Treatment of Depression Is Associated With Suppression of Nonspecific and Antigen-Specific T\textsubscript{H}1 Responses in Multiple Sclerosis

David C. Mohr, PhD; Donald E. Goodkin, MD; Janeen Islar, BS; Stephen L. Hauser, MD; Claude P. Genain, MD

**Objective:** To examine the relationship between depression, treatment of depression, and interferon gamma (IFN-\(\gamma\)) production by peripheral blood mononuclear cells in patients with comorbid diagnoses of relapsing-remitting multiple sclerosis (MS) and major depressive disorder.

**Design:** A randomized comparative outcome trial of three 16-week treatments for depression. Assessments were conducted at baseline, week 8, and treatment cessation.

**Setting:** An academic outpatient treatment and clinical research center.

**Patients:** Fourteen patients who met the criteria for relapsing-remitting MS and major depressive disorder.

**Interventions:** Individual cognitive behavioral therapy, group psychotherapy, or sertraline therapy.

**Main Outcome Measures:** Depression was assessed using the Beck Depression Inventory. Interferon gamma production by peripheral blood mononuclear cells was measured following stimulation with OKT3 or recombinant human myelin oligodendrocyte glycoprotein (MOG). Variability in immune assays was controlled using 8 nondepressed healthy subjects who were enrolled at times corresponding with the enrollment of MS patients.

**Results:** Results of the Beck Depression Inventory were significantly related to IFN-\(\gamma\) production stimulated with OKT3 or MOG at baseline (\(P\leq .03\) for all). Level of depression, OKT3-stimulated IFN-\(\gamma\) production, and MOG-stimulated IFN-\(\gamma\) production all declined significantly over the 16-week treatment period (\(P\leq .03\) for all). Among controls, there were no significant changes over time in OKT3-or MOG-stimulated IFN-\(\gamma\), or in depression (\(P\leq .25\) for all).

**Conclusions:** These findings suggest that the production of the proinflammatory cytokine IFN-\(\gamma\) by autoimmune T cells in relapsing-remitting MS is related to depression and that treatment of depression may decrease IFN-\(\gamma\) production. Thus, treatment of depression may provide a novel disease-modifying therapeutic strategy as well as a symptomatic treatment for patients with MS.

Arch Neurol. 2001;58:1081-1086

**MULTIPLE sclerosis** (MS) is the most common chronic demyelinating disease of the central nervous system in adulthood. Its cause is believed to be autoimmune.\(^1,2\) Depression, with a lifetime prevalence estimated at over 50%,\(^3\) is more common in MS than in other chronic illnesses\(^4,5\) or neurological disorders.\(^6,7\) The etiology of depression in MS is likely multifactorial and may be attributed to brain lesions,\(^8\) psychosocial losses,\(^9,10\) and/or immune dysregulation.\(^11,12\)

Studies examining the relationship between depression and immune dysregulation in MS have assumed that the causal relationship is unidirectional, with immune dysregulation causing depression.\(^11,12\) However, the psychoneuroimmunology literature suggests that this relationship may be more complex. Studies have consistently found depression to be associated with lowered natural killer cell activity and lowered numbers of natural killer, B, T-helper (T\textsubscript{H},) and suppressor-cytotoxic T cells.\(^13,14\) Depression may induce abnormalities of immune functions that are relevant to MS, in which a concerted immune attack by cellular and humoral factors directed against myelin constituents of the central nervous system results in tissue destruction.\(^2\)

In the animal disease model of MS, experimental allergic encephalomyelitis pathology is mediated by CD\(+\)CD8\(^-\) T\textsubscript{H}1 cells that are activated by the presentation of specific myelin antigens on major histocompatibility complex class II mol-
SUBJECTS AND METHODS

SUBJECTS

Fourteen patients with MS were enrolled over 12 months through the University of California, San Francisco, Multiple Sclerosis Center after signing informed consent documents. These patients were involved in a larger clinical trial of treatments for depression in MS26,27 and met the criteria for clinically definite relapsing-remitting MS28,29 and major depressive disorder using the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition.30 Patients were excluded if at screening they had experienced an MS exacerbation within the previous 30 days; had taken immunosuppressive medication within the last 14 days; were receiving any treatment for depression, including medication or psychotherapy; or met the criteria for dementia, defined as performing below the fifth percentile on 3 of the following tests: Digit Span Test,31 Symbol Digit Modalities Test,32 Rey Auditory Verbal Learning Test,33 7/24 Test,34 Controlled Oral Word Association Test,35 brief Boston Naming Test,36 and California Card Sort Test.37 Patients were not asked to participate in blood draws for this study if they were using disease-modifying medications (interferon beta-1a, interferon beta-1b, or copolymer I). Most patients in the larger clinical trial were using one of the disease-modifying drugs, and no patient ceased using MS medications for participation in the study.

