

Defective Production of Anti-Inflammatory Cytokine, TGF- β by T Cell Lines of Patients with Active Multiple Sclerosis¹

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Activated T lymphocytes play an important role in the pathogenesis of multiple sclerosis (MS). These T cells secrete both pro- and anti-inflammatory cytokines. We have studied the production of these two kinds of cytokines by PBL of patients with MS and compared it with normal controls and other autoimmune diseases (OAD). PBL of 29 patients with MS, 14 patients with OAD, and 14 healthy normal controls were cultured for 5 wk. PBL of MS patients produced more pro-inflammatory cytokines, IL-2, IFN- γ and TNF/lymphotoxin, and less anti-inflammatory cytokine, TGF- β , during wk 2 to 4 in culture than PBL of normal controls. PBL of MS patients also produced more IL-2 and TNF/lymphotoxin than PBL of OAD patients. Decreased TGF- β production by lymphocytes of patients with MS correlated directly with disease activity. MS patients with active disease produced less TGF- β than MS patients with stable disease. The cells producing TGF- β were primarily CD8⁺ T cells and CD45RA⁺ T cells. These findings emphasize the complexity of immune response in MS patients and suggest that the increased production of pro-inflammatory cytokines by lymphocytes of patients with MS, combined with the decreased production of TGF- β (anti-inflammatory cytokine), may play an important role in the mechanisms and manifestations of MS. *Journal of Immunology*, 1994, 152: 6003.

Multiple sclerosis (MS)³ is characterized by an inflammatory response within the central nervous system (CNS), and immune abnormalities in the peripheral blood, cerebrospinal fluid, and brain. In the blood, there is activation of T cells (1) and increased serum levels of IL-2 and IL-2R (2). IFN- γ (3), IL-1, IL-2, and IL-2R (4) and TNF (5) have been detected in cerebrospinal fluid of patients with MS. IFN- γ and TNF/LT production are both reported to play active roles in the pathology of MS. Although treatment with a preparation of IFNs potentiated the exacerbations of exacerbating relapsing MS (6), using an IFN inducer in the treatment of

chronic progressive MS appeared to be different and somewhat beneficial (7, 8). Increased TNF levels were observed in the cerebrospinal fluid and sera of patients with acute relapsing MS and in patients with Landry-Guillain-Barré syndrome (9, 10). TNF was present in the cerebrospinal fluid of patients with MS and other inflammatory neurologic diseases (5, 11) and its level correlated with severity and progression of MS (12). Immunohistochemical studies of MS brain have also indicated strong intrathecal immune activation and cytokine production (13–15). In brain lesions in MS, TNF was associated with astrocytes, macrophages and endothelial cells (16, 17).

Culture of PBL of normal human donors in the presence of IL-2 results in the generation of long term proliferating T cells that produce a variety of cytokines in vitro (18). Previous studies on the in vitro production of IFN- γ by lymphocytes of MS patients have yielded conflicting results, ranging from deficient (19, 20) to normal (21, 22) and elevated production (3), probably caused by different durations and conditions of culture. Some investigators have found low production of IFN- γ in short term cultures of PBL with PHA (23) and IL-2 (24), leading to the conclusion that T cells from MS patients were defective in production of IFN- γ .

Received for publication December 6, 1993. Accepted for publication March 22, 1994.

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¹ This work was supported, in part, by Grant R29NS24688 from The National Institutes of Health and by a grant from the Maimonides Research and Development Foundation.

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³ Abbreviations used in this paper: MS, multiple sclerosis; CNS, central nervous system; OAD, other autoimmune diseases; M ϕ , macrophage; CP, chronic progressive; ER, exacerbating relapsing; LT, lymphotoxin; EAE, experimental autoimmune encephalomyelitis; MBP, myelin basic protein; CM, culture medium.

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Measurement of anti-inflammatory cytokines

IL-4. IL-4 was measured by direct immunoassay. The sensitivity of this assay is 3.0 pg/ml. All reagents were purchased from R & D Systems. Murine mAb against human IL-4 was first coated on a polystyrene microtiter plate. Standards with known amounts of IL-4 and samples were then added to the coated wells and incubated. Horseradish peroxidase-conjugated polyclonal Ab against IL-4 and tetramethylbenzidine substrate were used to detect the IL-4 bound to the solid phase.

TGF-β. Active form of TGF-β was measured in the cell supernatants, without previous acidification. TGF-β was measured by its ability to inhibit the growth of mink lung epithelial cell line, Mv 1 Lu (ATCC). Mv 1 Lu cells were plated in a 96-well plate at 3500 per 100 μl of DMEM containing 10% FCS. Serial dilutions of each sample were added to triplicate wells 5 h after plating of cells, and incubated at 37°C for 72 h. [³H]TdR was then added and the cells were incubated for an additional 24 h.

Inhibition of growth was expressed as the percent decrease of [³H]TdR incorporation by cells receiving the sample when compared with incorporation by cells alone. Human rTGF-β1 standards (R & D Systems) (0.01 to 5 ng/ml) were run in each assay which produced 5 to 80% inhibition of Mv 1 Lu cell growth. The specificity of this bioassay was demonstrated by inhibition of the assay by using polyclonal rabbit anti TGF-β1 and 2 Ab (R & D Systems). Culture supernatants were treated with 0.5 μg/ml of anti-TGF-β Ab at a final dilution of 1/8 for 1 h at room temperature. This concentration of Ab completely neutralized 0.5 ng/ml of rTGF-β1.

