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The Lambert-Eaton myasthenic syndrome

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Chapter 10

HLA and smoking in prediction and prognosis of small cell lung cancer in autoimmune Lambert-Eaton myasthenic syndrome

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Abstract

Patients with small cell lung cancer (SCLC) survive longer if they have the antibody-mediated Lambert-Eaton myasthenic syndrome (LEMS), making this autoimmune disorder a prototype disease for studying cancer immunosurveillance. Patients with non-tumour LEMS (NT-LEMS) never develop SCLC, but are otherwise indistinguishable clinically. Therefore, we have compared immunogenetic factors in SCLC-LEMS and NT-LEMS and studied their role in the pathogenesis of LEMS and survival from SCLC. In 48 British and 29 Dutch Caucasian LEMS patients, we studied clinical symptoms, antibody titres, HLA-types and alleles at six nearby located microsatellite loci. Highly significant associations were found in NT-LEMS, which appeared strongest with HLA-B8, but also involved HLA-DQ2, -DR3 and six flanking microsatellite alleles. SCLC-LEMS patients were not different from controls. Smoking was a strong predictor of SCLC. In contrast, HLA-B8 positivity correlated with a decreased risk of SCLC even among the smokers. Moreover, in SCLC-LEMS patients, HLA-B8 positivity correlated with prolonged survival after LEMS onset. We propose that two distinct immunopathogenetic routes can lead to one clinically and serologically indistinguishable autoimmune myasthenic syndrome. HLA-DR3-B8 is strongly associated with LEMS in non-tumour patients only. In other LEMS patients, SCLC apparently provides a powerful autoimmunogenic stimulus that overrides HLA restrictions in breaking tolerance to calcium channels. Moreover, negativity for HLA-B8 combined with smoking behaviour points more strongly to an underlying SCLC, and predicts a worse prognosis in SCLC-LEMS patients.

Introduction

Less than 10% of patients with small cell lung cancer (SCLC) survive more than two years after tumour detection.¹ The median overall survival is only 10 months after diagnosis of these strongly smoking-related tumours. However, it reaches 17.4 months in SCLC patients who have the rare autoimmune Lambert-Eaton myasthenic syndrome (LEMS).² This highly informative antibody-mediated paraneoplastic disorder must hold clues to mechanisms of cancer immunosurveillance. Several studies of paraneoplastic neurological diseases confirm that the immune system can mount a strong response against the associated malignant tumour, resulting in its regression or even eradication.³⁻⁵ However, the neoplastic cells often cease to present key target molecules and subsequently escape recognition.

LEMS is a prototypic paraneoplastic disorder, with an unusually well defined target autoantigen, the P/Q-type voltage gated calcium channels (VGCC). An SCLC is found in approximately 50% of all LEMS patients,⁶ while 1-3 % of all SCLC patients develop LEMS (SCLC-LEMS).⁷ The SCLC cells express P/Q-type VGCC, and LEMS patients' IgG reduces Ca²⁺ flux into SCLC cell lines.⁸ Apparently, therefore, in SCLC-LEMS, the autoantibodies are initially provoked by tumour VGCC that cross-react with those at the nerve terminals; indeed, if the tumour can be removed or destroyed, the antibodies may wane,⁹ and LEMS often remits.¹⁰

Interestingly, another subgroup of typical LEMS patients never develop SCLC, even after prolonged follow-up, most of whom are non-smokers.⁶ In three small series of these non-tumour LEMS (NT-LEMS) patients, there are consistent associations with HLA-DQ2 and -DR3 in the class II region, and especially with B8 in class I.¹¹⁻¹³ These alleles belong to the same conserved HLA-DR3-B8-A1 haplotype that associates with several other autoimmune disorders, including early-onset myasthenia gravis (MG).¹⁴ More detailed analysis of the intervening class III and nearby class I regions using microsatellites has identified a highly conserved combination of linked alleles.¹⁵ In SCLC-LEMS cases, the evidence for associations with this HLA region is less clear. A marginally increased prevalence of HLA-B8 was noted only in one series of 14 LEMS patients with tumours.¹¹ In other paraneoplastic autoimmune disorders, such as the SCLC-related anti-Hu syndrome¹⁶ and thymoma-related MG,¹⁷ no clear associations have been reported.

