Circulating CD3+ CD4+ CD8+ T Lymphocytes in Multiple Sclerosis

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Triple-antibody flow cytometry was used to search for distinctive populations of peripheral blood lymphocyte immunophenotypes in multiple sclerosis (MS). Using monoclonal antibodies to the cell surface markers CD3, CD4, and CD8, T cell subsets were quantified on a cohort of 31 MS patients (not treated with corticosteroids for at least 6 months), 30 healthy donors, and 14 patients with other autoimmune diseases (also corticosteroid treatment-free for at least 6 months). Untreated MS patients displayed a significantly greater population of CD3+CD4+CD8+ circulating T cells than healthy donors (P = 0.023). Patients with other autoimmune diseases displayed mean populations of CD3+CD4+CD8+ cells greater than normal donors and less than MS, but not significantly different from either. An additional 45 MS patients who had received corticosteroid therapy within the previous 6 months were phenotyped. Treatment of symptomatic MS with corticosteroids was associated with a smaller population of circulating CD3+CD4+CD8+ cells. Some MS patients have significantly greater numbers of peripheral blood T lymphocytes simultaneously expressing CD3, CD4, and CD8 surface markers than healthy donors and this population of cells may be reduced by corticosteroids treatment. This triple positive phenotype may be a manifestation of a systemic immune abnormality in MS.

KEY WORDS: Multiple sclerosis; T lymphocytes; phenotype; CD3; CD4; CD8; flow cytometry; demyelinating disease; autoimmune diseases; corticosteroids.

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INTRODUCTION

A constituent of the cells mediating central nervous system (CNS) injury in MS are bone marrow-derived circulating lymphocytes that migrate across the blood–brain barrier to participate in the acute inflammatory response (1). The peripheral origin of these cells has prompted examination of circulating lymphocytes in efforts to identify MS specific immune abnormalities. Evidence supporting a systemic immune abnormality in MS includes a decrease in functional suppressor activity of circulating lymphocytes (2–5), abnormalities of effector cell function (6), defects in interferon production by peripheral blood lymphocytes (7), and abnormal numbers of circulating lymphocyte subsets determined by immunophenotyping (2, 8–10). Abnormalities in peripheral blood immunophenotypes have been found by dual-color flow cytometry in chronic progressive MS during periods of activity (2, 4) and in relapsing MS during exacerbations (9), but no single immunophenotype abnormality characteristic of this disease has been identified.

The development of three-color flow cytometry has afforded the ability to delineate subpopulations of leukocytes found in low numbers or not previously resolved by one- or two-antibody techniques. Since more combinations can be evaluated, unique populations specific to a disease process might be identified. Using monoclonal antibodies to CD3, CD4, and CD8 cell surface markers, three-color flow cytometry can resolve the major T-cell subsets CD3+CD4+CD8− and CD3+CD4−CD8+ as well as minor populations such as CD3+CD4+CD8+, CD3+CD4−CD8+. In this study, we compared populations of minor circulating T lymphocytes in MS patients with those of normal donors and patients with other autoimmune diseases.
MATERIALS AND METHODS

Patients. Three-antibody flow cytometry was performed on peripheral venous blood from 76 patients with clinically definite MS (11), 14 patients with other relapsing autoimmune diseases (systemic lupus erythematosus, n = 4; rheumatoid arthritis, n = 7; mixed connective tissue disease, n = 3), and 30 healthy donors. Of the 76 MS patients, 31 had not been treated with corticosteroids in the previous 6 months. The remaining 45 had received adrenocorticotropic hormone (ACTH), methylprednisolone, or prednisone within the previous 6 months. No treatments lasted longer than 19 days. None of the autoimmune patients had received corticosteroid therapy within the previous 6 months.

Of the MS patients studied, 42 (55%) had relapsing neurologic disease characterized by exacerbations followed by complete or partial remissions and 34 (45%) had chronic progressive illness characterized by nonrelapsing progressive neurologic disability. The clinical disability of the MS patients ranged from none to severe (requiring substantial assistance with normal activities of daily living). None of the patients had received cyclophosphamide, azathioprine, or other nonsteroid immunosuppressant.

Cell Phenotyping. Triple-antibody phenotyping was performed in the following manner. One milliliter of heparinized whole venous blood was washed twice with 14 ml of phosphate-buffered saline (PBS), then resuspended, and 50 μl of mouse polyclonal IgG (2 mg/ml) was added to block Fc and nonspecific antibody binding. After 10 min of incubation, aliquots of 50 μl of cell suspensions were added to tubes containing a premixed panel consisting of the fluoresceinated CD3 mAb [leu 4, Bectin Dickinson Immunocytometry (BDIS), San Jose, CA, Systems], the phycoerythrin-conjugated CD4 mAb (leu 3, BDIS), and the biotinylated CD8 mAb (leu 2, BDIS). Cell suspensions were incubated for 15 min, washed in a PBS/0.5% bovine serum albumin/0.1% azide sodium buffer, centrifuged, and resuspended in residual buffer. Cells were then incubated for 15 min with either 20 μl of Duochrome or 10 μl of properly tiered tandem conjugate (Southern Biotech, Birmingham, AL). Erythrocytes were lysed with 2 ml of 0.87% unbuffered ammonium chloride made fresh daily. After centrifugation, the cells were washed twice with 3 ml PBS and fixed in 1% paraformaldehyde.

Sample data acquisition was performed using a FACScan flow cytometer (BDIS). A sample of the patients blood was stained with FITC-CD45 (HRel, BDIS) and PE-CD14 (leu m3, BDIS) as recommended by the National Committee for Clinical Laboratory Standards (12) to define the lymphocyte region CD45+CD14-. The data were analyzed using Lysis software (BDIS). A sample gate that contained all mononuclear cells but excluded granulocytes was used to acquire data. To analyze the data, a gate was selected to include all CD3+ cells. This method, referred to as T-gating (13), includes both large and small T cells but not other lymphocytes or monocytes.

By using the CD3, CD4, and CD8 antibodies simultaneously, the following T-cell phenotypes were identified: (i) CD3+CD4+CD8- (helper/inducer), (ii) CD3+CD4-CD8+ (suppressor/cytotoxic), (iii) CD3+CD4+CD8+, and (iv) CD3+CD4-CD8-. Statistical analysis was performed using RS/1 software on a VAX 3300.

RESULTS

The bivariate distributions for the three antibodies in one of the MS patients (untreated for prior 6 months) is shown in Fig. 1. In Fig. 1A, CD3+ cells are delineated in region 1 (R1). Figure 1B shows the CD3+ cells from R1 as a bivariate plot of CD4 versus CD8 for the same MS patient. Figure 1C shows a plot identically gated to 1B but for a normal donor subject. The CD3+CD4+CD8+ cells are identified in region 2 (R2) in Figs. 1B and C. As can be seen by comparing R2 in Figs. 1B and C, the MS patient displays a larger population of CD3+CD4+CD8+ cells than the normal donor.

Table I shows the mean CD3+ subsets for the 31 MS patients not treated with steroids for the previous 6 months, the 30 healthy donors, and the 14 patients with other autoimmune diseases. The percentage of CD3+CD4+CD8+ lymphocytes was significantly higher in the MS patients than in the healthy donors (P = 0.023, t test unequal variances). The patients with autoimmune disease did not differ significantly in the percentage of CD3+CD4+CD8+ T lymphocytes from either the healthy donors (P = 0.30) or the MS patients (P = 0.23), (t test unequal variances). The mean percent of circulating CD3+CD4+CD8+ cells, when analyzed across all three groups, just failed to achieve significance (ANOVA one way, P = 0.054).

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