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Defective autologous mixed lymphocyte reactivity in multiple sclerosis

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SUMMARY

T cells from patients with multiple sclerosis (MS) and normal controls were assessed for their ability to respond in the autologous mixed lymphocyte reaction (AMLR). Cells from stable MS patients demonstrated a significant defect in their proliferative response to non-T cells in comparison to normal controls. Despite the defective AMLR response, T cells from MS patients reacted as well as T cells from normal controls to allogeneic stimuli. Furthermore, MS non-T-cells were fully capable of stimulating allogeneic MLR responses by normal and MS T cells. Since the $T4^+$ cell is the major subpopulation which proliferates in the AMLR, these studies suggest a functional defect in a subpopulation of $T4^+$ cells in MS patients. Since the AMLR may represent an important mechanism by which immune responses are regulated, a defect in the ability of MS T cells to respond to autologous cells could account for several of the autoimmune features of the disease.

Keywords autologous mixed lymphocyte reaction multiple sclerosis suppressor cells suppressor inducer cells

INTRODUCTION

In the autologous mixed lymphocyte reaction (AMLR), T cells respond to autologous non-T-cells (either B cells and/or macrophages) (Opelz *et al.*, 1975; Kuntz, Innes & Weksler, 1976). T cells activated in the autologous mixed lymphocyte reactions (AMLR) differentiate and proliferate, acquiring helper (Shin, Wang & Choi, 1981; Sakane & Green, 1979), suppressor (James *et al.*, 1981; Smolen *et al.*, 1982), and cytotoxic activities (Tomonari, 1980). Activation of T cells in the AMLR is a consequence of their recognition of products of the major histocompatibility locus (Dr antigens) on non-T cells (Palacios & Moller, 1981) and represent an *in vitro* model for immune regulation.

The majority of T cells which proliferate in AMLR bear the $T4^+$ surface phenotype (Damle & Gupta, 1982; Sakane *et al.*, 1983). During the first 3 to 4 days of an AMLR, T-helper cell activity is generated within the $T4^+$ cell population (Sakane *et al.*, 1983). After 5 to 7 days of culture, suppressor cell activity, within another subset of $T4^+$ cells is observed (Sakane *et al.*, 1983; Kotani, Takada & Ueda, 1984). Since suppressor as well as helper cell function is generated in the AMLR, activation of T cells in the AMLR may represent an important regulatory mechanism by which immune system function is controlled.

Among the many immunological abnormalities which have been described in patients with active multiple sclerosis (MS), is a depression of concanavalin A (Con A) inducible suppressor cells (Arnason & Antel, 1978; Antel, Arnason & Medof, 1979). Several studies have also suggested that the number of suppressor cells, as determined by surface markers on peripheral blood (Reinherz *et*

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al., 1980; Weiner *et al.*, 1984) or cerebrospinal fluid (Coyle *et al.*, 1980; Sandberg-Wollheim, 1983; Panitch & Francis, 1982) lymphocytes, is also decreased during active MS. However, this has not been a consistent finding (Kastrukoff & Paty, 1984; Rice *et al.*, 1984; Hirsch *et al.*, 1985).

Few studies have examined the ability of lymphocytes from MS patients to respond to allogeneic or autologous cells *in vitro*. Depressed responses of MS lymphocytes to allogeneic lymphoid cell lines have been demonstated (Knight *et al.*, 1975). However MS lymphocytes seem to respond well against normal allogeneic lymphocytes (Kallen, Low & Nilsson, 1975; Birnbaum & Kotilnek, 1981; Kam-Hansen, Anderson & Link, 1983). One study has reported that T cells, obtained from active MS patients, show increased reactivity in the AMLR (Birnbaum & Kotilnek, 1981). The increased AMLR response may have been a consequence of an increased capacity of the non-T-cells to stimulate T cell proliferation in the AMLR (Birnbaum & Kotilnek, 1981). However, a recent communication has reported decreased AMLR responses in active chronic progressive MS patients (Hafler *et al.*, 1985).

We report in this study that T cells from stable MS patients demonstrate a depressed AMLR in response to non-T-cells. Furthermore, the defective AMLR response is seen in the presence of normal allogeneic MLR responses. Since the $T4^+$ cell population is the major responding cell in the AMLR, these studies suggest functional abnormalities within the $T4^+$ cell population in MS patients.

MATERIALS AND METHODS

Patients. MS patients, with exacerbating/remitting disease, were seen in the University of Maryland MS Clinic. At the time of study all patients were stable, not having had an attack within the past 60 days, and were not taking any immunomodulating medication. Laboratory and staff personnel served as normal controls.