These findings suggest that distinct immunopathogenetic pathways could lead to an identical neurological and serological picture in paraneoplastic and idiopathic LEMS. Seeking clues to underlying mechanisms, we studied differences in HLA-genotypes between these LEMS subgroups in a large combined British and Dutch cohort.

Materials and methods

Patients

The 37 available Dutch LEMS patients were ascertained through a nationwide study from 1997 to 2000. Twenty-nine of them met our inclusion criteria, as did 48 of the 67 available British LEMS patients (seen in Oxford or London between 1983 and 1997) (Fig. 1). These criteria were Caucasian LEMS patients with a cytologically or histologically proven SCLC, or patients with no tumour plus at least three years follow-up after the diagnosis of LEMS.

LEMS was diagnosed if the patient had muscle weakness and either anti-P/Q-type VGCC serum antibodies or abnormal electromyography (or both), the latter comprising a reduced resting compound muscle action potential amplitude that increased by >100% following high-frequency repetitive nerve stimulation or maximal voluntary contraction. Two neurologists, one in Oxford and one in Leiden, examined all patients and evaluated (serially) their weakness and autonomic dysfunction, the drugs given to treat their LEMS and the presence of other immunological disorders (Table 1). They were considered smokers if they had consumed one or more cigarettes per day during at least one year. All sera were assayed for the presence of anti-P/Q-type VGCC antibodies in Oxford at diagnosis as described before.⁹

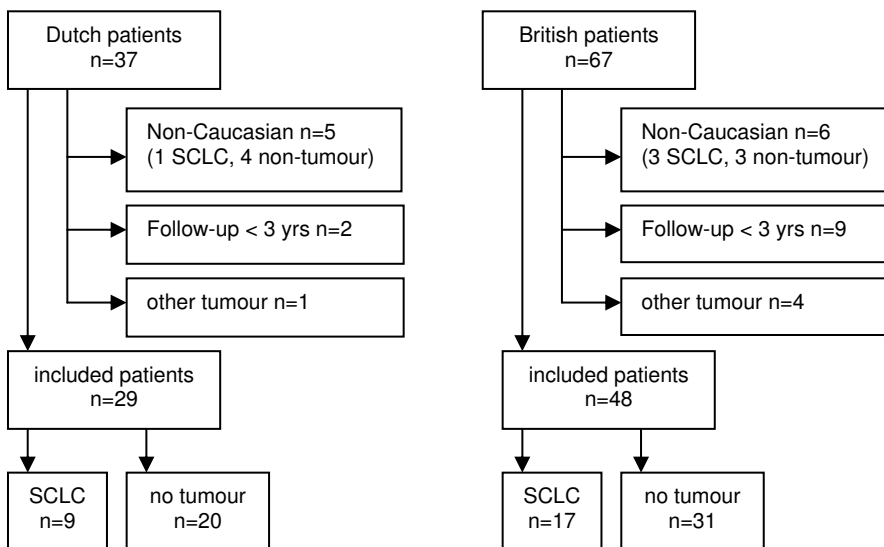


Figure 1. Flow-chart showing inclusion of Dutch or British patients.

Table 1. Clinical features of the patients included.

	Non-tumour associated LEMS (n=51)			Small cell lung cancer associated LEMS (n=26)			<i>p</i> ^a
	Dutch	British	Total	Dutch	British	Total	
Sex (male:female)	9:11	16:15	25:26	7:2	10:7	17:9	n.s.
Onset-age in years ^b	53 (11-69)	51 (16-74)	51 (11-74)	55 (35-73)	58 (43-72)	58 (35-73)	0.0004
Anti-VGCC-antibody							
patients positive (%)	18 (90%)	27 (87%)	45 (88%)	8 (89%)	15 (88%)	23 (88%)	n.s.
titre in pmol/l ^b	274 (0-738)	190 (0-1532)	217 (0-1532)	451 (36-741)	157 (0-2772)	237 (0-2772)	n.s.
Smoking history (%)	9 (45%)	11 (35%)	20 (39%)	9 (100%)	14/15 ^c (93%)	23/24 (96%)	1.6x10 ⁻⁶
Additional AID (%)	5 (25%)	10 (32%)	15 (29%)	0 (0%)	3 (18%)	3 (12%)	n.s.

LEMS=Lambert-Eaton myasthenic syndrome. VGCC= voltage gated calcium channel. n.s.= not significant. AID= autoimmune disorders.

