Short report

Suppressor T cell changes in active multiple sclerosis: analysis with three different monoclonal antibodies

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SUMMARY This study demonstrates a significant reduction in the number of both total T cells and suppressor T cells identified by monoclonal antibodies in multiple sclerosis patients in acute relapse but not in those in remission. The reduction in the number of suppressor T cells was shown by all three monoclonal antibodies used but was most clearly demonstrated using Leu 2a rather than either OKT 8 or OKT 5. These findings suggest that the choice of monoclonal antibody used in a study of suppressor T cell numbers will influence the results and may help explain the lack of agreement in previous studies.

Quantitative abnormalities of peripheral blood lymphocytes (PBL) and their subpopulations in patients with multiple sclerosis were originally described in the mid 1970s.^{1,2} Employing monoclonal antibodies, a reduction in the suppressor/cytotoxic T cell subset was demonstrated in multiple sclerosis patients in acute relapse or with progressive disease.^{3,4} This was supported by subsequent serial studies,^{5,6} though more recent work has yielded conflicting results.⁷⁻⁹

We have studied T lymphocytes and their subsets in multiple sclerosis and in particular the changes in the suppressor T cell subset during acute relapse. All patients in the study had clinically definite multiple sclerosis¹⁰ and factors which might influence the results were standardised. Our preliminary findings indicated that an additional factor (the choice of monoclonal antibody used to identify the suppressor cell subset) significantly affects the results obtained.

Patients, materials and methods

Peripheral blood T lymphocytes and their subsets were measured in 15 patients with clinically definite multiple sclerosis in acute relapse, in 26 patients in remission, and in 13 healthy laboratory controls. All patients satisfied McDonald's criteria for clinically definite multiple sclerosis.¹⁰ Acute relapse was defined as being within two weeks of the onset of symptoms persisting longer than 24 hours and remission as a definite improvement in symptoms over the previous month.¹¹ No patient was on steroids or other immunosuppressive therapy for at least six months

prior to the time of sampling. All blood sampling was performed between 8.30 and 11.00 a.m.

Venous blood was collected in 10 ml samples into heparinised evacuated tubes. Mononuclear cells were isolated by Ficoll-Hypaque density gradient centrifugation, washed twice in phosphate-buffered saline (PBS) and adjusted to a concentration of $5 \times 10^6 \text{ ml}^{-1}$. 100 μl aliquots were reacted with the appropriate monoclonal antibody; Leu 4 to measure the total T cells, Leu 3a the helper subset, and Leu 2a, OKT 8 and OKT 5 to measure the suppressor subset. (The Leu reagents were obtained commercially from Becton-Dickinson, OKT 8 was purchased from Ortho and OKT 5 was obtained as a gift from Dr Gideon Goldstein of Ortho). The cells were incubated for 30 min at 4°C, washed twice in PBS and then reacted with fluoresceinated goat antimouse IgG (Tago) for an additional 30 min. 200 cells were counted within 24 hours of sampling using a Leitz Dialux UV microscope. All slides were examined without knowledge of the monoclonal antibody or the patient under scrutiny to avoid bias. Differential white cell counts were performed on all samples to allow lymphocyte subset results to be expressed, assuming no selective loss of cells during handling, as absolute counts.

Statistical analysis was carried out using the non-parametric Wilcoxon Rank Sum Test and Wilcoxon Test for Pair Differences.

Results

The total mononuclear cell (MNC) count in patients with multiple sclerosis in relapse or in remission was not significantly different from controls (table). There was a significant reduction in total T cells identified by Leu 4 in multiple sclerosis patients in relapse (0.83 ± 0.13) compared with healthy con-

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Table Peripheral blood lymphocytes in multiple sclerosis patients and controls ($\times 10^9/l$)

