In Vitro Glatiramer Acetate Treatment of Brain Endothelium Does Not Reduce Adhesion Phenomena

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Despite the well-documented clinical effect of glatiramer acetate (GA) (copolymer-1; Copaxone) in multiple sclerosis (MS),[1,2] the mechanism(s) of action of this synthetic mixture of polypeptides has not been fully clarified. The proposed actions of GA include the generation of antigen-specific suppressor T cells[3,4] and/or competition with self-encephalitogenic antigens (ie, myelin basic protein, MOG, proteolipid protein) for binding with MHC class II antigen on the surface of antigen-presenting cells.[5] Neuroradiological follow-up studies[6,7] suggest that GA treatment leads to the reduction of active lesions as assessed by gadolinium-enhanced magnetic resonance imaging, in analogy to what has been shown to occur for interferon (IFN)-β.[8]

This observation leads to the investigation of the putative effects of GA on blood–brain barrier (BBB) permeability and/or transmigration of immune cells through the BBB into the brain parenchyma. In a recent study by Prat and coworkers,[9] 10 relapsing-remitting (RR) MS patients treated with GA for an average 6 months were evaluated for the ability of their peripheral blood T lymphocytes to migrate through a fibronectin monolayer over a Boyd chamber; it is interesting that the number of T lymphocytes recovered in the lower chamber after the 6-hour incubation period was lower than that detected in untreated RR MS patients; similar data were observed in 7 RR MS patients treated with IFNβ-1b (Betaseron) for an average of 24 months.[9] This reduction in transmigration was also observed after in vitro incubation of peripheral blood mononuclear cells (PBMNCs) from healthy donors with IFNβ-1b but not after incubation with GA. These findings suggest that IFN-β has a direct effect on immune cells, but the effect of GA on transmigration remains unexplained.

Bearing in mind these considerations, we performed a study aimed at evaluating the effect(s) of GA on the counterpart of immune cells at their diapedesis from blood to brain, ie, human brain microvascular endothelial cells (HBMECs). We studied the putative in vitro modulation of intracellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, ICAM-2, and HLA-DR by GA on HBMECs from 3 patients undergoing surgery for low-grade glioma or cerebral aneurysm (1 of whom was also affected by MS), alone or following coincubation (for 72 hours) with proinflammatory cytokines. The effects of HBMEC stimulation with GA, on the adhesion of PBMNCs from healthy controls, was also investigated. The effect of GA on endothelial cells was also assessed by the evaluation of β_2-microglobulin in HBMEC supernatants. District specificity was verified by the use of human umbilical vein endothelial cells (HUVECs). The results described in the Table show that GA enhanced the adhesion of PBMNCs to endothelial cells and increased the elevated adhesion of cells to stimulated HBMECs. However, GA did not induce the expression of adhesion molecules on MS-HBMECs (Fig), and, moreover, it did not affect the expression of adhesion molecules induced by proinflammatory cytokines (see Fig). When the experiments were performed with HUVECs, similar results were obtained.

Table Adhesion Assay and β2-Microglobulin Release

Stimuli	PBMNCs Adhered[a] (absolute numbers)	β2-Microglobulin (μg/L)[b]
Basal	789.4 ± 311.8	67.0
LPS (100 ng/mL)	1099.6 ± 510.8[c]	109.2
γ-IFN (250 U/mL)	2005.0 ± 546.5[c]	186.5
TNF-α (10 ng/mL)	2161.6 ± 573.2[c]	71.8
Cop-1 (25 μg/mL)	1399.0 ± 818.1[c]	59.8
Cop-1/LPS	1580.8 ± 748.2[d]	127.3
Cop-1/γ-IFN	2251.8 ± 691.9	200.2
Cop-1/TNF-α	2792.6 ± 773.1[e]	69.8

[a]Adhesion assay was performed according to Benschop's technique.[10] Adhesion was evaluated after 1-hour incubation of 2×10^5 PBMNCs over HBMECs, removal of nonadherent cells and trypsinization (6 healthy donors were studied).
[b]β2-Microglobulin levels were assessed only on MS-HBMECs supernatants;
Statistical analysis: Wilcoxon signed rank test: [c]significantly different as compared with basal values ($p < 0.027$); [d]significantly different as compared with LPS alone ($p < 0.027$); [e]significantly different as compared with TNF-α alone ($p < 0.027$).