^ap-values are given for comparisons between totals of patients with non-tumour associated versus small cell lung carcinoma associated LEMS.

^bmedian (range).

^cfor two British patients, no information about smoking history was available.

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The medical Ethical Committees of both hospitals approved the study and all patients gave informed consent.

HLA typing

All patients were HLA-typed for class I using PCR-SSO (Dynal Biotech) and for class II using standard PCR for sequence-specific polymorphisms (PCR-SSP).¹⁸ HLA typing of the Dutch control group was previously performed in Leiden.¹⁸ HLA data for British controls are from Haworth.^{19,20} The control group for microsatellite prevalences consisted of 324 unrelated healthy randomly selected Dutch individuals.

Genotyping for microsatellite alleles

Six microsatellite marker loci were studied; D6S1014, D6S273, TNFa in the MHC class III region of chromosome 6 and MIB, C1-2-5 and C1-3-2 nearby in the MHC class I region (Fig. 2). Microsatellite typing was performed as described previously.¹⁵

Statistical analysis

We compared clinical characteristics by standard t-test or Chi-square test when appropriate. Odds Ratios (OR) were calculated using Haldane's modification of Woolf's method. Differences in prevalences of alleles or allele combinations were tested by Fisher's exact test using the StatXact statistical package (Cytel Software, Cambridge, MA, USA). For extra rigor, p-values of HLA and microsatellite phenotype prevalences were corrected for multiple testing (using the sum of all informative alleles tested at each locus, $n=102$; p_c -value). Survival data were compared using a Log-rank test included in the SPSS software package (SPSS Inc., Chicago, Illinois, USA).

Results

Clinical characteristics

The NT-LEMS patients had significantly lower onset-ages than the SCLC-LEMS patients ($p=0.0004$) (Table 1). The modest increase in prevalence of other autoimmune diseases in NT-LEMS did not reach significance. We saw no differences in anti-P/Q-type VGCC antibodies. The proportion of smokers among the SCLC-LEMS patients was 96% compared to only 39% in the NT-LEMS patient group ($p=1.6 \times 10^{-6}$); the average in the general Dutch population for 1997 was 40% in males and 32% in female.²¹

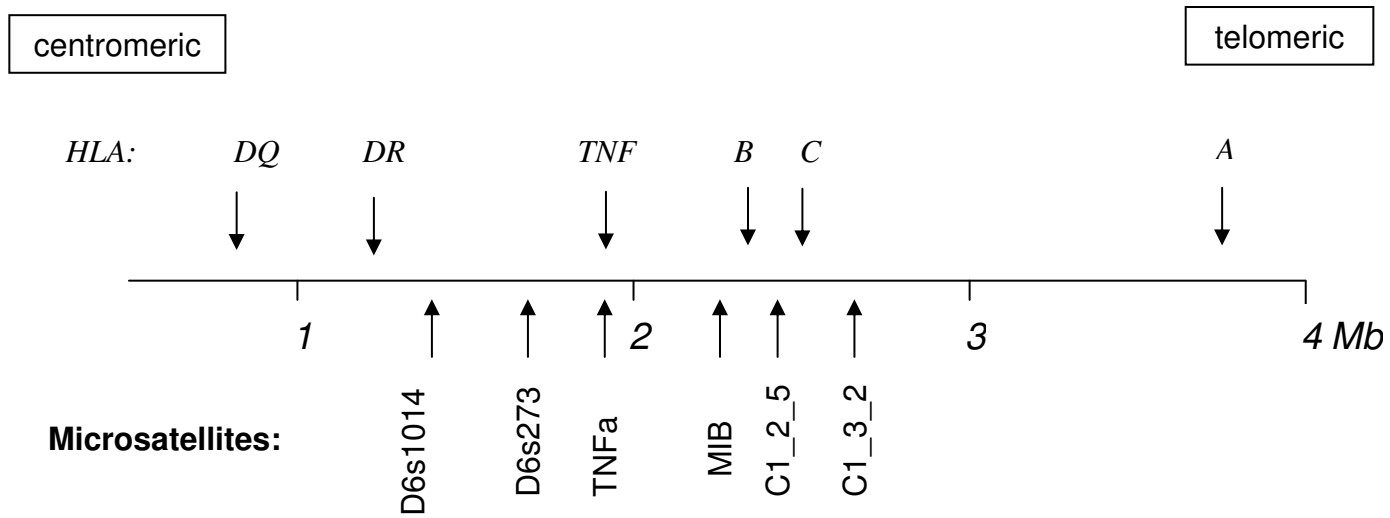


Figure 2. Map of MHC region on chromosome 6, showing the localization of HLA-loci and microsatellites.

