Circulating T cell subsets in the Lambert-Eaton myasthenic syndrome

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SUMMARY Peripheral blood T cell subsets were measured using monoclonal antibodies and a fluorescence activated cell sorter in 15 untreated patients with Lambert-Eaton myasthenic syndrome (nine with small cell carcinoma, one undifferentiated epithelial tumour (ca-LEMS)), five with no demonstrable tumour (non-ca-LEMS), 10 age-matched healthy controls and 10 patients with small cell carcinoma without neurological disease. OKT_8^+ (suppressor/cytotoxic) T cells were significantly decreased in ca-LEMS compared with non-ca LEMS ($p < 0.001$) ca-controls ($p < 0.01$) and healthy controls ($p < 0.001$). In one patient depressed OKT_8^+ T cells antedated clinically evident tumour by five months. OKT_3^+ (total) and OKT_4^+ (helper) T cells were similar in ca-LEMS, non-ca LEMS and controls. The mechanism underlying the loss of circulating OKT_8^+ T cells in ca-LEMS is unknown, but these changes may help to predict the presence of carcinoma in this disease.

The Lambert-Eaton myasthenic syndrome (LEMS) is now considered to be an autoimmune disorder, mediated by IgG autoantibodies to determinants concerned with acetylcholine release at the motor nerve terminal.¹ The disease is characterised by proximal muscle weakness² and autonomic disturbance.³ Two thirds of cases are associated with carcinoma, usually of the small cell type, and almost half have organ specific autoantibodies.⁴ Physiological⁵ and pharmacological⁶ studies on biopsied LEMS muscle have shown a reduction in the number of quanta of acetylcholine released from the nerve terminal, with consequent reduction in the amplitude of the evoked muscle action potential. Non-quantal release of transmitter ("molecular leakage") is also reduced.⁶ Freeze fracture studies have shown loss and disorganisation of presynaptic active zone particles, which may represent calcium channels.⁷ Plasma exchange causes a transient improvement in LEMS symptoms and immunosuppressive drug treatment in the non-carcinomatous cases leads to improvement or recovery.⁸ The immunological nature of disease in both its carcinomatous and

non-carcinomatous forms is established by the finding that its physiological features and the characteristic changes in presynaptic active zone particles can be induced in mice by daily injections of the IgG fraction of LEMS plasma.^{1,9-11}

The mechanism of induction of the disease is unknown. It has been suggested that autoimmunity arises when there is a disturbance in the balance between inducer and suppressor subsets within the human T cell circuit.¹² We therefore studied the distribution of peripheral blood T cell subsets, using monoclonal antibodies and a fluorescence activated cell sorter, in patients with recently diagnosed and untreated LEMS, both in its carcinomatous and non-carcinomatous form.

Subjects and methods

LEMS patients

Diagnosis of LEMS was based on typical clinical features (proximal muscle weakness, autonomic symptoms, depressed tendon reflexes, often with post-tetanic potentiation) and electromyographic findings. The latter consisted of a reduced initial compound muscle action potential in abductor digiti minimi in response to a supramaximal nerve stimulation, and an increment exceeding 25% after 15 seconds maximal voluntary contraction or (in case 10) during nerve stimulation at 50 Hz (table 1).

Ten patients (five men, five women; aged 53-72 years) had carcinoma (ca-LEMS). Small cell carcinoma was confirmed histologically in nine; biopsy of a mediastinal

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lymph node in the tenth (patient 2) showed undifferentiated epithelial carcinoma.

There were five patients (three men, two women; aged 45–68 years), in whom extensive screening had failed to disclose evidence of tumour (non-ca-LEMS). Clinical details for all 15 LEMS patients are given in table 1.

Controls

Ten patients (seven men, three women; aged 46–72 years) were studied with histologically proven small cell carcinoma but without clinical evidence of neurological disease; these served as controls for the ca-LEMS group. The clinical stage of carcinoma was similar in the LEMS and the control group; nine out of ten patients in each group subsequently underwent anti-tumour therapy.

Ten healthy subjects (five men, five women, aged 40–72 years), were controls for the non-ca-LEMS group.

Methods

All blood samples were taken before chemotherapy, radiotherapy, plasma exchange or, in the non-ca LEMS, immunosuppression with azathioprine and alternate day prednisolone. Samples were obtained from patients and controls at the same time of day wherever possible (usually between 14.00 and 16.00 hours).

Peripheral blood mononuclear cells (PBM) were isolated by Ficoll-Hypaque density centrifugation from 15–20 ml heparinised venous blood. The cells were washed twice in Hanks balanced salt solution and then frozen in liquid nitrogen to await simultaneous analysis with controls.

