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# T Lymphocyte Populations in Multiple Sclerosis

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**Summary.** In 36 patients representing different clinical stages of multiple sclerosis (MS) (9 patients with acute exacerbations; 21 patients in remission; 5 patients with chronic progressive MS) determinations of T lymphocyte populations using monoclonal antibodies against surface antigens (OKT3 (pan T cells), OKT4 (helper T cells), OKT8 (cytotoxic/suppressor T cells)) were performed. Compared to the control group (40 healthy individuals) a clear elevation of the T4/T8 ratio was found in acute exacerbations and to a lesser degree in patients with inactive phases of MS. Patients with chronic progressive disease did not show increased T4/T8 ratios. Serial determination of lymphocyte populations after corticosteroid therapy in 10 selected patients revealed no significant changes which could be attribted to this therapeutic modality.

Pathogenetic and clinical implications of the shifts in surface antigen expression of T lymphocyte populations mirroring the clinical course of MS are discussed.

**Key words:** Multiple sclerosis – Lymphocyte populations – T4/T8 ratio and therapy

## Introduction

Virological, genetic and immunological findings contribute to the current concept of etiology and pathogenesis of multiple sclerosis (MS). Studies on alterations of immune regulatory processes by Reinherz et al. and Bach et al. showed quantitative shifts in the balance of regulatory T lymphocyte populations in the peripheral blood of MS patients [3, 15]. As determined by analysis of cell surface markers these authors found a significant relative increase of $T4^+$ lymphocytes (lymphocytes with helper functions) in active phases of the disease accompanied by diminished relative numbers of $T8^+$ lymphocytes (lymphocytes with supressor/cytotoxic functions). In patients with inactive disease the ratio of the two antagonistic cell populations ($T4^+/T8^+$) was comparable to results obtained in normal healthy individuals.

Panitch and Francis reported similar changes in the T4/T8 ratio in lymphocytes from MS patients [13]. Other investigators have published observations about a relative increase in lymphocytes with helper cell phenotype in foci of demyelination in the CNS of MS patients [4, 18]. The congruent changes of expression of lymphocyte surface antigens in peripheral blood and CSF has not been confirmed by all investigators [6]. Nevertheless, the findings cited above led to the hypothesis that fluctuations of the helper/suppressor cell ratio in peripheral blood indicate changes of disease activity in the CNS.

Monitoring lymphocyte populations in patients with disseminated lupus erythematosus has suggested that in addition to disease related changes immunosuppressive therapy (high dosage corticosteroids) could modulate the expression of lymphocyte surface antigen in autoimmune disorders [7].

In this study we have determined changes in the expression of lymphocyte surface markers in patients with MS prior to and during therapy.

## Patients and Methods

In 36 patients with MS lymphocyte surface antigens were determined prior to specific immunosuppressive therapy. At the time of testing 9 patients had an acute exacerbation of disease, 5 patients were suffering from chronic progressive MS and 21 patients were in remission (i.e., no clinical deterioration for 6 months before test). In 10 patients (5 patients with active disease, 5 patients with chronic progressive disease) we repeated the determination of lymphocyte populations after 14 days of corticosteroid therapy (approximately 1000 mg prednisolone; 100 mg/day initially; decreasing dosage; 40 mg/day at day 14). The control group consisted of 40 individuals matched by age and sex.

In each test we determined the number of T lymphocytes ($T3^+$), T helper lymphocytes ($T4^+$), T suppressor/cytotoxic lymphocytes ($T8^+$) and B lymphocytes in heparinized peripheral blood samples collected 1 to 6 h before determination. The expression of T lymphocyte antigens was assessed in an indirect immunofluorescence assay described earlier [16] using monoclonal antibodies (OKT3, OKT4, OKT8) purchased from Ortho Diagnostics (Raritan, NJ 08869 USA). The B lymphocyte count was assessed by determining the percentage of lymphocytes which express membrane immunoglobulin according to the recommendations of the International Union of Immunological Societies [1].

## Results

Table 1 shows the results of the analysis of lymphocyte populations in individual patients before therapy. As an indicator

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**Table 1.** Lymphocyte populations in patients with MS