HLA analysis

No difference has been reported for HLA-DR3, -B8 or -A1 prevalences between Dutch and British healthy controls.¹⁸⁻²⁰ Our Dutch and British LEMS patients also showed very similar HLA prevalences without significant differences (Table 2, Fig. 3). Therefore, they have been combined here (Table 3). We found highly significant associations with the HLA-DR3, -B8 and -A1 alleles in NT-LEMS compared to both the controls and the SCLC-LEMS cases (Table 3, Fig. 3). The strongest was with HLA-B8 ($p_c=7.8 \times 10^{-10}$ versus controls, $p_c=2.2 \times 10^{-4}$ versus SCLC-LEMS). The associations with HLA-B8 and -DR3 could not be separated from each other by calculating either of them conditional on the other. However, it should be noted that DR3⁺-B8⁻ and DR3⁻-B8⁺ haplotypes are uncommon in Caucasians. In striking contrast, in SCLC-LEMS, these alleles showed no increases, whether individually or in combination; the apparent decreases are not significant.

Microsatellite analyses

The microsatellite combination D6S1014*143, D6S273*139, TNFa*99, MIB*350, C1-2-5*196 and C1-3-2*354 was seen in 45% of the NT-LEMS patients, versus 15% of the controls (Table 3, Fig. 3). These alleles are in strong linkage disequilibrium with the HLA-DR3-B8-A1 haplotype; collectively they are markers for a conserved haplotype.¹⁵ We confirmed that all six of these alleles were inherited together; in four of four families of Dutch NT-LEMS patients, the complete haplotype was present in all the HLA-DR3-B8⁺ first- degree family members that were tested, including at least one parent and one sibling (not shown). No significant differences in allele prevalences were found between SCLC-LEMS and controls.

Table 2. HLA-DR3, -B8 and -A1 prevalences in Dutch and British patients with non-tumour Lambert-Eaton myasthenic syndrome (NT-LEMS) and small cell lung carcinoma associated Lambert-Eaton myasthenic syndrome (SCLC-LEMS).

Locus	Allele	Dutch NT-LEMS (%) n=20	British NT-LEMS (%) n=31	p	Dutch SCLC-LEMS (%) n=9	British SCLC-LEMS (%) n=17	p
<i>HLA-phenotype prevalences</i>							
HLA-DRB1	0301	13 (65)	23 (74)	ns	2 (22)	3 (18)	ns
HLA-B	0801	14 (70)	21 (68)	ns	2 (22)	1 (6)	ns
HLA-A	0101	9 (45)	16 (52)	ns	1 (11)	1 (6)	ns

Figure 3. HLA and microsatellite phenotypes in each patient. Black/white boxes indicate positivity/negativity for each allele listed from left (centromeric) to right (telomeric): HLA-DRB1*0301; D6S1014*143; D6S273*139; TNF α *99; MIB*350; HLA-B*08; C1-2-5*196; C1-3-2*354 and HLA-A*01.