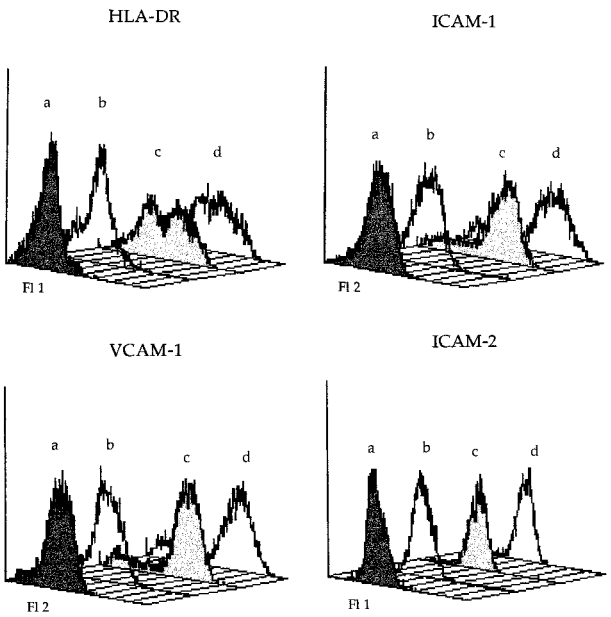

Fig. (a) Basal antiagen expression. (b) L-COP (25 μg/ml) stimulation. (c) Cytokines stimulation (IFN-γ [250 U/ml] for HLA-DR and TNF-α [10 ng/ml] and ICAM-1, VCAM-1, and ICAM-2). (d) L-COP (25 μg/ml) plus cytokine stimulation (IFN-γ [250 U/ml] for HLA-DR and TNF-α [10 ng/ml] for ICAM-1, VCAM-1, and ICAM-1). The pattern of adhesion molecules modulation in the other patients was similar to the one reported in the figure (not shown).

Adhesion phenomena are not necessarily of the same entity and direction as transmigration ones. In a cohort of 7 patients treated with IFNβ-1b for 6 months,[11] we observed a

reduction in PBMNC transmigration through HUVEC monolayers; this reduction paralleled an increase in adhesion.

Our results suggest that the reduction in transmigration in GA-treated individuals, reported by Prat and co-workers,[9] is not mediated via changes in ICAM-1, ICAM-2, VCAM-1, or HLA-DR.

Whether other actions are involved in obtaining this putative effect (such as modulation of matrix metalloproteinase release or BBB-disrupting cytokine synthesis within the brain), or whether a generalized peripheral shift to Th2 phenotype may lead to initially increased transmigration of Th2 cells followed by dampening of the local autoimmune process, remains to be investigated.

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Reply

Jack P. Antel, MD, and Alexandre Prat, MD

Dufour and colleagues, in their current letter, and in another recently published in *Annals,*[1] describe their studies of in vitro lymphocyte transmigration through a barrier formed by endothelial cells (ECs) grown on a membrane. In our initial studies of in vitro lymphocyte migration, we utilized a Boyden chamber assay, in which the chambers were separated by a fibronectin-coated membrane.[2] The EC system has the advantage of allowing one to address issues of chemoattraction, adherence, and transmigration. We have also grown human adult brain ECs on membranes[3] and confirm the results reported by Wong and associates,[4] that these cells do provide a barrier to lymphocyte migration.