Preparation of T- and non-T cells. Peripheral blood was collected in preservative free heparin (20 u/ml) and the mononuclear cell (MNC) fraction was obtained by centrifugation on Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, NJ, USA). Cells were then washed two times in phosphate buffered saline (PBS) and resuspended at 3×10^6 cells/ml in RPMI-1640, containing 20% fetal bovine serum (FBS). The adherent cell population was removed by incubating the MNC on FBS coated 100 mm tissue culture plates for 1 h at 37°C. The non-adherent cell population, containing both T and B cells was then removed and resuspended at 3×10^6 cells/ml in PBS containing 20% FBS. These cells were then mixed with 2-aminoethyl-isothiouronium-treated sheep red blood cells (SRBC) (Chandy *et al.*, 1984), centrifuged at 200 ***g*** for 5 min, and placed on ice for 1 h. The SRBC rosette forming cells were then separated from non-T-cells by centrifugation in Ficoll-Paque. T cells in the rosetted population were treated with NH_4Cl_2, to lyse the SRBC, washed, and counted, and resuspended in RPMI containing 5% FBS, 10 mM HEPES buffer, glutamine, and gentamicin (complete RPMI). Non-T-cells were similarly washed, counted, and resuspended in complete RPMI. A portion of the cells in each fraction was routinely analyzed to determine the percentage of T cells (OKT3 positive, Ortho Pharmaceutical Co., Raritan, NJ, USA), and the percentage B cells (Leu-12 positive). At least 5,000 cells in each fraction were analysed using a FACS IV, as previously described (Hirsch *et al.*, 1985). The T-cell enriched fraction contained average of $85{\cdot}6 \pm 2{\cdot}2\%$ $T3^+$ cells. The non-T-cell fraction contained $60{\cdot}9 \pm 15{\cdot}8\%$ Leu 12^+ cells and less than 5% $T3^+$ cells. In some experiments MNC were stained with additional monoclonal antibody reagents (OKT4, OKT8, and OKIa1, Ortho Pharmaceutical Co., Raritan, NJ, USA) to determine the percentage of these cell populations in peripheral blood MNC.

Mixed lymphocyte reactions. Non-T cells were counted and resuspended at 1×10^6 cells/ml in Hank's balanced salt solution (HBSS), containing calcium and magnesium. Cells were treated with 50 μg/ml mitomycin C (Sigma Chemical Co., St Louis, MO, USA) for 45 min. Cells were then washed twice with HBSS and resuspended at 1×10^6 cells/ml in complete RPMI.

Equal volumes (0·5 ml) of mitomycin treated non-T-cells were mixed with T cells at 1×10^6 cells/ml in 12×75 mm plastic tubes. Controls consisted of T cells diluted with an equal volume of complete RPMI. The mixtures were then plated into four or five replicate wells of flat bottom

Table 1. T cell markers on MNC from MS and normal subjects

	Positive cells (%) ± s.d.*			
	T3	T8	T4	Ia
MS (10)	60·6 ± 7·9	24·6 ± 4·4	43·9 ± 6·9	14·5 ± 7·9
Normal (8)	61·1 ± 10·7	25·1 ± 4·9	40·7 ± 6·8	12·1 ± 4·5

* Prior to separation by rosetting, portions of the MNC fraction were washed and treated with the above monoclonal antibodies. The percentage cells, within the lymphocyte peak, binding these antibodies was determined by counting 5,000 cells on a FACS IV. No significant differences were observed between cells obtained from MS and normal controls.

microtitre plates and cultured for 6 days at 37°C. During the last 16 to 18 h of culture, each well received 1 μCi ^{3}H-thymidine (^{3}H-TdR, specific activity 40 Ci/mM, Amersham, Arlington Heights, IL, USA). Cultures were then harvested onto glass fiber filters and prepared for scintillation counting (Hirsch *et al.*, 1981).

RESULTS

AMLRs were established by culturing enriched T cells with mitomycin C treated non-T-cells. These studies demonstrate that lymphocytes from MS patients exhibited a significantly lower ($P < 0{\cdot}05$, Student's t-test) AMLR response in comparison to normal controls (MS = 5,429 ± 2,683 ct/min/10^6 cells; controls = 13,071 ± 2,928 ct/min/10^6 cells). The individual values of the AMLR responses of MS patients and normal controls are shown in Fig. 1. The AMLR values for the combined groups were normally distributed and thus the results could be subjected to rank-sum analysis. This analysis showed that there was a high degree of relationship between group membership and ranking ($r_g = 0{\cdot}62$), and that the median value of the control group was significantly higher that that of the MS patient group ($Z = 2{\cdot}324$, $P = 0{\cdot}01$).