The freezing method has been described by Ludgate *et al.*¹⁴ Briefly, the PBM ($15\text{--}20 \times 10^6$) were pelleted and resuspended in 250 μ l of neat fetal calf serum (FCS) at 4°C. 250 μ l of ice cold dimethylsulphoxide: FCS (1:4)

were then added dropwise, the mixture was placed in freezing vials on ice and then transferred rapidly to a well insulated polystyrene container and kept at -70°C overnight before transfer to a liquid nitrogen storage flask the next day.

For analysis, vials of frozen cells were thawed rapidly in a water bath at 37°C and, once the last crystal of ice had melted, transferred to Roswell Park Memorial Institute (RPMI) 1640 culture medium at room temperature, in which they were left for 20 minutes. The cells were then washed twice in RPMI 1640, counted and viability determined by trypan blue dye exclusion. Aliquots of 1×10^6 cells were pelleted and resuspended in 50 μ l of phosphate buffered saline containing 2.0% FCS and 0.1% sodium azide (PBS-SA). 5 μ l of a 1 in 5 dilution in PBS-SA of each of the following mouse monoclonal antibodies were added and incubated for five minutes at room temperature: OKT₃ (total T cells), OKT₄ (helper T cells) and OKT₈ (suppressor/cytotoxic T cells) (Ortho Diagnostics). The cells were then washed in PBS-SA and treated for 5 mins at room temperature with 5 μ l of a 1 in 5 dilution in PBS-SA of fluorescein labelled goat anti-mouse immunoglobulin (Gm FITC). After a further wash in PBS-SA the cells were resuspended in 1 ml PBS-SA and kept on ice until counting.

All samples were coded and each assay included samples from patients, controls and a reference control. Analysis was performed using a fluorescence activated cell sorter (Ortho System 50H cytofluorograph). A minimum of 2000 PBM with the light scattering properties of lymphocytes were analysed per sample. Background staining was measured in a sample stained with Gm FITC only and subtracted from the test sample to give the net percentage of cells positive for each monoclonal antibody out of the total PBM analysed.

The data were analysed by Student's unpaired *t* test.

Table 1 Clinical details of LEMS patients

Case	Age (yr) sex	Duration years	Tumour	Other diseases	Organ-specific autoantibodies	Muscle action potential amplitude*	
						Resting mV	Increment† %
1	72/F	0.1	SC	Thyroidectomy	—	6.4	190
2	70/M	0.7	AN	—	—	7.6	136
3	62/M	0.3	SC	—	GPC+	1.0	200
4	58/F	0.7	SC	Lymphosarcoma	TM 1:1/600	3.2	333
5	56/F	0.4	SC	Mammary carcinoma	—	1.8	333
6	60/M	1.7	SC	—	—	1.3	1169
7‡	53/M	1.0	SC	—	—	4.0	180
8	66/M	0.7	SC	Vitiligo	—	8.2	95
9	65/F	1.1	SC	—	—	6.6	188
10	65/F	1.1	SC	—	—	7.3	>64
11	45/M	2.3	—	—	—	6.0	167
12	66/F	4.3	—	—	GPC+	0.75	1966
13	61/M	11.3	—	—	—	1.5	933
14	56/M	0.8	—	—	—	7.7	146
15	68/F	3.0	—	Pernicious anaemia	GPC++ TM 1:28600	0.8	112
Healthy controls > 8.3§							<25§

*Measured in abductor digiti minimi.

†Measured 3 seconds after maximum voluntary contraction or during nerve stimulation at 50 Hz (case 10).

‡See Compston and Hughes.¹³

§See Newsom-Davis and Murray.⁸

GPC Gastric parietal cell antibodies.

SC small cell.

TM Thyroid microsomal antibodies.

AN Anaplastic carcinoma.

Table 2 Peripheral blood T cell subsets in LEMS and controls

	(mean % $\pm$ SEM)		
	OKT ₃ ⁺	OKT ₄ ⁺	OKT ₈ ⁺
Healthy Controls n = 10	75.2 $\pm$ 2.9	52.7 $\pm$ 3.2	26.4 $\pm$ 1.5
Non-ca-LEMS n = 5	70.8 $\pm$ 3.8	44.3 $\pm$ 4.2	25.9 $\pm$ 1.2
Ca-Controls n = 10	64.7 $\pm$ 4.3	47.2 $\pm$ 3.4	25.0 $\pm$ 2.2
Ca-LEMS n = 10	69.2 $\pm$ 4.5	55.5 $\pm$ 3.9	16.5 $\pm$ 1.7*

*p < 0.01 cf ca-controls, p < 0.001 cf non-ca-LEMS, p < 0.001 cf healthy controls.