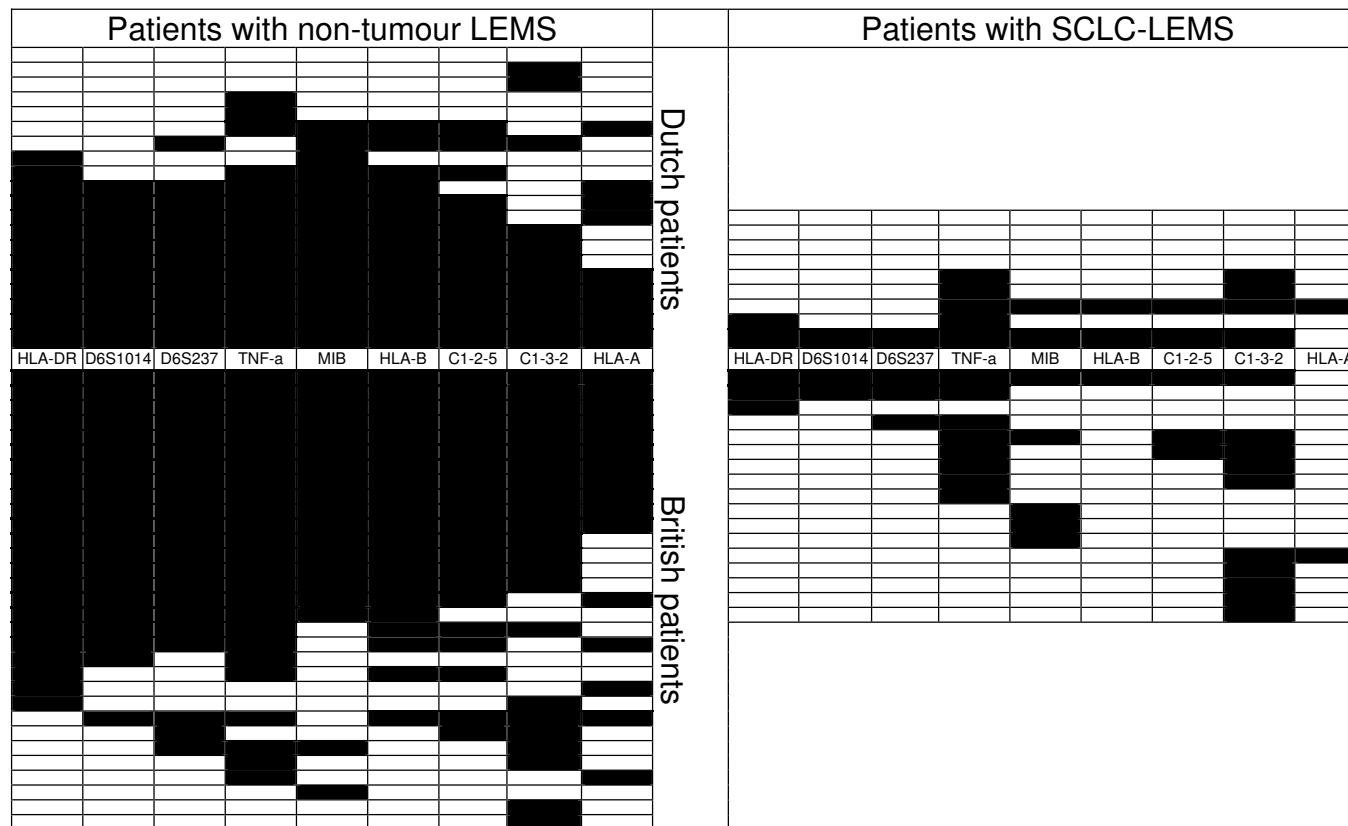


Table 3. HLA and microsatellite phenotype prevalences in non-tumour Lambert-Eaton myasthenic syndrome (NT-LEMS), small cell lung carcinoma associated Lambert-Eaton myasthenic syndrome (SCLC-LEMS) and healthy controls.