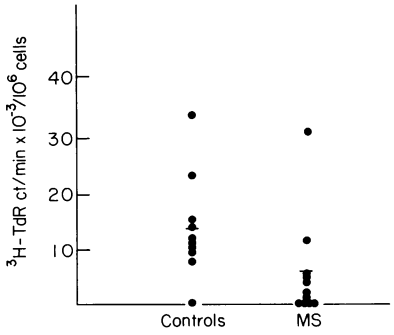

Fig. 1. Individual values of AMLR responses. Enriched T cells were cultured with mitomycin C treated autologous non-T cells (1 : 1 responder to stimulator ratio) for 6 days. The incorporation of ^{3}H-TdR, during the final 16 h of culture, was used to determine lymphoproliferation in the AMLR. The median value of the MS group response is significantly lower than that of the normal control group. The solid bars represent the mean responses.

Table 2. Allogeneic MLR response of normal and MS T cells to normal and MS B cells*

	^{3}H-TdR ct/min/10^6 cells (^{3}H-TdR W ct/min/10^6 cells)					
Stimulator B-cells:	Normal			MS		
	1	2	3	4	5	6
Responder T cells						
Normal						
1	**9,070**	86,170	—	129,500	195,520	—
2	99,570	**9,150**	—	39,680	84,620	—
3	—	—	**23,110**	—	—	96,030
MS						
4	84,920	70,300	—	**4,800**	76,910	—
5	94,170	78,760	—	27,330	**11,760**	—
6	—	—	51,140	—	—	**2,060**

* Enriched T cells were cultured with mitomycin C treated autologous or allogeneic non-T-cells at 2:1 responder to stimulator ratio. After 6 days, cultures were labelled with ^{3}H-TdR for 16 h, harvested, and prepared for scintillation counting. Values in bold represent autologous responses. Data on subjects 1, 2, 4 and 5 were determined in one experiment and that for subjects 3 and 6 in a second experiment.

Since others have demonstrated that T4$^+$ cells are the major cell population which proliferate in AMLR (Sakane *et al.*, 1983), the distribution of T cell subsets in the MNC population was analysed. There were no significant differences between MS patients and normal controls in the percentages of T3, T4, T8 and Ia positive lymphocytes (Table 1). These results suggest that there is a functional defect in the ability of cells from MS patients to respond in the AMLR.

Previous studies suggested that the degree to which MS patients respond in the AMLR is a function of the stimulator cell population (Birnbaum & Kotilnek, 1981). Thus, it was possible that the B cells of MS patients were not sufficiently stimulating their own T cells. Thus, additional experiments were performed in which normal and MS non-T-cells were used to stimulate normal and MS allogeneic T cells. These results (Table 2) demonstrate that both normal and MS T cells were responsive to allogeneic non-T-cells from either normal or MS subjects. When the MS patient T cell response to normal non-T-cells was compared to the normal T cell response to MS non-T-cells, no significant differences were observed (MS T *v* normal non-T, $82{,}038 \pm 8{,}105$; control T *v* MS non-T, $109{,}070 \pm 29{,}019$). Thus, T cells from MS patients responded well in the allogeneic MLR and, non-T-cells from MS patients functioned well as stimulators for both MS and normal T cells.

DISCUSSION

These studies demontrate that T cells from stable MS patients have a significant defect in their ability to proliferate in an AMLR in comparison to cells from normal controls. Although we have only investigated one stimulator to responder cell ratio, a recent report, using a 5:1 ratio, showed that active MS patients also had depressed AMLR responses (Hafler *et al.*, 1985). These same investigators, however, reported that the AMLR was depressed, though not significantly, in inactive MS patients. The fact that our stable patients had significantly depressed AMLR reactivity whereas Hafler's patients did not could be due to the fact that our patients had not had an attack for 2 months whereas Hafler's patients had not had an attack for 9 months. Thus, our 'stable' patients

may more closely approximate Hafler's active patients. A previous study showed that active MS patients had increased AMLR responsiveness (Birnbaum & Kotilnek, 1981), and that the increased responses were a function of a more active stimulator B cell population. We have also addressed the possibility that the B cells from MS patients may be aberrant in their ability to stimulate an allogeneic MLR response. Our results show that MS T cells are responsive to allogeneic stimuli and that MS non-T cells are capable of stimulating both normal and MS T cells to a similar extent.