Results

No differences were observed in the percentages of circulating OKT₃⁺ and OKT₄⁺ T cells in ca-LEMS and non-ca LEMS compared with controls (see table 2). By contrast, there was a significant reduction in circulating OKT₈⁺ T cells in the ca-LEMS group (16.5 $\pm$ 1.7% (mean $\pm$ SEM)) compared with non-ca-LEMS (25.9 $\pm$ 1.2%, p < 0.001), with ca controls (25.0 $\pm$ 1.5%, p < 0.01), and with healthy controls (26.4 $\pm$ 1.5%, p < 0.001) (see fig).

There was no significant correlation between numbers of circulating OKT₈⁺ T cells in the LEMS patients and duration of symptoms (r = 0.49) or

severity of disease, as measured by initial compound muscle action potential in abductor digiti minimi (r = 0.319).

In patient 1 initial screening for occult malignancy was negative. However the proportion of circulating OKT₈⁺ T cells was very low (14.4%), more than three standard deviations below the mean for the non-ca-LEMS group. Five months later, in the absence of any immunosuppressive therapy, a paratracheal mass became evident on chest radiographs and was found on biopsy to be a small cell carcinoma.

Discussion

The results show a highly significant reduction in the proportion of circulating OKT₈⁺ T cells in tumour-associated Lambert-Eaton myasthenic syndrome. This abnormality is not observed in patients with the non-tumour form of the disease. Furthermore, in one patient a reduction in circulating OKT₈⁺ T cells antedated the clinical detection of tumour by five months.

Several potential sources of error in this type of study have been eliminated. With the finding that lymphocytes may be successfully frozen and thawed without loss of T cells markers¹⁴ we chose to store frozen samples from patients and controls for subsequent assay together, thus avoiding errors introduced by serial assays (unpublished observations). T cells have been shown to have a circadian rhythm,¹⁵⁻¹⁷ we therefore obtained samples from patients and controls at the same time of day whenever possible, to allow for valid comparison. Furthermore T cell subsets have been shown to vary with advancing age;¹⁸ we therefore selected controls with a similar age distribution to the LEMS cases. Finally, samples were coded and analysed automatically using a fluorescence activated cell sorter, thus avoiding observer bias that might otherwise occur.

The mechanism of the selective loss of OKT₈⁺ T cells is open to speculation. Reduction of OKT₈⁺ T cells has been observed in other autoimmune diseases,¹⁹⁻²³ and in acute polyradiculoneuritis²⁴ and active multiple sclerosis.^{25, 26} However there is con-

Fig Peripheral blood OKT₈⁺ lymphocytes (%) in LEMS and controls. (The mean of each group is given $\pm$ SEM)

siderable debate as to whether this represents a primary event (for example genetically determined loss of suppressor activity) or a secondary effect of the disease (for example sequestration of OKT_8^+ T cells in target organs or lymphoid tissue, or modulation of the cell surface marker). Interpretation of the findings is further complicated by the fact that functional heterogeneity may exist within a phenotypically defined subset, cells of the OKT_8^+ phenotype having been shown to exert suppressor as well as helper activity.²⁷⁻²⁹

Loss of circulating OKT_8^+ lymphocytes in LEMS appears to be selective for the tumour form of the disease. One possible explanation is that sequestration of OKT_8^+ T cells occurs at the tumour site or in local lymph nodes. It has been shown that early in the development of spontaneous mammary carcinomas in C3H/HEJ mice large numbers of T cells bearing the phenotype $\text{lyt } 1^{-2+}$ (suppressor/cytotoxic T cells) may be found within the tumour, thymus and regional draining nodes.³⁰ However, were sequestration the only explanation for low OKT_8^+ T cells in ca-LEMS, one would expect similar changes in the tumour control group. This was generally not the case, though two patients in this group did have relatively low circulating OKT_8^+ T cells (see fig), the patient with the lowest value being the only one of the ten with such extensive metastatic disease that anti-tumour therapy was not considered appropriate. Further studies of larger numbers of patients would be of interest, particularly if homing of T cell subsets to the tumour or lymphoid sites were studied. Whether such findings could be construed as cause or effect of neoplasia would, nevertheless, still be open to debate.³¹

We found that low circulating levels of OKT_8^+ T cells antedated clinical detection of the tumour in one LEMS patient by five months. Measuring this subset in LEMS patients may thus have practical value in providing an indicator of occult malignancy, and perhaps also of tumour recurrence following therapy. Further serial studies will be needed to confirm this.

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