To control for variability in the immunological assays, 8 nondepressed healthy control subjects were enrolled corresponding with the enrollment of MS patients.

INTERVENTIONS

Patients were randomly assigned to 1 of 3 commonly used 16-week treatments for depression: (1) individual cognitive behavioral therapy26,27 designed to improve coping skills, (2) group psychotherapy28 designed to provide social support and facilitate emotional expression, or (3) psychopharmacological therapy with the commonly prescribed antidepressant sertraline.37

ASSESSMENT OF DEPRESSION

Level of depression were measured using the Beck Depression Inventory (BDI).38

IMMUNOLOGICAL ASSAYS

Heparinized venous blood was collected at baseline, week 8, and treatment cessation. Blood was processed within 4 hours to obtain PBMCs (Ficoll Hypaque; Pharmacia Biotech, Uppsala, Sweden).

Nonspecific OKT3-stimulated production of IFN-γ was measured with and without interleukin 10 (IL-10) suppression.23 Peripheral blood mononuclear cells (10⁷/mL) were cultured in RPMI-1640 medium ( Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (BioWhittaker, Walkersville, Md), 4mM l-glutamine, 25mM Hepes buffer, 50-U/mL penicillin, and 50-µg/mL streptomycin. After 2 days, cells were resuspended in 1 mL of serum-free X-VIVO-10 medium (BioWhittaker) with 1 µg of OKT3 antibody, and supernatants were collected after an additional 2 days. Suppression of IL-10 was achieved in a duplicate set of tubes by adding 1 µg of IL-10.

Antigen-stimulated production of IFN-γ and T-cell proliferative responses were assessed by standard 3-day proliferation assays39 using 2 × 10⁵ PBMCs in 96-well round-bottom plates after the following additions: no antigen (control); myelin basic protein (MBP), 50 pg/mL; a recombinant protein corresponding to the N-terminus domain of human myelin oligodendrocyte glycoprotein (MOG) aa 1-125, 10 pg/mL; and phytahemagglutinin, 2.5 µg/mL. After 48 hours, 100 µl of supernatant was removed from each well and stored at −80°C for cytokine assays. After medium replacement, 0.5 µCi of tritiated thymidine was added. The wells were harvested 18 hours later. Stimulation index values were calculated as ratios of counts per minute in stimulated wells over counts per minute in unstimulated (control) wells.

The concentrations of IFN-γ and IL-4 in supernatants were measured using enzyme-linked immunosorbent assay kits according to the manufacturer’s instructions (Biosource International, Camarillo, Calif). The production of each cytokine was calculated as the difference between the concentration in stimulated supernatant culture and that in unstimulated cultures. Concentrations below the threshold of detection for the assay (5 pg/mL) were arbitrarily set at 0.

STATISTICAL ANALYSES

All IFN-γ production variables are reported in picograms per milliliter and were analyzed as continuous variables, except those in which MOG was used as an antigen. Because approximately 70% of all MS patients do not react to MOG at any given point in time,37 the IFN-γ response to MOG was dichotomized at each assessment point as “response” or “no response.” Response was defined as MOG-stimulated IFN-γ production that exceeded 3 SDs above the mean production of unstimulated cells (19.1 pg/mL).

For T-cell proliferative responses, a stimulated index value of 2.5 or greater was considered a positive response. Associations between 2 continuous variables were analyzed using Pearson correlations. Comparisons of continuous variables across 2 groups were analyzed using t tests. Analyses of change over time were performed using analysis of variance for continuous variables or the Cochran Q test for dichotomous variables.

Interferon gamma (IFN-γ) is the main proinflammatory cytokine produced by activated Tc1 cells and is regarded as a major effector mechanism in the pathogenesis of MS.19 Administration of IFN-γ has been reported to trigger exacerbations.20 Increased production of IFN-γ has been shown to precede both MS exacerbations21 and the development of new magnetic resonance
imaging brain lesions, it may also be associated with an acceleration of the disease course. Increased IFN-γ production has also been associated with severe depression in psychiatric inpatients. However, the nature of the relationship between depression and the production of IFN-γ by myelin-specific T cells has not been examined in MS. Furthermore, the relationship between IFN-γ and more common forms of depression that do not require inpatient treatment remains unclear.