Separation of T cell subsets

Separation of T cell subsets on the basis of CD4, CD8, CD45RA, or CD29 expression was performed after first removing macrophage (Mφ)/monocytes and Ig positive cells (B cells) from the cell suspension by panning technique (28). Cell cultures were depleted of Mφ/monocytes by incubation on FCS-coated plates, and B cells were removed by incubation on petri dishes precoated with purified goat anti-human IgG Ab (Southern Biotechnology Associates, Inc., Birmingham, AL). The Ig-negative cell population was then incubated separately with each mouse anti-human CD4, CD8, CD45RA, and CD29 mAb (Coulter Immunology, Hialeah, FL) for 30 min on ice. The Ab-T cell complexes were then washed free of Ab and added to prewashed magnetic beads with covalently attached goat anti-mouse IgG (Advanced Magnetics). The number of beads to cell ratio was 50:1. The cells were incubated with the beads in RPMI 1640 supplemented with 10% FCS for 30 min on ice with gentle shaking every 10 min. After this incubation, the cell-mAb-bead complexes were separated from unlabeled cells in a strong magnetic field using BioMag Separators (Advanced Magnetics) for 5 min. This procedure was repeated once to ensure removal of all mAb-labeled cells. T cells in CD8-depleted population were >95% CD4⁺, and CD4-depleted population contained >90% CD8⁺ T cells. CD45RA depleted T cells were >96% CD29⁺, and CD29 depleted T cells were >93% CD45RA⁺, as demonstrated by indirect FACS analysis. The negatively selected population of T cell subsets was cultured in CM for 4 days and supernatants were harvested as above.

Statistical analysis

The significance of the results was determined by Student's *t*-test and was confirmed by nonparametric Wilcoxon signed rank test. Statistical analyses were performed with Apple Macintosh plus/Statview 512 software. All probability values were two-tailed.

Results

Production of pro-inflammatory cytokines

IL-2 production by lymphocytes of MS and OAD patients was low and similar to that of normal controls at the end of 1 wk of culture (Fig. 1A). IL-2 production by lymphocytes of MS patients then steadily increased during the 5 wk tested, was significantly higher than IL-2 production by lymphocytes of normal controls during the second through the fifth week of culture (*p* < 0.05, *p* < 0.03, *p* =

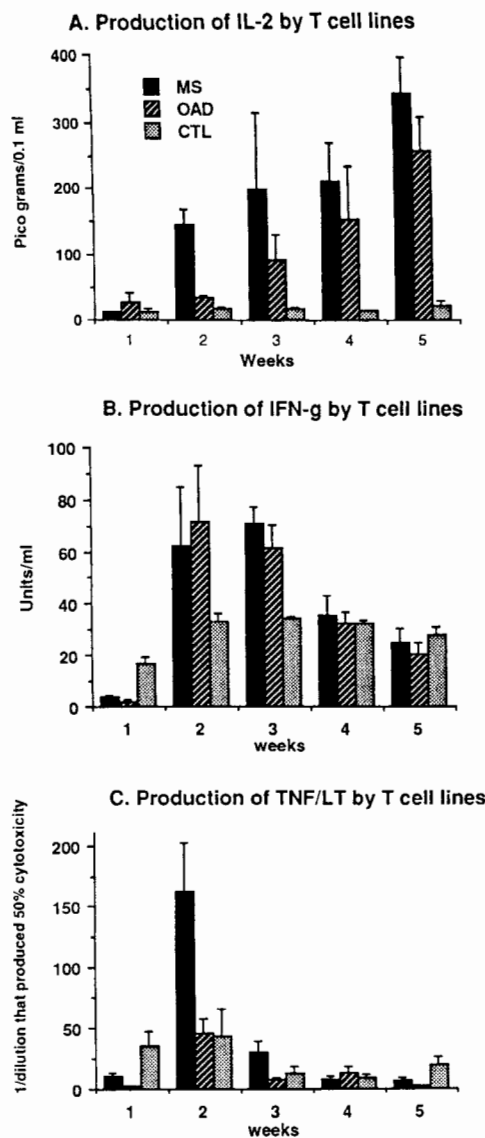


FIGURE 1. Culture supernatants of IL-2-activated T cell lines from patients with MS, OAD, and normal controls (CTL) were harvested at 5 weekly intervals and assayed for pro-inflammatory cytokines. Values represent the mean ± SD of 29 MS patients, 14 OAD patients, and 14 normal controls. A. Production of IL-2 was measured by ELISA in duplicate wells for each sample. Results are expressed as pg/0.1 ml, as determined from standard curve. Before IL-2 assay, T cell lines were washed twice in CM without IL-2. The T cells were then incubated in IL-2-free medium for 48 h and supernatants collected for IL-2 determination. *p* < 0.05, *p* < 0.03, *p* < 0.03, and *p* < 0.002, MS vs CTL for wk 2 through 5; *p* < 0.03 and *p* < 0.05, MS vs OAD for wk 2 and wk 3. *p* < 0.05, *p* < 0.03, and *p* < 0.001, OAD vs CTL for wk 3 through wk 5. B. Production of IFN-γ was measured by RIA in duplicate wells for each sample. Results are expressed as U/ml, as determined from standard curve. *p* < 0.03, MS and OAD vs CTL for wk 1; *p* < 0.02, MS and OAD vs CTL for both wks 2 and 3. C. Production of TNF/LT was measured by bioassay in serial dilutions of each sample. The results are expressed as 1/dilution which produced 50% cytotoxicity on WEHI 164 cell lines. *p* = 0.05, MS vs CTL and *p* < 0.02, OAD vs CTL for wk 1. *p* < 0.01, MS vs CTL and OAD for wk 2.