In our initial study, we attributed the increased migration rate of lymphocytes derived from patients with active multiple sclerosis to intrinsic properties of the lymphocytes because no blood–brain barrier cellular components were present in our assay system.[5] Migration could be partially inhibited with inhibitors of metalloproteinases, which is consistent with observations by others, that multiple sclerosis patient–derived lymphocytes produce high levels of metalloproteinases.[6,7] We used this assay to show that patients receiving either interferon-β (IFN-β) or glatiramer acetate (GA) therapy had reduced migration rates compared with untreated patients.[8] We postulated that IFN-β had a direct effect on the molecular mechanisms underlying migration, because we could reproduce the effect by adding IFN-β in vitro. The basis for the GA effect seemed less direct because the effect was not reproduced by adding GA in vitro.

Consistent with our results using the fibronectin-coated assay system, Corsini and colleagues[1] show that IFN-β inhibits lymphocyte migration through ECs. Lou and associates[9] also reported that IFN-β inhibits activated leukocyte migration through human brain microvessel EC monolayers. In their letter, Corsini and colleagues[1] do not provide any data regarding the effect of GA on either migration of lymphocytes through ECs or expression on lymphocyte of LFA-1 and VLA-4 adhesion molecules. They indicate that GA enhances lymphocyte adhesion to ECs but without increasing adhesion molecule expression on these cells. Thus, our initial observations regarding reduced in vitro migration rates of lymphocytes derived from GA-treated patients remain to be confirmed and explained. The emerging experimental techniques to study lymphocyte–vascular interactions will, hopefully, lead to a clearer understanding of the overall molecular events involved in lymphocyte transmigration into the central nervous system and how therapeutic agents can modulate this process.

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The Role of Inheritance in Sporadic Parkinson's Disease

Christopher Hawkes, MD, FRCP

The recent publication of Piccini and colleagues[1] is an interesting twin-based study with some statements and results that are potentially misleading.

The latency of concordance in twins with Parkinson's disease (PD) is cited as "approaching three decades." This is an extreme value and unrepresentative. A useful figure is that given in a large population-based study of 193 male twins,[2] in which the mean for monozygous twins (MZ) was 8.6 years (range, 2–28 years) and for dizygous (DZ) was 9.7 years (range, 2–31 years). The opportunity for chance concordance in a common disease such as PD will increase in an elderly population when the latency for clinical concordance is high.

The initial sample obtained by the authors in 1991 was recruited from three countries (UK, US, and Italy), which would introduce both genetic and environmental bias.

The proportion of MZ and DZ twins in which both sexes are recruited is wrong, ie, 18 MZ and 16 DZ. The MZ/DZ ratio should be 1:2. Males are overrepresented (ratio, 17:10); the sex ratio should be equal.

The cohort that was rescanned 7 years later is even more imbalanced, ie, 10 MZ and 9 DZ. Details of the 8 MZ and 7 DZ dropouts are not given, apart from 3 twins who died. There would be concern in the re-evaluation study that the apparently unaffected co-twins might be self-selected and would be more likely to volunteer for rescanning. For example, a patient with minimal symptoms not disclosed to or detected by the clinician, such as intermittent tremor or clumsiness, would be more likely to volunteer for the second scan. Whatever the mechanism, the existence of substantial numbers of dropouts is likely to produce unpredictable and unreliable data.

Three of 34 twin pairs had a family history of PD. This will tend to increase concordance and these patients should have been excluded.

The principles verifying the reliability of a twin sample (not referred to in the present study), in terms of proportionof MZ/DZ twins and males versus females, were established by Weinberg[3] in 1901. Furthermore, volunteer-based studies such as this tend to attract more MZ and females, the so-called rule of two thirds.[4] This bias is, in part, exemplified here except for a surprising excess of males. Sampling characteristics must be correct before any conclusion about genetic influence can be drawn.

It would not be denied that there is a genetic contribution in the development of PD, the magnitude of which is still unclear. Nearly all volunteer-based twin studies (such as the current study) overstate the genetic aspect to a given disease[5]; hence, the genetic component to PD is probably strongly overstated here.