Locus	Allele	Controls (%)	NT-LEMS (%) n=51	SCLC-LEMS (%) n=26	NT-LEMS vs. Controls p/p _c -value	NT-LEMS vs. SCLC-LEMS p/p _c -value	SCLC-LEMS vs. Controls p/p _c -value
<i>HLA-phenotype prevalences</i>							
HLA-DRB1	0301	599/2395 (25)	36 (71)	5 (19)	1.9x10 ⁻¹¹ / 2.0x10 ⁻⁹	2.6x10 ⁻⁵ / 2.8x10 ⁻³	ns
HLA-B	0801	554/2440 (23)	35 (69)	3 (12)	7.4x10 ⁻¹² / 7.8x10 ⁻¹⁰	2.1x10 ⁻⁶ / 2.2x10 ⁻⁴	ns
HLA-A	0101	747/2439 (31)	25 (49)	2 (8)	8.5x10 ⁻³ / ns	3.0x10 ⁻⁴ / 3.1x10 ⁻²	9.2x10 ⁻³ / ns
DR3+B8		426/2131 (20)	32 (63)	2 (8)	7.0x10 ⁻¹¹ / 7.4x10 ⁻⁹	2.3x10 ⁻⁶ / 2.5x10 ⁻⁴	ns
B8+A1		416/2201 (19)	22 (43)	1 (4)	9.1x10 ⁻⁵ / 9.6x10 ⁻³	2.0x10 ⁻⁴ / 2.1x10 ⁻²	ns
DR3+B8+A1		343/2103 (16)	21 (41)	0	2.9x10 ⁻⁵ / 3.0x10 ⁻³	3.2x10 ⁻⁵ / 3.4x10 ⁻³	1.5x10 ⁻² / ns
<i>Microsatellite marker phenotype prevalences</i>							
D6S1014	143	51/324 (16)	32 (63)	4 (15)	9.9x10 ⁻¹² / 1.0x10 ⁻⁹	8.8x10 ⁻⁵ / 9.3x10 ⁻³	ns
D6S273	139	48/324 (15)	34 (67)	5 (19)	6.2x10 ⁻¹⁴ / 6.5x10 ⁻¹²	8.9x10 ⁻⁵ / 1.0x10 ⁻²	ns
TNFA	99	136/324 (42)	40 (78)	14 (54)	1.4x10 ⁻⁶ / 1.5x10 ⁻⁴	3.6x10 ⁻² / ns	ns
MIB	350	84/324 (26)	34 (67)	8 (31)	2.6x10 ⁻⁸ / 2.7x10 ⁻⁶	3.7x10 ⁻³ / ns	ns
C1-2-5	196	62/324 (19)	34 (67)	6 (23)	2.2x10 ⁻¹¹ / 2.3x10 ⁻⁹	5.9x10 ⁻⁴ / ns	ns
C1-3-2	354	101/324 (31)	34 (67)	14 (54)	2.6x10 ⁻⁶ / 2.8x10 ⁻⁴	ns	2.8x10 ⁻² / ns
Conserved haplotype ^a		39/254 (15)	23 (45)	2 (8)	1.1x10 ⁻⁵ / 1.2x10 ⁻³	7.6x10 ⁻⁴ / ns	ns

^aThe conserved haplotype includes the alleles: D6S1014*143, D6S273*139, TNFA*99, MIB*350, C1-2-5*196, and C1-3-2*354. P-values are shown before (p) and after correction (p_c) for the total number of alleles tested (correction factor = 102).

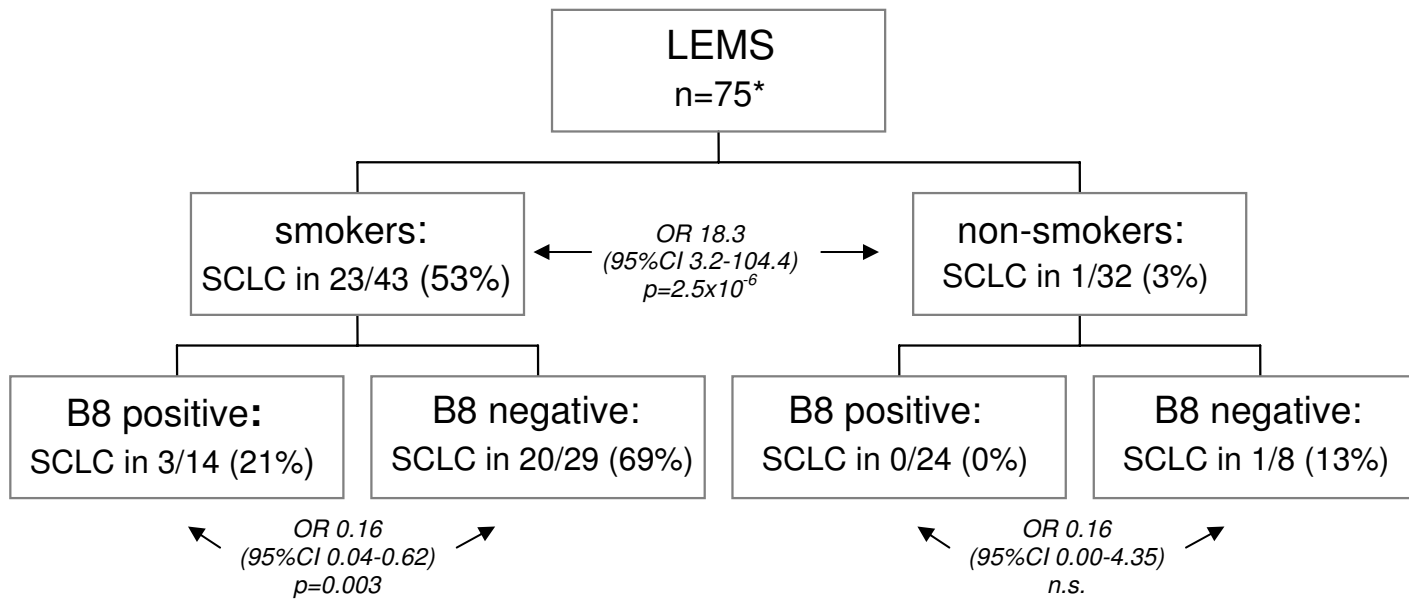


Figure 4. Risks of SCLC by smoking behaviour and presence of HLA-B8.