0.03 and $p < 0.002$), and also significantly higher than that of OAD patients during the second and third weeks of culture ($p < 0.03$ and $p < 0.05$). IL-2 production by lymphocytes of OAD patients was significantly higher than IL-2 production by lymphocytes of normal controls during the third through the fifth week of culture ($p < 0.05$, $p < 0.03$, $p < 0.001$) (Fig. 1A). IL-2 production by lymphocytes of normal controls remained at constant low levels during all 5 wk tested.

IFN- γ production after 1 wk of culture of lymphocytes from MS and OAD patients was significantly lower than from normal controls ($p < 0.03$ for both MS and OAD) (Fig. 1B). IFN- γ production by lymphocytes of MS and OAD patients increased sharply and reached its peak at the end of wk 2 and 3 for MS and wk 2 for OAD patients, and then declined gradually during the fourth and fifth weeks. In cultures of lymphocytes from normal controls, IFN- γ production also increased, but remained significantly lower than those of MS and OAD patients ($p < 0.02$ and $p < 0.02$ respectively) during the second and third weeks of culture (Fig. 1B). In the fourth and fifth weeks, production of IFN- γ by lymphocytes of MS and OAD patients and normal controls were not significantly different.

TNF/LT production, like IFN- γ production, after 1 wk of culture of lymphocytes from MS and OAD patients was lower than from normal controls ($p = 0.05$ for MS and $p < 0.02$ for OAD) (Fig. 1C). Unlike IFN- γ , TNF/LT production by lymphocytes of OAD and normal controls only marginally increased at the end of the second week. TNF/LT production in cultures of lymphocytes of MS patients was significantly higher than those of OAD and normal controls only at the end of the second week of culture ($p < 0.01$) (Fig. 1C). TNF/LT production by lymphocytes of MS patients declined sharply during wk 3 to 5. Likewise, the lymphocytes of OAD patients and normal controls did not produce significant amounts of TNF/LT during wk 3 to 5 in culture (Fig. 1C). TNF/LT bioactivity was found to be caused entirely by the production of LT and not TNF- α : the positive samples were all retested, in separate experiments by immunoassays for LT and for TNF- α , and they were found to be positive for LT but not for TNF- α .

Production of anti-inflammatory cytokines

IL-4. No significant amount of IL-4 was detected in any of the lymphocyte cultures of MS, OAD, and normal controls, as tested by ELISA and compared with the standard curve prepared from IL-4 standards (data not shown).

TGF- β . TGF- β production after 1 wk was low in lymphocyte cultures of MS and OAD patients and normal controls. Production of TGF- β after 2 wk in cultures of lymphocytes of normal controls rose to significantly higher levels than those of MS ($p < 0.05$) and OAD patients ($p < 0.05$) (Fig. 2). Production of TGF- β in cultures of normal controls was still higher, although not significantly, than those of MS in the third week of culture. In the

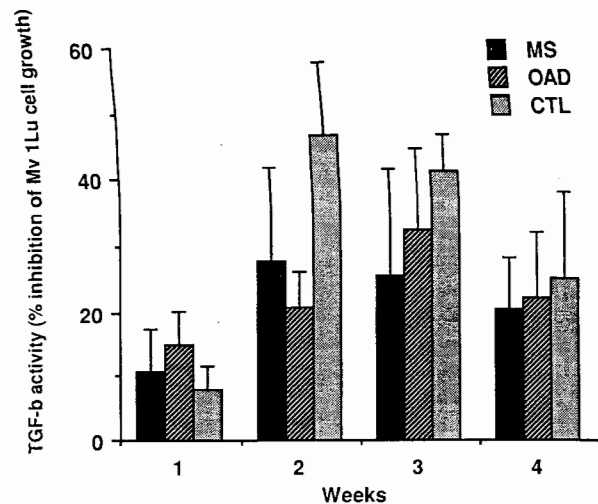


FIGURE 2. Production of active form of TGF- β by IL-2-activated T cell lines from 14 patients with MS, 4 with OAD, and 7 normal controls (CTL) at 4 weekly intervals. Values represent the mean \pm SD of the percentage of growth inhibition of Mv 1 Lu cell line in triplicate wells for each sample. $p < 0.05$, CTL vs MS and OAD for wk 2.

fourth week of culture, production of TGF- β in all three groups was comparable and started to decline. The specificity of this bioassay was tested by neutralization with the specific Ab. Ab to TGF- β 1 and 2 neutralized this bioactivity when tested on four different TGF- β positive samples (data not shown).