The present study demonstrates the existence of subclinical concordance in PD twins. Unfortunately, it cannot contribute to the genetic versus environmental debate because of flaws in the methodology that are cited above.

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Association of Primary Central Nervous System Lymphoma with Long-Term Azathioprine Therapy for Myasthenia Gravis?

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The association of mild immunosuppression, such as azathioprine therapy for autoimmune disease, with the development of lymphoma is unclear. In the present study, we analyzed all 229 patients with myasthenia gravis (MG) seen at our department between 1983 and 1998 for the development of lymphoma. One hundred fifty-nine patients were treated with azathioprine, initially 2 mg/kg/day, for a median of 3.4 years (range, 1 day to 18.8 years) with 906.3 patient-years of follow-up after the start of azathioprine treatment.

With one pancreatic carcinoma, two lung cancers, one breast cancer, and two multiple basaliomas, the incidence of

solid tumors in patients with azathioprine was not significantly increased as compared with a general population with the same follow-up ($p > 0.05$, χ^2 test). Two patients, aged 60 and 66 years old, developed primary central nervous system lymphoma (PCNSL) after 6 and 12 years of azathioprine and without cyclosporine treatment. Epstein-Barr virus (EBV) latent membrane protein-1 expression was detected in both biopsy specimens. The occurrence of PCNSL in 2 of 159 patients treated with azathioprine (1.5%) is significantly increased ($p < 0.01$, χ^2 test) as compared with a general population with an incidence of 0.43:100,000 and identical follow-up.

The increased incidence of PCNSL in our MG patients is likely due to azathioprine treatment, since PCNSL is not associated with autoimmune diseases in general and since both tumors were positive for an EBV marker that is highly associated with PCNSL in immunosuppressed patients and rarely found in PCNSL of immunocompetent patients. Regarding autoimmune diseases other than MG, an increased incidence of lymphoma was reported for azathioprine treatment in rheumatoid arthritis[1] but not for azathioprine treatment in multiple sclerosis[2] or inflammatory bowel disease.[3] Such differences may be explained by the fact that patients with MG (median age, 53.9 years; this study) or rheumatoid arthritis were 20 years older at onset of azathioprine treatment than patients with inflammatory bowel disease or multiple sclerosis. Also, duration of treatment with azathioprine was much shorter for patients with inflammatory bowel disease in one previous study (12.5 months)[3] than for patients with MG (3.4 years) in our study.

Azathioprine has clearly improved the long-term prognosis of severe MG.[4] The relapse rate after discontinuation of azathioprine has been analyzed so far in only one study: 8 of 15 patients experienced relapse on discontinuation of azathioprine after 2.2 years (median) of treatment.[5]

In conclusion, the observed association of PCNSL with long-term azathioprine treatment should alert us that in every patient with MG, particularly patients older than 50 years, an attempt should be made to discontinue azathioprine therapy after several years in stable remission of MG.

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14-3-3 Cerebrospinal Fluid Protein and Creutzfeldt-Jakob Disease

Martin Zeidler, MRCP

The 14-3-3 cerebrospinal fluid (CSF) protein has been shown to be an accurate test for sporadic Creutzfeldt-Jakob disease (CJD) when used for patients suspected to have this diagnosis.[1,2] In Saiz and colleagues' recent study,[3] the 14-3-3 CSF protein was detected in 10 of 80 patients with paraneoplastic neurological disorders. They state that since these disorders may mimic CJD, "if the neurologist considers CJD as the first diagnosis and orders the 14-3-3 protein assay instead of testing for anti-neuronal antibodies, a positive result could lead to the wrong conclusion that the patient has probable CJD according to new criteria." Actually, reviewing the results in Saiz and co-workers' article and other studies shows that this would be extremely unlikely if the cited World Health Organization (WHO) criteria[4] are strictly followed. Of the 10 cases reported in the article with paraneoplastic neurological syndromes and a positive 14-3-3 result, most, if not all, would not fulfil the last two lines of the WHO criteria (Table). Five would not be considered to have CJD, since CSF pleocytosis is not a feature of this disorder. Of the remaining 5 cases without CSF pleocytosis, 2 had a duration greater than 2 years and 2 more had positive paraneoplastic antibodies (which presumably would have been routinely tested in a center with the sophistication to order 14-3-3 protein testing). In the single case with a paraneoplastic neurological syndrome and a short clinical duration and negative antibodies, the clinical features are not stated, so it is not possible to tell whether they would fulfil the WHO criteria.