	Total MNC	Leu 4 Total	Leu 3a Helper	Leu 2a	OKT 8 Suppressor subset	OKT 5
Controls (13)	1.99* ± 0.15	1.20 ± 0.15	0.79 ± 0.08	0.42 ± 0.05	0.48 ± 0.06	0.34 ± 0.04
Multiple sclerosis Remission (26)	2.05 ± 0.17	1.10 ± 0.09	0.79 ± 0.07	0.39 ± 0.03	0.44 ± 0.04	0.32 ± 0.03
Multiple sclerosis Relapse (15)	2.02 ± 0.19	0.83 ± 0.13 $p < 0.05^\dagger$	0.63 ± 0.10	0.17 ± 0.04 $p < 0.01$	0.28 ± 0.04 $p < 0.02$	0.18 ± 0.02 $p < 0.05$

* = Mean $\pm$ Standard error of mean.
 $\dagger$ = Significance of difference from control values.

trols (1.20 ± 0.15 , $p < 0.05$) while these were normal in patients in remission. The number of helper T cells (identified by Leu 3a) in both groups of multiple sclerosis patients was not significantly different from controls although some reduction was apparent in patients in relapse.

A reduction in suppressor T cells in patients in relapse compared with controls was detected by all three antibodies (Leu 2a, $p < 0.01$; OKT 8, $p < 0.02$; OKT 5, $p < 0.05$). The number of cells recog-

nised by Leu 2a (0.17 ± 0.04) was significantly lower than that detected using OKT 8 (0.28 ± 0.04 , $p < 0.05$) (fig). There was no significant difference in the number of suppressor T cells in either multiple sclerosis patients in remission or controls using either Leu 2a or OKT 8. Measurement of suppressor T cells with OKT 5 in controls and in multiple sclerosis patients in remission yielded significantly lower results than those obtained using the other two antibodies ($p < 0.05$) and fell during relapse to the same level as that measured by Leu 2a. No significant change occurred in the number of B lymphocytes or monocytes.

Discussion

A significant reduction in the number of both total T cells and suppressor T cells identified by monoclonal antibodies occurs in multiple sclerosis patients in acute relapse but not in remission. The reduction in the number of suppressor T cells was shown by all three antibodies used but was most marked using Leu 2a rather than either OKT 5 or OKT 8.

The results of this study support the findings of Reinherz³ and other workers^{4,12} but are in disagreement with several more recent reports.⁷⁻⁹ We have taken care to exclude a number of variables which might affect the results obtained. Only patients with clinically definite disease¹⁰ were studied and strict clinical definitions¹¹ were adhered to. No patients were receiving steroids or other immunosuppressive therapy likely to disturb lymphocyte subpopulations.¹³ Circadian variations in lymphocyte populations¹⁴ were avoided by taking samples at a fixed time each day. Cells were examined using UV microscopy which correlates well with automated cytofluorometry¹⁵ and changes in suppressor T cells in multiple sclerosis have been demonstrated using both techniques simultaneously.¹⁶

Conflicting results using different antibodies to identify suppressor T cells have also been reported by Paty who has shown a reduction in Leu 2a expression not revealed by OKT 8 in patients with

Fig Suppressor T cell numbers in multiple sclerosis patients in acute relapse and controls measured by three monoclonal antibodies: Leu 2a, OKT 8 and OKT 5 (Mean $\pm$ standard error).

progressive multiple sclerosis.⁷ In a more recent serial study however he found OKT 5 to be the most sensitive gauge of disease activity in multiple sclerosis.¹⁷ The reasons for these differences are not clear since all three antibodies recognise determinants present on the same cell surface glycoprotein present on suppressor T cells.^{18,19} It now appears that monoclonal antibodies recognising the helper T cell subset may also yield conflicting results in certain conditions.^{20,21}

The changes observed in active multiple sclerosis may reflect a reduction in the density of this molecule on the cell surface or other conformational changes rather than a change in the size of the suppressor T cell subset.²² The absence in our study of a simultaneous decrease in the total mononuclear cell count or an increase in the numbers of B cells or monocytes would support this. The consequences of alterations in the expression of the surface glycoprotein used to identify suppressor T cells on the function of these cells remains unknown, particularly since no direct correlation has been found between the apparent number and function of suppressor T cells in individual multiple sclerosis patients.²³

In conclusion, this study demonstrates that the choice of monoclonal antibody used to identify suppressor T cells in patients with multiple sclerosis is another factor which must be considered when undertaking a study of lymphocyte changes in multiple sclerosis.

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