Cytokine production at different stages of disease in MS patients

Initially, 15 MS and 10 OAD patients and 7 normal controls were tested for the production of pro-inflammatory cytokines. No significant differences were noted in the generally high level of pro-inflammatory cytokines produced by lymphocytes of MS patients at different stages of disease. However, when the production of anti-inflammatory cytokine TGF- β was measured in another 14 MS patients, 4 OAD patients, and 7 normal controls, significant differences in the level of TGF- β production was observed among MS patients at different stages of disease. The average TGF- β production (as indicated by percentage of inhibition of indicator cell growth) by lymphocytes of 7 MS patients who had active disease (11.2%) was significantly lower ($p < 0.002$) than by lymphocytes of 7 MS patients with stable disease (33.4%) and by lymphocytes of 7 normal controls (44.5%) ($p = 0.002$) (Fig. 3). The average TGF- β production by lymphocytes of 4 OAD patients was 20.7% and statistically was not significantly different from the average production by lymphocytes of MS patients with active or stable disease (Fig. 3). One MS patient, who had been in remission for over 1 yr at the time of blood sampling, had an inhibition rate of 48.5%.

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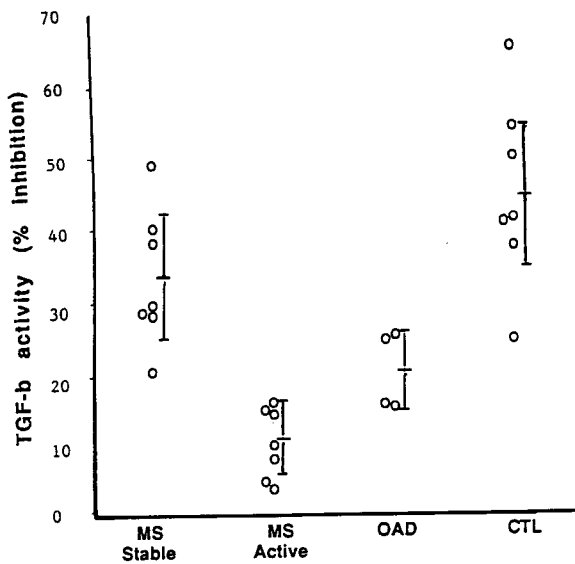


FIGURE 3. Production of active form of TGF- β by IL-2 activated T cell lines from seven patients with stable and seven patients with active MS, four with OAD, and seven normal controls (CTL). Supernatants were harvested on day 14 after culture and TGF- β was assayed, as described in *Materials and Methods*. Patients with stable MS had no apparent worsening of disease since their last 3-mo visit. Patients with active MS had progression of disease since the last 3-mo visit. $p < 0.002$, active MS < stable MS, and $p < 0.002$, active MS < CTL.

TGF- β production by subsets of T cells

To determine the subsets of T cells producing TGF- β , T cell lines from three MS patients and one normal control were separated and purified after the second week in culture. The resulting T cell subsets, always in the same number of T cells/ml, were cultured and the supernatants were analyzed for TGF- β production. Supernatants collected from unseparated T cell cultures of two MS patients with active disease (patients 1 and 2) and one with stable disease (patient 3), produced 17.0%, 3.5%, and 20.4% inhibition of growth of Mv1 Lu cell line, respectively (Fig. 4), whereas supernatant from T cells of one normal control showed 24.4% inhibition. Supernatants from CD8⁺ T cell depleted cultures of the three MS patients and one normal control produced comparable TGF- β activity of 7%, 6.9%, 8.6%, and 7.2% inhibition, respectively, which was much lower than supernatants from their corresponding unseparated T cells. Supernatants from CD4⁺ T cell depleted cultures showed TGF- β activity of 39%, 32.4%, 29.7%, and 35.6% inhibition for patients 1, 2, 3, and normal control, respectively. The level of TGF- β production was no longer different in the separated T cell subsets. CD4⁺ T cell-depleted cultures produced a much higher level of TGF- β than CD8⁺ T cell-depleted cultures, indicating that the T cells producing TGF- β were therefore primarily CD8 and not CD4 T cells (Fig. 4).

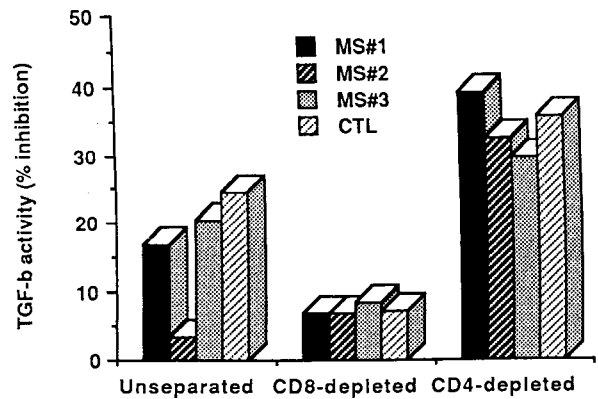


FIGURE 4. Production of active form of TGF- β by unseparated and CD4⁺- and CD8⁺-depleted subsets of T lymphocytes from three MS patients and one normal control (CTL). PBL were first cultured in IL-2 medium for 14 days, at which time T cell subsets were separated by magnetic beads and cultured in IL-2 medium for another 4 days. The supernatants were harvested for measurement of TGF- β activity by using Mv 1 Lu cell growth inhibition assay. Each set of bars represents the values for unseparated and subset-depleted T cells from each of three MS patients (patients 1, 2, and 3) and one CTL.