*For two patients, no information about smoking behaviour was available.

Prediction of SCLC in LEMS patients

As expected, smoking conferred a significantly increased risk of SCLC in our LEMS series (Fig. 4); 23 of the 43 smokers developed SCLC versus only 1 of the 32 non-smokers (OR 18.3; $p=2.5 \times 10^{-6}$). On the other hand, among all our 38 LEMS patients with HLA-B8, SCLC was detected in only 3 (8%; all were smokers) versus 21 of the 37 without -B8 (57%; 20 were smokers) (OR 0.08; $p=5.1 \times 10^{-6}$). Even among the LEMS smokers, SCLC was less common in those with HLA-B8 (OR 0.16; $p=0.003$). Consistently, the only non-smoking patient with SCLC was HLA-B8⁻.

Prognosis of SCLC

We determined the 'diagnostic interval' – the period between LEMS onset and the diagnosis of SCLC – in 23 of the 26 SCLC-LEMS patients, the median value being 5 months (range –2 to 213 months). In 74% (17/23) of the patients, the tumour was diagnosed within two years of the first symptoms of LEMS. We could also determine the 'tumour survival' for 17 of the 26 SCLC-LEMS patients. Their overall median survival was at least 14 months (range 0-122) after tumour detection versus 10 months for SCLC patients without LEMS.² Survival after onset of LEMS, i.e. 'diagnostic interval' and 'tumour survival', was significantly longer in the three HLA-B8⁺ (median 71 months) than in the 14 HLA-B8⁻ SCLC-LEMS patients (median 24, range 4-194 months; $p=0.047$).

Discussion

We report a striking contrast in HLA-associated LEMS-susceptibility between patients with and without SCLC in independent Dutch and UK cohorts. The alleles from the conserved HLA-DR3-B8 haplotype all showed substantial increases in the NT-LEMS cases, whereas no significant differences were found between the SCLC-LEMS patients and controls.

Comparing HLA associations in LEMS

The majority of the NT-LEMS patients expressed the HLA-DR3-B8 haplotype. In Caucasians, this highly conserved haplotype associates with many immunopathological diseases.¹⁴ Our results from six microsatellites very strongly suggest that they are also highly conserved in our NT-LEMS patients, their 'core' being inherited *en bloc*. Despite rigorous correction, the HLA-B8 association in NT-LEMS is very strong and is very reminiscent of that in early-onset MG,²² where other autoimmune disorders are again common. As in LEMS, this haplotype is not increased or even slightly decreased in MG with tumour (thymoma).¹⁷ A recent study stressed the relationship between HLA-B8 and thymic hyperplasia but not thymoma in MG.¹⁷ This analogy suggests that

thymoma and SCLC play similar roles in pathogenesis, autosensitizing by expressing relevant antigens in a 'dangerous' autoimmunogenic environment.

In both MG and LEMS, paraneoplastic and idiopathic subgroups are clinically and serologically indistinguishable: autoantibody profiles can be almost identical in MG patients with and without thymoma. Therefore, the autoimmunity-prone conserved HLA-DR3-B8 haplotype appears to be a decisive factor in responsiveness in patients without tumour, possibly affecting early stages in cellular activation and/or the cytokine balance.¹⁴ An increased spontaneous release of TNF- α is very characteristic of Caucasians with the HLA-DR3-B8-A1 haplotype,²³ and was also noted in first-degree relatives of our patients with NT-LEMS, although it was not confined to those with HLA-DR3-B8.²⁴

Interestingly, our present study could not confirm the weak positive association with HLA-B8 that we noted previously in 14 patients with tumours.¹¹ This partly reflects our strict exclusion now of cases with tumours other than SCLC; if these were coincidental, they would belong to the NT-LEMS group.