| Patient | $T3^+$ cells (%) | $T4^+$ cells (%) | $T8^+$ cells (%) | B cells (%) | T4/T8 ratio |
|---|---|---|---|---|---|
| Clinically active disease (acute exacerbation) | | | | | |
| 1: (F; 33 years) | 61 | 49 | 12 | 10 | 4.1 |
| 2: (F; 26 years) | 71 | 61 | 20 | 20 | 3.1 |
| 3: (F; 45 years) | 57 | 50 | 7 | 11 | 7.1 |
| 4: (M; 37 years) | 52 | 45 | 6 | 11 | 7.5 |
| 5: (M; 29 years) | 48 | 41 | 7 | 15 | 5.7 |
| 6: (M; 44 years) | 67 | 58 | 5 | 10 | 11.6 |
| 7: (F; 40 years) | 48 | 40 | 4 | 12 | 10.0 |
| 8: (F; 35 years) | 64 | 52 | 11 | 17 | 4.7 |
| 9: (F; 36 years) | 57 | 44 | 10 | 8 | 4.4 |
| Chronic progressive disease | | | | | |
| 10: (F; 24 years) | 63 | 43 | 15 | 11 | 2.9 |
| 11: (F; 30 years) | 71 | 49 | 18 | 4 | 2.7 |
| 12: (M; 44 years) | 51 | 39 | 18 | 11 | 2.2 |
| 13: (M; 36 years) | 49 | 33 | 14 | 6 | 2.4 |
| 14: (F; 47 years) | 53 | 26 | 12 | 18 | 2.2 |
| Inactive disease (remission) | | | | | |
| 15: (M; 53 years) | 71 | 48 | 36 | 9 | 1.3 |
| 16: (M; 44 years) | 73 | 48 | 31 | 9 | 1.5 |
| 17: (M; 51 years) | 74 | 56 | 31 | 6 | 1.8 |
| 18: (F; 35 years) | 42 | 29 | 15 | 10 | 1.9 |
| 19: (F; 30 years) | 67 | 36 | 22 | 12 | 1.6 |
| 20: (F; 23 years) | 63 | 34 | 20 | 10 | 1.7 |
| 21: (F; 22 years) | 78 | 59 | 27 | 10 | 2.7 |
| 22: (M; 41 years) | 69 | 57 | 26 | 13 | 2.2 |
| 23: (F; 42 years) | 58 | 44 | 17 | 8 | 2.6 |
| 24: (M; 39 years) | 59 | 44 | 15 | 17 | 2.9 |
| 25: (F; 37 years) | 70 | 47 | 24 | 8 | 2.0 |
| 26: (F; 40 years) | 78 | 64 | 19 | 13 | 3.4 |
| 27: (F; 38 years) | 80 | 59 | 17 | 10 | 3.5 |
| 28: (F; 29 years) | 69 | 57 | 12 | 14 | 4.8 |
| 29: (F; 46 years) | 79 | 48 | 11 | 10 | 4.4 |
| 30: (M; 33 years) | 63 | 49 | 9 | 8 | 5.4 |
| 31: (M; 56 years) | 57 | 46 | 7 | 17 | 6.6 |
| 32: (M; 53 years) | 56 | 56 | 7 | 11 | 8.0 |
| 33: (F; 49 years) | 79 | 66 | 9 | 10 | 7.3 |
| 34: (M; 35 years) | 58 | 52 | 6 | 10 | 8.7 |
| 35: (F; 50 years) | 70 | 64 | 6 | 11 | 10.6 |

for the balance between lymphocytes with helper cell characteristics and lymphocytes with suppressor cell characteristics we included the quotient of $T4^+$ and $T8^+$ lymphocytes referred to as T4/T8 ratio. The distribution of T4/T8 ratios in patients with different courses of the disease in comparison to the control group is presented in Fig. 1. In 7 of 9 patients (78%) with acute disease a clear elevation of the T4/T8 ratio (above mean + 2 SD of the control group) was noted, whereas patients with chronic progressive disease invariably showed T4/T8 ratios in the normal range. Clearly elevated ratios were found in 27% of patients in remission.

Figure 2 shows T4/T8 ratios prior to and after 2 weeks of corticosteroid therapy. There is no indication for a uniform effect of high dosage corticosteroids on the T4/T8 ratio in MS. Longitudinal determinations of T4/T8 ratios in healthy individuals in our laboratory showed intraindividual fluctuations up to 0.5 over a 6-month period.

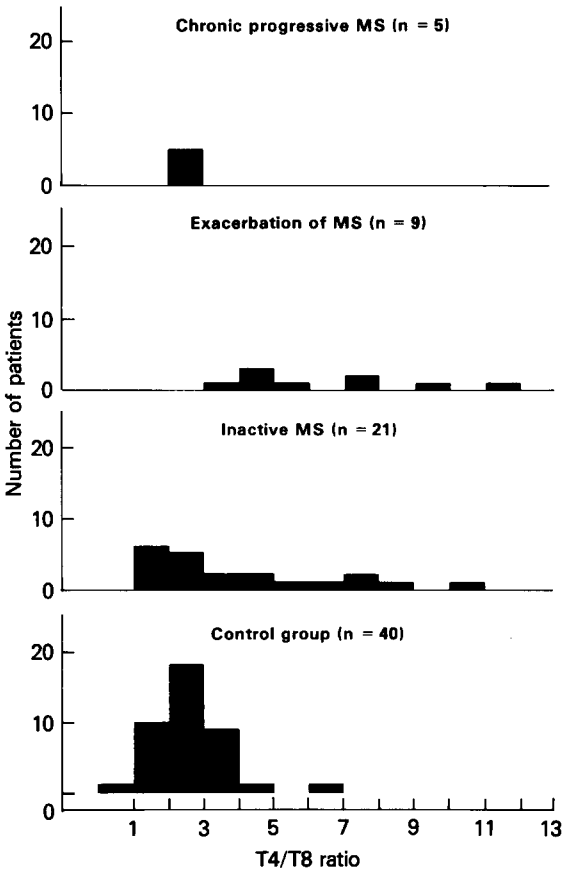


**Fig. 1.** T4/T8 ratios in patients with MS. Increased T4/T8 ratios were found in acute disease and to a lesser degree in inactive disease. All patients with chronic progressive disease showed normal T4/T8 ratios