TGF- β activity in unseparated T cells of the two MS patients with active disease (patients 1 and 2) was lower than that of a patient with stable disease (patient 3) and of a normal control (Fig. 4, left column). However, when the concentration of CD8⁺ T cells was increased by depletion of CD4⁺ T cells from each culture, but maintaining the same concentration of total T cells, TGF- β activity of both patients with active disease rose to a greater degree than that of the MS patient with stable disease and the normal control (Fig. 4, right column).

Separation of T cells of one MS patient and one normal control into CD29⁺(CD45RA⁻) and CD45RA⁺ subsets indicated that supernatants of CD29⁺ T cell-depleted cultures produced 33% and 29% inhibition, whereas CD45RA⁺ T cell-depleted cultures produced 1% and 7.8% inhibition of TGF- β activity (Fig. 5). The T cells producing TGF- β were therefore primarily CD45RA⁺ T cells.

When the supernatants from the above T cell subsets were also tested for the production of IFN- γ , no significant differences in the levels of this cytokine were noted (data not shown).

Discussion

MS is believed to be a T cell-mediated autoimmune demyelinating disease of the CNS (29). The presence of activated T cells in MS PBL and cerebrospinal fluid, as indicated by the expression of HLA-DR Ag, and of IL-2R-bearing T cells in PBL, cerebrospinal fluid, and brain tissue (2, 15, 30), suggests that the disease may be initiated by autoreactive T cells. We have previously shown that the precursor frequency of

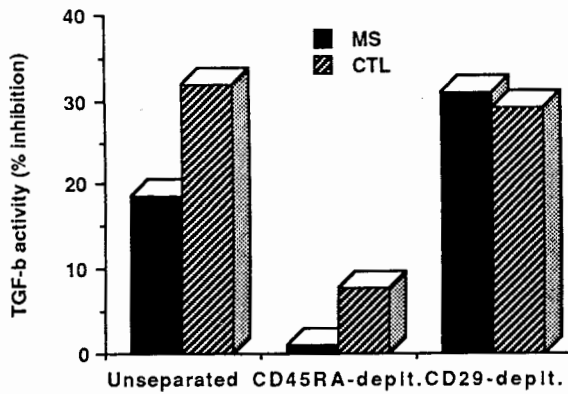


FIGURE 5. Production of active form of TGF- β by unseparated and CD45RA⁺- and CD29⁺-depleted subsets of T lymphocytes from one MS patient and one normal control (CTL). See legend of Figure 4 for culture conditions and assay. Each set of bars represents the values for unseparated and subset-depleted T cells from one MS patient and one CTL.

IL-2-activated T cells is much higher in the PBL of MS patients than of normal controls (31).

In the present study, T cells of MS and OAD patients produced more pro-inflammatory cytokine IL-2, IFN- γ and TNF/LT than those of normal controls, and T cells of MS patients produced more IL-2 during the second through the fifth weeks of culture than the T cells of OAD patients or normal controls. It appears that T cells of MS patients are more activated and produce more IL-2 than T cells of OAD patients. Other studies have found increased levels of IL-2 and IL-2R in the sera (2) and cerebrospinal fluid (4) of MS patients. These findings support the presence of a systemic T cell activation (32). It has also been suggested (33) that an excess of IL-2 may trigger autoreactive effector cells and break autotolerance.

Although during the first week of culture the T cells of MS and OAD patients produced less IFN- γ than those of normal controls, during the next 4 wk they produced significantly more. In other studies, short term cultures of lymphocytes of MS patients with PHA or IL-2 induced lower than normal production of IFN- γ in vitro (23, 24). Our findings confirmed the previous reports of low IFN- γ production during the first week of culture. This may be caused by refractoriness or feed back inhibition induced by IL-2 activation of these already activated cells (27, 31). This low IFN- γ production, however, did not last longer than the first week of culture, and by the end of the second week a significantly increased production was observed by T cells of MS and OAD patients compared with normal controls. IFN- γ is known to induce expression of class II MHC Ag (30). Production of IFN- γ by PBL may therefore be followed by increased Ag presentation and immunologic activity in the CNS (15) and is likely to induce tissue damage and demyelination, as observed in an experimental autoimmune encephalomyelitis (EAE) model (34). A significant increase in the exacerbation rate was reported

after systemic administration of IFNs to MS patients (6). Increased production of both IFN- γ and TNF was also found preceding onset or exacerbation of clinical symptoms in MS patients (35). Increased levels of TNF have been found in the sera of MS patients (10). The level of TNF- α in the cerebrospinal fluid of MS patients correlated with the progression of disease (12). Our data also suggest high production and likely involvement of TNF/LT in the pathogenesis of MS. TNF/LT production by T cells of MS patients was higher than by T cells of OAD patients. This cytokine is cytotoxic to other cells (36) and is found to be involved in demyelination in an in vitro system (9). The type of pathology seen in the CNS of animals with EAE, a well known model for MS, is reminiscent of the type of pathology produced by TNF in the in vitro system.