A role for HLA alleles in immunosurveillance

The low prevalence of HLA-DR3-B8 haplotype in SCLC-LEMS is intriguing. We previously demonstrated that SCLC patients with LEMS have an improved survival after detection of the tumour,² though they mostly die eventually from tumour complications. This accords with the concept of 'cancer immunoediting', whereby an immune attack on tumour cells initially controls their growth, but often also selects for less sensitive escape variants.⁵ In several of our patients more than 20 months elapsed before tumours were detected, conceivably because the immune response can control the tumour in an early stage by reacting against its VGCC (and other antigens) expressed by the SCLC in this 'dangerous' immune-stimulating environment. The presence of the conserved haplotype containing HLA-B8 and linked MHC loci in our SCLC-LEMS patients evidently resulted in an additional survival advantage. Conversely, it cannot be excluded that some HLA-B8⁺ smokers included in the NT-LEMS group already have eradicated a developing SCLC before their LEMS presented. As predicted before,²⁵ such immunity is inevitably hard to substantiate.

Our evidence also supports previous deductions that autoimmunization must begin when the tumours are still very small,^{5,25} and that their 'dangerous' microenvironment must provide powerful stimuli that can overcome the usual HLA restrictions. Indeed, tumour macrophage infiltration was greater in SCLC-LEMS than in patients with SCLC alone.²⁶ In time, SCLC may escape immune recognition or elimination, for example by deficiencies in HLA expression.²⁶

Independent evidence implicates HLA alleles in SCLC – notably an increase in HLA-B44.²⁷ We noted a similar, but non-significant trend in our UK patients (data not shown). There is an intriguing parallel in the CD8⁺ T cell response to Epstein-Barr virus antigen EBNA-3A.²⁸ Healthy subjects with HLA-B8 nearly all respond to one particular epitope, their T cells using T cell receptors with highly restricted α and β sequences that happen to cross-recognize HLA-B4402 (without EBNA-3). Moreover, these T cells are undetectable (deleted) if the donor also has B4402, and any responding cells now use different T cell receptors. One could speculate that, also in patients with SCLC and LEMS, protective responses to SCLC can be restricted to HLA-B8 and prevented by coincident B4402.

The beneficial effects of immunosurveillance were evidently not confined to HLA-B8⁺ cases. One of the B8 negative patients who has survived >10 years achieved a complete clinical remission of LEMS after chemotherapy. We previously reported a second patient with a tumour-free survival of >7 years without symptoms of LEMS or drug treatment,¹⁰ and a third who became seronegative for anti-VGCC antibodies after chemotherapy.⁹ Thus, not only can these tumours be completely eliminated in the absence of the HLA-DR3-B8 haplotype: the resulting decline in the anti-VGCC antibodies and clinical remission of LEMS without further immunosuppressive therapy strongly suggests that the immunogenicity of the tumour cells is the overriding factor in SCLC-associated autoimmunity.

Early detection of SCLC in LEMS patients

In 50-60% of LEMS patients, SCLC is detected within two years of diagnosis of LEMS, but this interval can sometimes be much longer, up to almost 6 years.⁶ A significant clinical concern is how frequently to monitor for tumours when following new LEMS cases who smoke. It is accepted that patients with SCLC-associated and idiopathic LEMS do not show any clearly distinguishing clinical or serological differences.^{6,29} Smoking- or tumour-associated traits such as a higher onset-age, a male bias, more weight loss at the time of diagnosis, and a higher ESR, either appear late in the course of disease or lack specificity, and cannot therefore be used as early predictors of an occult SCLC. Interestingly, the absence of HLA-B8 proved to be highly predictive of SCLC even in LEMS patients who smoked. By combining the presence of HLA-B8 with a non-smoking habit, we could correctly predict the absence of an underlying SCLC (Fig. 4). These early pointers at the time of diagnosis of LEMS could be valuable in saving patients unnecessary investigations and stress.

Conclusion

We hypothesize that two different aetio-pathologies can lead to LEMS. In ~50% of cases, provocation is mysterious but the autoimmunity-prone HLA-B8 haplotype is

clearly one important contributor. In the others, LEMS is provoked by 'dangerous' exposure of VGCC by SCLC cells, leading to temporary loss of tolerance and symptoms that remit if the tumour is eradicated. Here, HLA-DR3-B8 or linked alleles can act like a two-edged sword by favouring autoimmune reactions while also enhancing immune surveillance against the SCLC and improving survival; their absence should also help clinicians to anticipate an underlying SCLC.

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