In the current study, we used a longitudinal design to examine the relationship between immune system reactivity as measured by nonspecific and myelin antigen–stimulated production of IFN-γ in peripheral blood mononuclear cells (PBMCs) and a standardized measurement of depression in patients with relapsing-remitting MS. Depression was manipulated through outpatient treatment using either psychotherapy or sertraline. It was hypothesized that depression would be positively correlated with nonspecific and specific antigen-stimulated IFN-γ production, that depression and IFN-γ production would decline during the course of treatment for depression, and that changes in IFN-γ production would be related to changes in depression.

## RESULTS

### PATIENT CHARACTERISTICS

Of the 14 relapsing-remitting MS patients enrolled in the study, 10 (71%) were women and 4 (29%) were men. The average age was 47.4 years (range, 29-69 years). The mean Expanded Disability Status Scale score was 3.6 (range, 0-6.5). The mean time since diagnosis was 11.3 years (range, 10 months–19.8 years). The mean (SD) BDI at baseline was 21.9 (6.35). Three patients experienced clinical exacerbations: 1 patient at week 3, 1 patient at week 6, and 1 patient at week 8, coincident with the scheduled assessment and blood draw.

### BASELINE ANALYSES

#### Dependent Variables (BDI and Immune Variables) and Demographic or Disease Variables

Age was related to BDI (r = -0.65, P = .01) and OKT3-stimulated IFN-γ (r = -0.69, P = .006). Dependent variables were otherwise unrelated to demographic and disease variables, including age, sex, marital status, Expanded Disability Status Scale, or time since diagnosis (P = .09 for all).

#### Depression and Immune Variables

Baseline BDI was significantly related to OKT3-stimulated IFN-γ production (r = 0.64, P = .01). Five patients showed a positive IFN-γ response to MOG at baseline. Baseline BDI was significantly related to IFN-γ response to MOG, with nonresponders significantly less depressed (mean [SD] BDI, 19.2 [4.9]) than responders 26.8 [6.1]). Baseline BDI was strongly correlated with IFN-γ production (r = 0.87, P = .05). The BDI was not significantly related to IL-4 production, MBP-stimulated variables, proliferative responses, or IL-10 suppression of IFN-γ production (r < 0.47, P = .10 for all).

### EFFECTS OF TREATMENT (WITHIN-SUBJECT CHANGE)

**Table 1** displays the values of the dependent variables over the course of treatment for depression. Because both BDI and OKT3-induced IFN-γ production were related to age, the effects of age were covaried out of outcome analyses for these measures.

**Depression**

The BDI dropped significantly over the 16 weeks of treatment for depression (F (2,26) = 6.19, P = .006). The age covariate was significant, with younger patients showing greater improvement (Rao R = 5.95, P = .01). There was no differential effect for treatment modality (P = .86).

#### OKT3-Stimulated Production of IFN-γ

Non-specific IFN-γ production decreased significantly over the course of treatment (F (2,24) = 3.92, P = .03). The age covariate was marginally related to change in IFN-γ over time, with younger patients showing a greater decline in IFN-γ production (Rao R = 3.58, P = .05). Interleukin-10 suppression of OKT3-stimulated production of IFN-γ increased during treatment (F (2,26) = 6.88, P = .005). There was no significant differential effect for treatment modality (P = .55).

#### MOG-Stimulated Production of IFN-γ

Values of MOG-stimulated IFN-γ for responders during treatment are displayed in the figure. Five patients (36%) showed an IFN-γ response to MOG at baseline while 9 (64%) did not. Three patients (21%) showed an IFN-γ response to MOG at week 8 (these were different patients than those who showed a response at baseline), and no patients showed an IFN-γ response to MOG at treatment cessation (1 patient who showed a response to MOG at week 8 was experiencing an exacerbation at the time of the blood draw and was subsequently treated.
with prednisone). The reduction in the number of patients showing a response to MOG over the course of treatment was significant (Cochran Q, 5.0; P = .03). All patients who responded to MOG also showed significant IL-10 suppression (Cochran Q, 5.0; P = .03). Because there were no responders to MOG at treatment cessation, evaluation of differential effects for treatment modality would only reflect pretreatment differences. As this would be meaningless, these calculations were not performed.