Although production of pro-inflammatory cytokines in the cultures of lymphocytes of MS patients was higher than that of normal controls, there was no correlation between the level of production and the clinical condition of MS patients. As discussed by Trotter et al. (37), correlation with strictly clinical data could be complicated by the possible occurrence of subclinical events in MS.

A possible explanation for T cell activation and the higher frequency of IL-2-activated T cell clones in MS patients may be that lymphocytes of MS patients produce lower amounts of inhibitors of cell proliferation or anti-inflammatory cytokines. Production of anti-inflammatory cytokine TGF- β is associated with inhibition of EAE in natural recovery (38) and in mice orally fed with MBP (39, 40). TGF- β exerts suppressor activity on proliferation of MBP and down-regulates the production of inflammatory cytokines in the brain (40). Studies using mice orally tolerized with MBP have shown TGF- β to be the effector cytokine in the induction of tolerance. CD8⁺ T cells were found to produce TGF- β in both natural recovery and oral tolerance in EAE. In this study we found that PBL of normal controls produced more TGF- β than MS patients after activation by IL-2. Furthermore, patients with MS produced significantly less TGF- β at the time of active disease (exacerbation/progression). Only the production of the active form of TGF- β was found to be different among MS patients at different stages of disease, and between the MS patients and controls, whereas the latent form of TGF- β was not different (data not shown). Also studies of EAE in rats have indicated that suppressor T cells released TGF- β in vitro, which suppressed IL-2 and IFN- γ production (38). Our failure to detect IL-4 in the supernatants of T cell cultures does not preclude the production of this cytokine in small quantities. Our finding is consistent with that of Lewis et al. (41), who reported very low production of IL-4 mRNA, and secretion of protein, by activated human PBL cultures. Like our cultures, these activated PBLs produced high levels of IFN- γ and IL-2. This difference was found to be caused by the low frequency of CD4⁺ T cells expressing IL-4 mRNA (<5%), compared with those expressing IFN- γ and IL-2 (33% and 60%, respectively).

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Our study suggests that TGF- β producing CD8⁺ T cells may play a role in down-regulating the activation of T cells in the peripheral blood. It is not clear whether in MS patients a defect in high production of pro-inflammatory cytokines or in low production of anti-inflammatory cytokines, or both, results in activation of disease. The higher/lower production of pro-/anti-inflammatory cytokines may be caused by different percentages of T cell subsets in the PBL of MS patients compared with normal controls. In fact, a reduction in the number of CD8⁺ (suppressor) T cells during exacerbation of MS has been suggested (32), but this issue remains controversial. A decrease in suppressor inducer CD45RA⁺ T cells in the PBL (27) and in the CNS (42) of MS patients has been described. CD4⁺CD45RA⁺ T cells were decreased in PBL of patients with active SLE and with rheumatoid arthritis (43). Studies on autologous mixed lymphocyte reaction have indicated that CD45RA Ag is involved in the generation of suppressor signals by CD4⁺CD45RA⁺ T cells, and of suppressor function of CD8⁺CD45RA⁺ T cells (44, 45). Taken together, these studies strongly suggest a defect in the down-regulation of immune response in patients with MS, as evidenced by a reduction in the expression of CD45RA Ag. We have further observed that although the production of TGF- β resided mostly in CD8⁺ T cells and only in CD45RA⁺ T cells, IFN- γ was produced by CD4⁺, CD8⁺, CD45RA⁺ and CD29⁺(CD45R⁻) T cells (data not shown). This study showed that during progression or exacerbation of MS (active disease), production of anti-inflammatory cytokine TGF- β declined below normal. This is possibly caused by a lower number of CD8⁺CD45RA⁺ T cells, as suggested by our and several other studies (27, 32, 42–45), and may prove to be a mechanism for potentiation of disease. We have found that although unseparated T cells of active and stable MS patients and normal controls made variable amounts of TGF- β , after purification of CD8⁺ T cells and CD45RA⁺ T cells and culture of the same number of cells, the level of TGF- β production among patients and controls was no longer different (Figs. 4 and 5). This further suggests that the lower production of TGF- β by unfractionated T cells of MS patients with active disease is caused by the lower frequency of TGF- β -producing CD8⁺CD45RA⁺ T cells. Studies of in situ hybridization are in progress to determine the percentage of TGF- β -producing CD8⁺CD45RA⁺ T cells in MS patients and normal controls.

The current study and other reports, taken together, suggest that in MS, probably as a result of immunoregulatory abnormalities in the PBL, the migration of increased inflammatory and immune cells to the CNS and the production of increased levels of pro-inflammatory cytokines and decreased levels of anti-inflammatory cytokines results in damage to the blood brain barrier and to the myelin sheath. Efforts must be made to devise therapeutic strategies that effectively interfere with these mechanisms.