A decreased AMLR response has been demonstrated in other diseases of suspected autoimmune etiology (Sakane *et al.*, 1983; Chandy *et al.*, 1984). A depressed AMLR response in these diseases may have major significance to the pathogenesis or progression of disease. First, in the type of AMLR studied here, in which non-T-cells are used as stimulator cells, the majority of the responding cells have the $T4^+$ phenotype (Damle & Gupta, 1982). Furthermore, different subsets of $T4^+$ cells which proliferate in the AMLR develop both helper/inducer and suppressor cell activity. Since MS patients have depressed suppressor T cells (T_s) function, especially during active disease (Antel *et al.*, 1979), it is possible that the decreased proliferative activity seen in the AMLR represents the inability of the MS $T4^+$ cell to proliferate and generate suppressor cell function. Since the previously reported decreases in $T8^+$ cells in active MS patients (Reinherz *et al.*, 1980; Weiner *et al.*, 1984) have not been confirmed in several laboratories (Rice *et al.*, 1984; Kastrukoff & Paty, 1984; Hirsch *et al.*, 1985), it is possible that the depression of Con A induced T_s activity in active MS is associated with a population of cells other than $T8^+$ cell. Interestingly, in systemic lupus erythematosus, decreases in the AMLR induced proliferation of $T4^+$ cells are independent of disease activity and have been correlated with a decrease in the development of $T4^+$ suppressor cell but not helper cell activity (Sakane *et al.*, 1983). In addition, other investigators have reported increased helper cell activity (Levitt, Griffin & Egan, 1980) and IgG production (Goust, Hougan & Arnaud, 1982) by MS lymphocytes *in vitro*. These studies indicate that there may be additional abnormalities within the $T4^+$ cell population in MS patients.

Patients with MS have many abnormalities of immune function which suggest that the progression of disease has an autoimmune component. Thus, it is possible that the depressed AMLR responses represent the inability of cells from these patients to down regulate immune responses *in vivo*. There is some evidence, from the evaluation of cellular immune responses to influenza vaccine, that the immune system of MS patients is defective in its ability to regulate responses. After vaccination with inactivated influenza virus, the proliferative responses of MS patients' cells to purified virus remained detectable for a longer period than that of controls (Brooks *et al.*, 1979). Although it is possible that the reduced AMLR reactivity in MS patients represents an inherent immunologic defect of an autoimmune disease, it is also possible that autoreactive T cells in MS migrate to the central nervous system. In fact, cells from the cerebrospinal fluid have been recently reported to have increased AMLR reactivity (Birnbaum *et al.*, 1984).

Cytotoxic cells with natural killer (NK) cell characteristics are also induced by the AMLR (Tomonari, 1980). Although we have not examined AMLR induced NK activity in this study, endogenous NK activity is depressed in MS (Hirsch & Johnson, 1985). Since NK cells may have important immunosuppressive (Abruzzo & Rowley, 1983; Brieva, Targan & Stevens, 1984) as well as antiviral activities, further study of the defective AMLR response in MS may suggest additional defects other than in the $T4^+$ cell population, of immune regulation.

We have not yet had the opportunity to study active MS patients. However, since conflicting results have been reported with respect to AMLR responses in this group of patients further studies are warranted. In addition, studies on the activation of suppressor and suppressor-inducer $T4^+$ cells in MS patients are necessary. Such studies would directly determine if the low level of proliferation in the AMLR is indicative of depressed $T4^+$ cell suppressor cell function in MS.

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REFERENCES

ABRUZZO, L.V. & ROWLEY, D.A. (1983) Homeostasis of the antibody response: Immunoregulation by NK cells. *Science*, **222,** 581.

ANTEL, J.P., ARNASON, B.G.W. & MEDOF, M.E. (1979) Suppressor cell function in multiple sclerosis—correlation with clinical disease activity. *Ann. Neurol.* **5,** 338.

ARNASON, B.G.W. & ANTEL, J. (1978) Suppressor cell function in multiple sclerosis. *Ann. Immunol. (Paris)* **129C,** 159.

BIRNBAUM, G. & KOTILINEK, L. (1981) Autologous lymphocyte proliferation in multiple sclerosis and the effect of intravenous ACTH. *Ann. Neurol.* **9,** 439.

