
A2	11 (58)	530 (50)	1.376	0.562 - 3.366	0.4975	1.0000
A3	4 (21)	285 (27)	0.798	0.277 - 2.299	0.7942	1.0000
A9	4 (21)	199 (19)	1.267	0.439 - 3.659	0.7672	1.0000
A11	2 (11)	126 (12)	1.065	0.280 - 4.062	0.7672	1.0000
A10	0 (0)	84 (8)	0.299	0.018 - 4.997	0.3903	1.0000
A29	1 (5)	53 (5)	1.539	0.285 - 8.312	1.000	1.0000
A31	1 (5)	46 (4)	1.738	0.329 - 9.660	0.5713	1.0000
A32	2 (11)	80 (7)	1.754	0.457 - 6.730	0.6489	1.0000
A28	2 (11)	115 (11)	1.181	0.309 - 4.505	1.000	1.0000
B5	1 (5)	105 (10)	0.741	0.139 - 3.962	1.000	1.0000
B7	3 (16)	266 (25)	0.640	0.200 - 2.044	0.5908	1.0000
B8	14 (74)	290 (27)	7.074	2.627 - 19.052	0.0000	0.0025 ‡
B12	2 (11)	297 (28)	0.371	0.098 - 1.405	0.1208	0.9997
B13	0 (0)	39 (4)	0.669	0.040 - 11.280	1.0000	1.0000
B14	0 (0)	49 (5)	0.529	0.031 - 8.883	1.0000	1.0000
B15	1 (5)	137 (13)	0.550	0.103 - 2.931	0.4961	1.0000
B16	0 (0)	79 (7)	0.319	0.019 - 5.341	0.3901	1.0000
B17	4 (21)	95 (9)	2.963	1.016 - 8.640	0.0863	0.9966
B18	1 (5)	79 (7)	1.010	0.188 - 5.418	1.0000	1.0000
B21	0 (0)	37 (3)	0.706	0.042 - 11.916	1.0000	1.0000
B22	2 (11)	55 (5)	2.611	0.675 - 10.098	0.2622	1.0000
B27	1 (5)	71 (7)	1.132	0.211 - 6.082	1.0000	1.0000
B35	3 (16)	191 (18)	0.973	0.304 - 3.117	1.0000	1.0000
B37	2 (11)	40 (4)	3.631	0.930 - 14.175	0.1646	1.0000
B40	2 (11)	165 (15)	0.781	0.205 - 2.968	0.7542	1.0000
DR1	5 (26)	232 (22)	1.359	0.504 - 3.667	0.5831	1.0000
DR2	2 (11)	292 (27)	0.378	0.100 - 1.435	0.1218	0.9997
DR3	13 (68)	315 (30)	4.940	1.920 - 12.710	0.0006	0.0252 ‡
DR4	4 (21)	271 (25)	0.849	0.294 - 2.448	0.7950	1.0000
DR7	7 (37)	232 (22)	2.151	0.859 - 5.380	0.1570	1.0000
DR8	1 (5)	61 (6)	1.324	0.245 - 7.132	0.6766	1.0000
DR9	0 (0)	25 (2)	1.045	0.061 - 17.793	1.0000	1.0000
DR10	1 (5)	41 (4)	1.989	0.366 - 10.812	0.5331	1.0000
DR11	1 (5)	180 (17)	0.398	0.075 - 2.116	0.2283	1.0000
DR12	0 (0)	45 (4)	0.350	0.021 - 5.849	0.6318	1.0000
DR13	3 (16)	289 (27)	0.565	0.177 - 1.806	0.4333	1.0000
DR14	0 (0)	72 (7)	0.350	0.021 - 5.849	0.6318	1.0000
DQ1	10 (53)	742 (70)	0.481	0.198 - 1.1695	0.1318	0.9999
DQ2	16 (84)	449 (42)	6.465	2.027 - 20.6218	0.0003	0.0126 ‡
DQ3	8 (42)	556 (52)	0.676	0.276 - 1.6556	0.4886	1.0000
DQ4	1 (5)	53 (5)	1.510	0.279 - 8.1563	1.0000	1.0000

*determined by Fisher's exact test; †corrected for 42 informative comparisons; ‡frequency difference significant ($P < 0.05$)

Table 2. Clinical characteristics of patients with Lambert-Eaton myasthenic syndrome without associated tumor