References

1. Hafler, D. A., M. Buchsbaum, D. Johnson, and H. L. Weiner. 1985. Phenotypic and functional analysis of T cells cloned directly from the blood and cerebrospinal fluid of patients with multiple sclerosis. *Ann. Neurol.* 18:451.
2. Gallo, P., M. G. Piccinno, B. Tavalato, and A. Siden. 1991. A longitudinal study on IL-2, sIL-2R, IL-4 and IFN-gamma in multiple sclerosis CSF and serum. *J. Neurol. Sci.* 101:227.
3. Hirsch, R. L., H. S. Panitch, and K. P. Johnson. 1985. Lymphocytes from multiple sclerosis patients produce elevated levels of interferon γ in vitro. *J. Clin. Immunol.* 5:386.
4. Sharief, M. K., and E. J. Thompson. 1993. Correlation of interleukin-2 and soluble interleukin-2 receptor with clinical activity of multiple sclerosis. *J. Neurol. Neurosurg. Psychiatry* 56:169.
5. Hauser, S. L., T. H. Doolittle, R. Lincoln, R. H. Brown, and C. A. Dinarello. 1990. Cytokine accumulations in CSF of multiple sclerosis patients: frequent detection of interleukin-1 and tumor necrosis factor but not interleukin-6. *Neurology* 40:1735.
6. Panitch, H. S., R. L. Hirsch, A. A. Haley, and K. P. Johnson. 1987. Exacerbations of multiple sclerosis in patients treated with γ -interferon. *Lancet* 1:893.
7. Bever, C. T., H. S. Panitch, H. B. Levy, D. E. McFarlin, and K. P. Johnson. 1991. γ -interferon induction in patients with chronic progressive MS. *Neurology* 41:1124.
8. Kastrukoff, L. F., J. J. Oger, W. W. Tourtellotte, S. L. Sacks, J. Berkowitz, and D. W. Paty. 1991. Systemic lymphoblastoid interferon therapy in chronic progressive multiple sclerosis. II. Immunologic evaluation. *Neurology* 41:1936.
9. Brosnan, C. F., K. Selmaj, and C. S. Raine. 1988. Hypothesis: a role for tumor necrosis factor in immune-mediated demyelination and its relevance to multiple sclerosis. *J. Neuroimmunol.* 8:87.
10. Tsukada, N., K. Miyagi, M. Matsuda, N. Yanagisawa, and K. Yone. 1991. Tumor necrosis factor and interleukin-1 in the CSF and sera of patients with multiple sclerosis. *J. Neurol. Sci.* 104:230.
11. Maimone, D., S. Gregory, B. G. Arnason, and A. T. Reder. 1991. Cytokine levels in the cerebrospinal fluid and serum of patients with multiple sclerosis. *J. Neuroimmunol.* 32:67.
12. Sharief, M. K., M. Phil, and R. Hentges. 1991. Association between tumor necrosis factor- α and disease progression in patients with multiple sclerosis. *N. Engl. J. Med.* 325:467.
13. Hofman, F. M., R. I. von Hanwehr, C. A. Dinarello, S. B. Mizel, D. Hinton, and J. E. Merrill. 1986. Immunoregulatory molecules and IL-2 receptors identified in multiple sclerosis brain. *J. Immunol.* 136:3239.
14. Cuzner, M. L., G. M. Hayes, J. Newcombe, and M. N. Woodroffe. 1988. The nature of inflammatory components during demyelination in multiple sclerosis. *J. Neuroimmunol.* 20:203.
15. Traugott, U., and P. Lebron. 1988. Interferon- γ and Ia antigen are present on astrocytes in active chronic multiple sclerosis lesions. *J. Neurol. Sci.* 84:257.
16. Hofman, F. M., D. R. Hinton, K. Johnson, and J. E. Merrill. 1989. Tumor necrosis factor identified in multiple sclerosis brain. *J. Exp. Med.* 170:607.
17. Selmaj, K., C. S. Raine, B. Cannella, and C. F. Brosnan. 1991. Identification of lymphotoxin and tumor necrosis factor in multiple sclerosis lesions. *J. Clin. Invest.* 87:949.
18. Green, L. M., M. L. Stern, D. L. Haviland, B. J. Mills, and C. F. Ware. 1985. Cytotoxic lymphokines produced by cloned human cytotoxic lymphocytes. *J. Immunol.* 135:4034.
19. Neighbour, P. A., A. E. Miller, and B. R. Bloom. 1981. Interferon response of leukocytes in multiple sclerosis. *Neurology* 31:561.
20. Vervliet, G., H. Carton, E. Meulepas, and A. Billiau. 1984. Interferon production by cultured peripheral leukocytes of MS patients. *Clin. Exp. Immunol.* 58:116.
21. Santoli, D., W. Hall, L. Kastrukoff, R. P. Lisak, B. Perussia, G. Trinchieri, and H. Koprowski. 1981. Cytotoxic activity and interferon production by lymphocytes from patients with multiple sclerosis. *J. Immunol.* 126:1274.