BIRNBAUM, G., KOTILINEK, L., SCHWARTZ, M. & STERNAD, M. (1984) Spinal fluid lymphocyte responsiveness to autologous and allogeneic cells in multiple sclerosis and control individuals. *J. clin. Invest.* **74,** 1307.

BRIEVA, J.A., TARGAN, S. & STEVENS, R.H. (1984) NK and T-cell subsets regulate antibody production by human *in vivo* antigen-induced lymphoblastoid B-cells. *J. Immunol.* **132,** 611.

BROOKS, B.R., HIRSCH, R.L., JUBELT, B., COLYE, P.K., SEAY, A.R., JOHNSON, R.T., O'DONNELL, P., KARASANYI, N., MADDEN, C.L. & SEVER, J.L. (1980) Effect of steroids on appearance of antigen reactive peripheral blood mononuclear cells following influenza immunization in multiple sclerosis patients. *Neurology*, **30,** 448 (abstract).

CHANDY, K.G., CHARLES, A.M., KERSHNAR, A., BUCKINGHAM, B., WALDECK, N. & GUPTA, S. (1984) Autologous mixed lymphocyte reaction in man. X. Cellular and molecular basis of deficient autologous mixed lymphocyte response in insulin-dependent diabetes mellitus. *J. clin. Immunol.* **4,** 424.

COYLE, P.K., BROOKS, B.R., HIRSCH, R.L., COHEN, S.R., O'DONNELL, P., JOHNSON, R.T. & WOLINSKY, J.S. (1980) Cerebrospinal fluid lymphocyte populations and immune complexes in active multiple sclerosis. *Lancet*, **ii,** 229.

DAMLE, N.K. & GUPTA, S. (1982) Autologous mixed lymphocyte reaction in man. V. Functionally and phenotypically distinct human T-cell subpopulations respond to non-T and activated T-cells in AMLR. *Scand. J. Immunol.* **16,** 59.

GOUST, J.M., HOGAN, E.L. & ARNAUD, P. (1982) Abnormal regulation of IgG production in multiple sclerosis. *Neurology*, **32,** 228.

HAFLER, D.A., BUCHSBAUM, M. & WEINER, H.L. (1985) Decreased autologous mixed lymphocyte reaction in multiple sclerosis. *J. Neuroimmunol.* **9,** 339.

HIRSCH, R.L., MOKHTARIAN, F., GRIFFIN, D.E., BROOKS, B.R., HESS, J. & JOHNSON, R.T. (1981) Measles virus vaccination of seropositive individuals suppresses lymphocyte chemotactic factor production and proliferation. *Clin. Immunol. Immunopathol.* **21,** 341.

HIRSCH, R.L. & JOHNSON, K.P. (1985) The effect of recombinant α_2-interferon on defective natural killer cell activity in multiple sclerosis. *Neurology*, **35,** 597.

HIRSCH, R.L., ORDONEZ, J., PANITCH, H.S. & JOHNSON, K.P. (1985) T8 antigen density on peripheral blood and lymphocytes remains unchanged during exacerbations of multiple sclerosis. *J. Neuroimmunol.* **9,** 391.

JAMES, S.P., YENOKIDA, G.G., GRAEFF, A.S., ELSON, C.O. & STROBER, W. (1981) Immunoregulatory function of T-cells activated in the autologous mixed lymphocyte reaction. *J. Immunol.* **127,** 2605.

KALLEN, B. & NILSSON, O. (1971) Mixed leucocyte reaction in multiple sclerosis. *Nature (New Biol.)*, **229,** 91.

KALLEN, B., LOW, B. & NILSSON, O. (1975) Mixed leukocyte reaction and HLA specificity at multiple sclerosis. *Acta. Neurol. Skandinav.* **51,** 184.

KAM-HANSEN, S., ANDERSSON, R. & LINK, H. (1983) Cerebrospinal fluid lymphocytes from patients with multiple sclerosis and aseptic meningoencephalitis respond in mixed lymphocyte culture. *J. Neuroimmunol.* **5,** 67.

KASTRUKOFF, L.F. & PATY, D.W. (1984) A serial study of peripheral blood T lymphocyte subsets in relapsing-remitting multiple sclerosis. *Ann. Neurol.* **15,** 250.

KNIGHT, H., TAKADA, S. & UEDA, Y. (1984) Activation of immunoregulatory circuits along OKT4$^+$ cells by autologous mixed lymphocyte reactions. *Clin. exp. Immunol.* **56,** 390.