**MBP-Stimulated Production of IFN-γ**

There was no significant change over time in MBP-stimulated IFN-γ (P = .15), nor was there any significant change in IL-10 suppression of MBP-stimulated IFN-γ (P = .49). There were no significant differential effects for treatment modality (P = .53 for all).

**Production of IL-4**

There was no significant change over the course of treatment in OKT3-, MOG-, or MBP-stimulated IL-4 (P = .22 for all). There were no significant differential effects for treatment modality (P = .55).

**T-Cell Proliferation Assays**

There were no significant changes in proliferative responses over time using MBP, phytohemagglutinin, or MOG (P > .16 for all) (**Table 2**). There were no significant differential effects for treatment modality (P > .56 for all).

**RELATIONSHIP OF CHANGE IN DEPRESSION TO CHANGE IN IMMUNE FUNCTION**

Using slopes analysis, the relationship between change in BDI and change in immune function was examined for those variables for which significant within-subject changes had occurred. The change in BDI was significantly related to the change in OKT3-stimulated IFN-γ, (F[3,12] = 3.75, P = .01). Change in BDI was not significantly related to change in IL-10 suppression of OKT3-stimulated IFN-γ (P = .74). The relationship between change in MOG-stimulated IFN-γ and change in BDI was not examined because there was no variability in IFN-γ response to MOG at treatment cessation (ie, no patient showed an IFN-γ response to MOG).

**ANALYSIS OF CONTROL SUBJECTS**

Of the 8 control subjects in the study, 5 (62%) were women and 3 (38%) were men, which was not significantly different from the MS patients (P = .12). The control subjects were significantly younger (mean, 31.2 years; range, 23-41 years) than the MS patients (t = 3.75, P = .001). Table 1 displays the BDI and IFN-γ values for control subjects. There were no significant changes over time in the BDI, nonspecific or antigen-specific IFN-γ responses, IL-10-suppressed IFN-γ responses, or IL-4 responses (P = .25 for all). With respect to proliferative responses, there was a nearly significant increase in phytohemagglutinin-stimulated proliferation (F[3,12] = 3.16, P = .06). No other proliferative responses showed significant changes over the course of the study (P = .24 for all). These control subjects were used solely to control for variability in the assays. Because control subjects were not depressed and did not have MS, no analyses were performed comparing controls and MS patients for BDI or immunological variables.

**T-Cell Proliferation Assays**

There were no significant changes in proliferative responses over time using MBP, phytohemagglutinin, or MOG (P > .16 for all) (**Table 2**). There were no significant differential effects for treatment modality (P > .56 for all).

**RELATIONSHIP OF CHANGE IN DEPRESSION TO CHANGE IN IMMUNE FUNCTION**

Using slopes analysis, the relationship between change in BDI and change in immune function was examined for those variables for which significant within-subject changes had occurred. The change in BDI was significantly related to the change in OKT3-stimulated IFN-γ, (F[3,12] = 3.75, P = .01). Change in BDI was not significantly related to change in IL-10 suppression of OKT3-stimulated IFN-γ (P = .74). The relationship between change in MOG-stimulated IFN-γ and change in BDI was not examined because there was no variability in IFN-γ response to MOG at treatment cessation (ie, no patient showed an IFN-γ response to MOG).

**ANALYSIS OF CONTROL SUBJECTS**

Of the 8 control subjects in the study, 5 (62%) were women and 3 (38%) were men, which was not significantly different from the MS patients (P = .12). The control subjects were significantly younger (mean, 31.2 years; range, 23-41 years) than the MS patients (t = 3.75, P = .001). Table 1 displays the BDI and IFN-γ values for control subjects. There were no significant changes over time in the BDI, nonspecific or antigen-specific IFN-γ responses, IL-10-suppressed IFN-γ responses, or IL-4 responses (P = .25 for all). With respect to proliferative responses, there was a nearly significant increase in phytohemagglutinin-stimulated proliferation (F[3,12] = 3.16, P = .06). No other proliferative responses showed significant changes over the course of the study (P = .24 for all). These control subjects were used solely to control for variability in the assays. Because control subjects were not depressed and did not have MS, no analyses were performed comparing controls and MS patients for BDI or immunological variables.
thermore, over the course of treatment for depression, decreases in levels of depression were associated with decreases in levels of IFN-γ production. The stability of the IFN-γ production in the control subjects rules out the possibility that these findings were caused by variability in the assays. Thus, these results support the hypothesis that depression is associated with increased IFN-γ production, and that IFN-γ production can be down-regulated by treating depression. The observed relationship between IFN-γ production, depression, and treatment of depression may have particular salience for MS patients as IFN-γ has been implicated as a major factor in the pathophysiology of this disease.