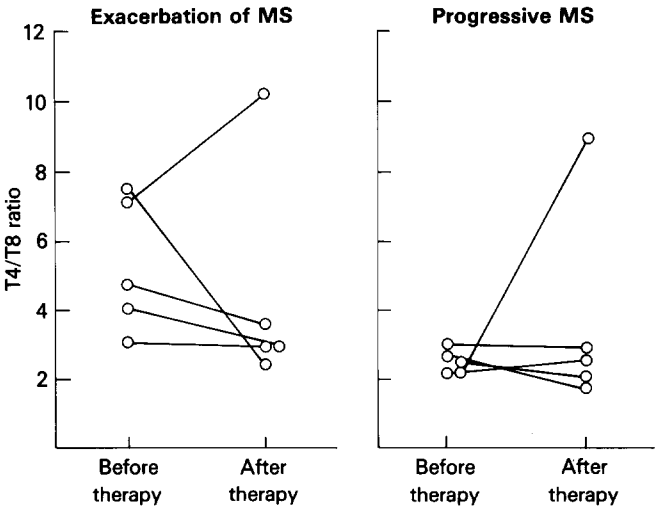


**Fig. 2.** T4/T8 ratios in patients with MS prior to and after 14 days of high dosage corticosteroid therapy (1000 mg prednisolone in 14 days)

## Discussion

Reports on quantitative changes in lymphocyte populations in MS are controversial. Elevated [10, 11] as well as unaltered [9] relative B lymphocyte counts have been observed. In exacerbations of disease slightly decreased [15], decreased [10] and normal relative T lymphocyte counts [17] have been found.

Apart from methodological modifications and differences in the selection of patients this diversity might be attributed to the fact that distinct membrane structures were employed to identify the same lymphocyte population. B lymphocyte counts were determined using the expression of complement receptor or alternatively the expression of surface immunoglobulin; T lymphocyte counts were assessed applying erythrocyte rosetting assays or by determination of the T3 surface antigen. Using a panel of monoclonal antibodies to T lymphocyte antigens Reinherz and Schlossman showed that the expression of these antigens depends on the grade of differentiation and the functional status of corresponding cells [14]. Thus, a decrease in the expression of membrane proteins is not necessarily associated with a numerical decrease, but could reflect disease-associated changes in differentiation or function of a given population. This could explain a differential expression of complement receptors and membrane immunoglobulins in B lymphocytes or the T3 antigen and receptors for sheep erythrocytes in T lymphocytes.

Antel et al. reported in 1982 that even the in vitro incubation of lymphocytes with a monoclonal antibody against surface antigens (T3;T8) induced significant but reversible changes in expression of these antigens without changes in cell number. Autoantibodies directed against lymphocyte antigens in MS patients might cause similar antigenic modulations in vivo [8]. The findings of crossreactivities between $T8^+$ lymphocytes and oligodendrocytes [12] lends support to the hypothesis that autoantibodies influence the expression of lymphocyte membrane antigens independently from etiopathologically more relevant effects on oligodendrocytes. According to this assumption disease-associated changes in the expression of the T8 antigen would indicate antibody-mediated autoimmune reactions in the CNS. A similar mechanism could be responsible for the occurrence of complement-dependent cold-reacting auto-lymphocytotoxins (CoCoCy) in MS patients [8].

In this study we confirmed the findings of Reinherz et al. [15], Bach et al. [3], and Compston [5] regarding the elevation of T4/T8 ratios in patients with acute exacerbations of MS. We observed a less pronounced increase in the average T4/T8 ratio in the patient group in remission. Only 6 out of 22 patients (27%) showed a clearly decreased relative proportion of $T8^+$ lymphocytes and consecutively elevated T4/T8 ratios. Compston reported similar findings in 25% of patients with clinically inactive disease [5].

According to Compston increasing T4/T8 ratios without new clinical signs of disease activity indicate asymptomatic fresh lesions in the CNS. In combination with refined diagnostic procedures (i.e., determination of evoked potentials, assessment of new lesions by nuclear magnetic resonance tomography) longitudinal measurements of lymphocyte populations in individual patients could contribute to the diagnosis of these clinically "silent" exacerbations.

The relative numbers of B lymphocytes in peripheral blood of patients included in this study was similar to the values obtained in normal healthy controls (B lymphocyte count in control group 10%–20% of peripheral blood lymphocytes; data not shown).

It is difficult to draw any conclusion from our data as to whether high dosage corticosteroid therapy has an effect on T4/T8 ratios in MS patients. In 10 patients who responded to therapy and showed symptomatic improvement 3 had a decrease, 2 an increase, and 5 no significant change in the T4/T8 ratio. There seems to be no uniform effect of this therapeutic modality on the expression of lymphocyte surface antigens in MS patients. The therapeutic effect might be limited to the antiinflammatory action of corticosteroids.

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