KUNTZ, M.M., INNES, J.B. & WEKSLER, M.E. (1976) Lymphocyte transformation induced by autologous cells. IV. Human T-lymphocyte proliferation induced by autologous or allogeneic non-T cells. *J. exp. Med.* **143,** 1042.

LEVITT, D., GRIFFIN, N.B. & EGAN, M.L. (1980) Mitogen-induced plasma cell differentiation in patients with multiple sclerosis. *J. Immunol.* **124,** 2117.

OPELZ, G., KIUCHI, M., TAKASUGI, M. & TERASAKI, P.I. (1975) Autologous stimulators of human lymphocyte subpopulations. *J. exp. Med.* **142,** 1327.

PLACIOS, R. & MOLLER, G. (1981) HLA-DR antigens render resting T-cells sensitive to interleukin 2 and induce production of the growth factor in the autologous mixed lymphocyte reaction. *Cell. Immunol.* **63,** 143.

PANITCH, H.S. & FRANCIS, G.S. (1982) T-lymphocyte subsets in cerebrospinal fluid in multiple sclerosis. *N. Engl. J. Med.* **307,** 560.

REINHERZ, E.L., WEINER, H.L., HAUSER, S.L., COHEN, J.A., DISTASO, J.A. & SCHLOSSMANN, S.F. (1980) Loss of suppressor cells in active multiple sclerosis—Analysis with monoclonal antibodies. *N. Engl. J. Med.* **303,** 125.

RICE, G.P.A., FINNEY, D., BRAHENY, S.L., KNOBLER, R.L., SIPE, J.C. & OLDSTONE, M.B.A. (1984) Disease activity markers in multiple sclerosis—another look at suppressor cells defined by monoclonal antibodies OKT4, OKT3, and OKT5. *J. Neuroimmunol.* **6,** 75.

SAKANE, T. & GREEN, I. (1979) Specificy and suppressor function of human T-cells responsive to autologous non-T cells. *J. Immunol.* **123,** 584.

SAKANE, T., KOTANI, H., TAKADA, S., MURAKAWA, Y.

& UEDA, Y. (1983) A defect in the suppressor circuits among OKT4+ cell populations in patients with systemic lupus erythematosus occurs independently of a defect in the OKT8+ suppressor T-cell function. *J. Immunol.* **131,** 753.

SAKANE, T., STEINBERG, A.D. & GREEN, I. (1978) Failure of autologous mixed lymphocyte reactions between T and non-T cells in patients with systemic lupus erythematosus. *Proc. natn. Acad. Sci. USA.* **75,** 3364.

SANDBERG-WOLLHEIM, M. (1983) Lymphocyte populations in the cerebrospinal fluid and peripheral blood of patients with multiple sclerosis and optic neuritis. *Scand. J. Immunol.* **17,** 575.

SHIN, H.-S., WANG, C.-Y. & CHOI, Y.S. (1981) Activation of autologous reactive helper T lymphocytes for differentiaton of human B lymphocytes. *J. Immunol.* **126,** 2485.

SMOLEN, J.S., CNUSED, T.M., NOVOTNY, E.A. & STEINBERG, A.D. (1982) The human autologous mixed lymphocyte reaction. III. Immunocircuits. *J. Immunol.* **129,** 1050.

TAKADA, S., UEDA, Y., SUZUKI, N., MURAKAWA, Y., HOSHINO, T., GREEN, I., STEINBERG, A.D., HOROWITZ, D.A. & SAKANE, T. (1985) Abnormalities in autologous mixed lymphocyte reaction-activated immunologic processes in systemic lupus erythematosus and their possible correction by interleukin 2. *Eur. J. Immunol.* **15,** 262.

THOMAS, Y., ROGOZINSKI, L., IRIGOYN, O.H., SHEN, H.H., TALLE, M.A., GOLDSTEIN, G. & CHESS, L. (1982) Functional analysis of human T-cell subsets defined by monoclonal antibodies. V. Suppressor cells within the activated OKT4+ population belong to a distinct subset. *J. Immunol.* **128,** 1386.

TOMONARI, K. (1980) Cytotoxic T cells generated in the autologous mixed lymphocyte reaction. I. Primary autologous mixed lymphocyte reaction. *J. Immunol.* **124,** 1111.

WEINER, H.L., HAFLER, D.A., FALLIS, R.J., JOHNSON, D., AULT, K.A. & HAUSER, S.L. (1984) Altered blood T-cell subsets in patients with multiple sclerosis. *J. Neuroimmunol.* **6,** 115.