Patient	age at onset*	sex	HLA			anti-VGCC antibodies†	autonomic dysfunction‡	other autoimmune diseases
			B8	DR3	DQ2			
1	11	f	+	+	+	-	+	
2	18	m	+	+	+	+	-	
3	25	f	+	+	+	+	+	
4	30	f	+	+	+	+	+	hyperthyroid
5	31	f	+	+	+	+	-	
6	33	m	+	-	+	-	+	RA
7	41	f	+	+	+	-	+	
8	42	m	+	+	+	+	-	
9	46	f	+	+	+	-	+	MG
10	53	m	-	-	+	+	+	IDDM
11	54	f	+	+	+	+	+	IDDM
12	54	f	+	-	-	+	+	hyperthyroid
13	55	m	-	-	-	+	+	
14	60	m	+	+	+	+	+	
15	61	f	+	+	+	+	+	
16	62	m	-	+	+	+	+	
17	64	m	-	-	+	+	+	
18	64	m	+	+	+	+	-	
19	69	f	-	-	-	+	+	

*age at onset of LEMS in years; †anti-VGCC antibody titer positive (+) or negative (-); ‡presence (+) or absence (-) of one or more signs of autonomic dysfunction (e.g. dry mouth, constipation, sexual impotence)
m = male; f = female; RA = reumatoid arthritis; MG = myasthenia gravis; IDDM = insulin-dependent diabetes mellitus

only 5 of 9 males (56%). The four anti-VGCC seronegative patients were all HLA-B8(+).

Discussion

In this study we show that LEMS without associated tumor is significantly associated with alleles on both HLA-class I (i.e. HLA-B8) as well as -class II (i.e. HLA-DR3 and –DQ2) and that HLA-B8(+) patients with NT-LEMS have younger age at onset than HLA-B8(-) patients and tend to be female.

Table 3. Clinical comparison between HLA-B8(+) and HLA-B(-) patients

Parameter	HLA-B8(+) (n=14)	HLA-B8(-) (n=5)	p
Sex (M:F)	5:9	4:1	ns*
Median age at onset of LEMS (years)	53	62	0.018†
Anti-VGCC antibody positive (%)	10/14 (71)	5/5 (100)	ns*
Autonomic dysfunction (%)	10/14 (71)	5/5 (100)	ns*
Immunosuppressive therapy (%)	7/14 (50)	2/5 (40)	ns*
Wheelchair dependence (%)	5/14 (36)	1/5 (20)	ns*

*determined by Fisher's exact test; †determined by Mann Whitney test

M = male; F = female; VGCC = voltage-gated calcium channel; ns = not significant

The association of LEMS with HLA-B8 and with -DR3 and -DQ2 has been described in two separate studies.[1,2] In the first study, a highly significant association of nontumor LEMS was found with HLA-B8. Furthermore, the frequency of HLA-DR3 was increased in these cases, but this did not reach significance, probably because the study contained only 10 nontumor cases.[1] In the second study, HLA-typing of only class II was performed and demonstrated an association with DR3 and DQ2.[2] We confirm these associations in one and the same group of patients with NT-LEMS.

In myasthenia gravis (MG), another antibody mediated neuromuscular disorder, distinct clinical subgroups have different HLA associations. In Caucasian MG female patients with young onset and without thymoma, the same association with HLA-B8, -DR3 and -DQ2 exists.[4-7] The relation of HLA-B8 with young age at onset and female gender in LEMS without associated carcinoma indicates an interesting analogy with MG without thymoma. This suggest that factors in the HLA-region contributing to the pathogenesis of both diseases are involved in the regulation of the general immune reactivity rather than of antigen-specific immune processes.

One patient in our study was diagnosed with both MG and LEMS. She initially had oculobulbar weakness with antibodies against the acetylcholine receptor (496 nM) and a decremental response at low frequency repetitive nerve stimulation, and was therefore diagnosed with young-onset MG. Ten years after onset she developed a prominent proximal weakness of the extremities, dryness of the mouth and a low CMAP amplitude with an increment of 300% at high-frequency stimulation, thereby confirming LEMS. No anti-VGCC-antibodies were found. HLA-typing of this patient showed HLA-B8-DR3-DQ2, which supports the idea of common HLA-related factors for both disorders.

In LEMS, the pathogenic antibodies are directed against P/Q-type VGCC's. These antibodies can be demonstrated in 76%-91% of LEMS patients without associated tumor.[8,9] In this study, in 14 NT-LEMS patients (79%) anti-P/Q-type VGCC antibodies were detected. The four seronegative patients were all B8(+) and had young age at onset of LEMS (<50 years). Except for age at onset and possibly gender no other clinical features were related to HLA-haplotype in NT-LEMS.
The results of this study implicate the presence of susceptibility genes in the HLA-B-DR region. Further mapping of genes in these region is needed for identification of loci contributing to the development of NT-LEMS.

Acknowledgements
We thank Peter de Lange for statistical analysis of HLA typing, and Willem Verduijn for his work in the HLA-typing laboratory. This study was supported by the Prinses Beatrix Fonds.

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