22. Tovell, D. R., I. A. McRobbie, K. G. Warren, and D. L. J. Tyrell. 1983. Interferon- γ production by lymphocytes from multiple sclerosis and non-MS patients. *Neurology* 33:640.
23. Papiha, S. S., J. Boddy, D. F. Roberts, and D. Bates. 1989. PHA-induced interferon in multiple sclerosis: association between γ interferon and clinical and genetical variables. *Acta Neurol. Scand.* 80:145.
24. Brakman, E., A. Van Tunen, A. Meager, and C. J. Lucas. 1986. Natural cytotoxic activity in multiple sclerosis patients: defects in IL-2/interferon- γ regulatory circuit. *Clin. Exp. Immunol.* 66:285.
25. Poser, C. M., D. W. Paty, L. Scheinberg, W. I. McDonald, F. A. Davis, G. C. Ebers, K. P. Johnson, W. A. Sibley, and D. H. Silberberg. 1983. New diagnostic criteria for multiple sclerosis guidelines for research protocols. *Ann. Neurol.* 13:227.
26. Kurtzke, J. F. 1981. A proposal for a uniform minimal record of disability in multiple sclerosis. *Neurol. Scand. (Suppl. 87):110.*
27. Mokhtarian F., M. Pino, W. Oforu-Appiah, and D. Grob. 1990. Phenotypic and functional characterization of T cells from patients with myasthenia gravis. *J. Clin. Invest.* 8:2099.
28. Wysocki, L. J., and V. L. Sato. 1978. 'Panning' for lymphocytes: a method for cell selection. *Proc. Natl. Acad. Sci. USA* 75:2844.
29. McFarlin, D. E., and H. F. McFarland. 1982. Multiple sclerosis. *N. Engl. J. Med.* 307:1183.
30. Fontana, A., P. Erb, H. Pircher, R. Zinkernagel, E. Weber, and W. Fierz. 1986. Astrocytes as antigen presenting cells. II. Unlike H-2K-independent cytotoxic T cells, H-2A-restricted T cells are only stimulated in the presence of interferon. *J. Neuroimmunol.* 12:15.
31. Oforu-Appiah, W., F. Mokhtarian, A. Miller, and D. Grob. 1991. Characterization of in vivo-activated T cell clones from peripheral blood of multiple sclerosis patients. *Clin. Immunol.* 58:46.
32. Hafler, D. A., and H. L. Weiner. 1989. MS: a CNS and systemic autoimmune disease. *Immunol. Today* 10:104.
33. Kroemer, G., and G. Wick. 1989. The role of interleukin 2 in autoimmunity. *Immunol. Today* 10:246.
34. Sun, D., and H. Wekerle. 1986. Ia-restricted encephalitogenic T lymphocytes mediating EAE lyse autoantigen-presenting astrocytes. *Nature* 320:70.
35. Beck, J., P. Rondot, L. Catinot, E. Falcoff, H. Kirchner, and J. Wietzerbin. 1988. Increased production of interferon γ and tumor necrosis factor precedes clinical manifestation in multiple sclerosis: do cytokines trigger off exacerbations? *Acta Neurol. Scand.* 78:318.
36. Ruddle, N. H. 1987. Tumor necrosis factor and related cytotoxins. *Immunol. Today* 8:129.
37. Trotter, J. L., R. C. van der Veen, and D. B. Clifford. 1990. Serial studies of serum interleukin-2 in chronic progressive multiple sclerosis patients: occurrence of bursts and effect of cyclosporin. *J. Neuroimmunol.* 28:9.
38. Karpus, W. J., R. H. Swanborg. 1991. CD4⁺ suppressor cells inhibit the function of effector cells of experimental autoimmune encephalomyelitis through a mechanism involving transforming growth factor β . *J. Immunol.* 146:1163.
39. Miller, A., O. Lider, A. B. Roberts, M. B. Sporn, and H. L. Weiner. 1992. Suppressor T cells generated by oral tolerization to myelin basic protein suppress both in vitro and in vivo immune responses by the release of transforming growth factor beta after antigen-specific triggering. *Proc. Natl. Acad. Sci. USA* 89:421.
40. Khoury, S. J., W. W. Hancock, and H. L. Weiner. 1992. Oral tolerance to myelin basic protein and natural recovery from experimental autoimmune encephalomyelitis are associated with downregulation of inflammatory cytokines and differential upregulation of transforming growth factor beta, interleukin 4, and prostaglandin E expression in the brain. *J. Exp. Med.* 176:1355.
41. Lewis, D. B., K. S. Prickett, A. Larsen, K. Grabstein, M. Weaver, and C. B. Wilson. 1988. Restricted production of interleukin 4 by activated human T cells. *Proc. Natl. Acad. Sci. USA* 85:9743.
42. Sobel, R. A., D. A. Hafler, E. E. Castro, C. Morimoto, and H. L. Weiner. 1988. The 2H4 (CD45R) antigen is selectively decreased in multiple sclerosis lesions. *J. Immunol.* 140:2210.
43. Raziuddin, S., M. A. Nur, and A. A. Alwabel. 1989. Selective loss of the CD4⁺ inducers of suppressor T cell subsets (2H4⁺) in active systemic lupus erythematosus. *J. Rheumatol.* 16:1315.
44. Takeuchi, T., C. E. Rudd, S. Tanaka, D. M. Rothstein, S. F. Schlossman, and C. Morimoto. 1989. Functional characterization of the CD45R (2H4) molecule on CD8 (T8) cells in the autologous mixed lymphocyte reaction system. *Eur. J. Immunol.* 19:747.
45. Takeuchi, T., C. E. Rudd, T. F. Tedder, S. F. Schlossman, and C. Morimoto. 1989. Amplification of suppressor inducer pathway with monoclonal antibody, anti-2H4, identifying a novel epitope of the common leukocyte antigen/T200 antigen. *Cell. Immunol.* 118:68.

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