Behavioral and pharmacological treatments for depression were used in this study. Because several antidepressants, including sertraline, have been shown to reduce IFN-γ secretion in PBMCs, we analyzed the data to determine if treatment assignment was related to IFN-γ production. Although the possibility of a relationship between treatment modality and IFN-γ production cannot be formally excluded, these data suggest that such a relationship is unlikely.

Consistent with previous studies, we found that a substantial proportion of patients with MS exhibited positive reactivity to MOG at baseline. Although some patients, including one who had an exacerbation, showed an IFN-γ response to MOG at week 8, all the patients became nonresponders by the end of treatment. This finding is potentially relevant to treatment strategies for MS. Both clinical studies and experimental studies using animal models of experimental allergic encephalomyelitis suggest that heightened immune response to MOG may trigger autoimmune demyelination in the central nervous system. Thus, our observation that treatment of depression is associated with decreased T-cell responsiveness to MOG suggests that amelioration of depression could be an important factor for down-regulating autoaggressive T cells and therefore may be an important component in the management of patients with MS.

Although the manipulation of depression through treatment supports the argument that depression can cause changes in IFN-γ production, these findings do not rule out the possibility that IFN-γ production can cause depression. It has been suggested that immune dysregulation in MS may cause depression, and the increased incidence of depression during disease exacerbation is consistent with this argument. Thus, the present findings might better be interpreted as supporting the notion that the relationship between immune dysregulation and depression in MS is dynamic and reciprocal.

While our finding that a reduction in levels of depression is associated with a reduction in IFN-γ production is consistent with the existing literature, potential mediating mechanisms are not understood. Ability to regulate cortisol may be one potential mechanism. Successful suppression of cortisol following the administration of dexamethasone is one of the most consistent biological markers of depression. Suppression of cortisol following dexamethasone administration is also negatively related to IFN-γ production. Epinephrine and norepinephrine, the absence of which is associated with increased depression, have been shown to down-regulate IFN-γ production. However, serotonin has been shown to stimulate, rather than reduce, IFN-γ production. Thus, the effect of depression and its treatment on IFN-γ is likely the final result of multiple and conflicting pathways, which will require further investigation.

Several limitations must be considered in interpreting the data of our study: (1) Confidence in our findings may have been limited by the small sample size. However, the effect sizes were robust, and the statistical methods employed controlled for the effects of chance based on sample size. Furthermore, our findings are consistent with those of previous cross-sectional and longitudinal studies. (2) We considered placebo treatment of major depressive disorder unethical. In the absence of a placebo control condition, it is possible that all patients showed unusually high autoimmune reactivity before treatment and that the observed changes reflect the natural course of such reactivity independent of treatment for depression. This interpretation, while not consistent with the existing literature, cannot be ruled out. (3) Because this study was of short duration, we do not know if observed changes in IFN-γ production are of clinical significance. It will be of considerable interest to examine whether the treatment of depression and reduction in IFN-γ production are related to clinical and/or imaging measures of progression and exacerbation.

None of the limitations invalidate the primary findings that depression is associated with IFN-γ production in MS and that treatment of depression is associated with a reduction in nonspecific and antigenspecific IFN-γ production. These novel findings suggest that treating depression may be an important disease-modifying component in the treatment and management of relapsing-remitting MS.

Accepted for publication December 11, 2000.

This research was supported by grant RG2719 A1/2 from the National Multiple Sclerosis Society, New York, NY; by grant R01 MH59708 from the National Institute of Mental Health, Bethesda, Md (Dr Mohr); by an award from the Osher Center for Integrative and Complementary Medicine, University of California, San Francisco (Dr Mohr); and by Harry Weaver Neuroscience Scholarship JF 2087-A-2 from the National Multiple Sclerosis Society (Dr Genain).

Corresponding author and reprints: David C. Mohr, PhD, Veterans Affairs Medical Center (116-A), 4150 Clement St, San Francisco, CA 94121 (e-mail: dmoht@itsa.ucsf.edu).

REFERENCES