The WHO report cited by Saiz and associates states that, "as the 14-3-3 protein may be present in the CSF of patients with other conditions, the test is not useful as a general screening test for CJD, and should be reserved for use in cases where the diagnosis of CJD is considered a reasonable possibility." However, Saiz and colleagues' study "screened"

Table. World Health Organization Clinical Diagnostic Criteria for Sporadic Creutzfeldt-Jakob Disease

Progressive dementia
and at least two of the following four clinical features:
Myoclonus
Visual or cerebellar disturbance
Pyramidal/extrapyramidal dysfunction
Akinetic mutism
and
A typical electroencephalogram during an illness of any duration *and/or*
A positive 14-3-3 CSF assay and a clinical duration to death <2 years
Routine investigations not suggestive of an alternative diagnosis.

their cases and does not state in how many of these CJD was considered a possibility.

In the two published studies of 14-3-3 protein testing as part of national CJD surveillance,[1,2] no case with a paraneoplastic syndrome and a positive 14-3-3 result was detected among the 161 CJD suspects that turned out to have an alternate diagnosis.

For the WHO criteria for probable sporadic CJD to be useful, they must be strictly followed, and 14-3-3 CSF protein testing should not be requested indiscriminately.

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Reply

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We agree with Dr Zeidler that the 14-3-3 protein assay, or any other test in medicine, should not be requested indiscriminately. However, physicians usually order this and other tests early in the evolution of a given neurological disorder to confirm the initial clinical diagnosis. In our experience as a referral center for the 14-3-3 protein assay, the test is frequently requested as part of the early workup of patients with acute or subacute confusional or ataxic syndromes after the usual causes are initially ruled out. Paraneoplastic neurological disorders (PNDs) are different from Creutzfeldt-Jakob disease (CJD) when the full-blown clinical picture is established. However, PNDs with predominant symptoms of limbic encephalitis or cerebellar disease may share common symptoms with CJD. At the onset of the disease, magnetic resonance imaging, cerebrospinal fluid assays, or antineuronal antibodies are not always helpful,[1,2] and the short evolution has prevented the development of other typical features of CJD. In this setting, a positive 14-3-3 result may give the neurologist who orders the test the false security that the correct diagnosis is indeed CJD when it is not as we demonstrated in our article. We agree with Dr Zeidler that this is an unusual situation. Fortunately, both CJD and PND are rare disorders, but this may explain why doctors erroneously made an initial diagnosis of CJD when in fact the patient had a PND and vice versa, as we explained early in our article. At the end of the study, we found that only 1 of the PND patients had a positive 14-3-3 assay, defined as a single band in the immunoblot, so our results further support the utility of the 14-3-3 assay in the diagnosis of CJD.

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Circulating Antiganglioside Antibodies Are Not Associated with the Development of Progressive Disease or Cerebral Atrophy in Patients with Multiple Sclerosis

Gavin Giovannoni, PhD, Patricia R. Morris, BSc, and Geoffrey Keir, PhD

In a cross-sectional study, Sadatipour and co-workers[1] recently reported increased circulating antiganglioside antibodies in patients with primary and secondary progressive multiple sclerosis. They proposed that the elevated levels of these antibodies may be involved in the process of axonal damage, which occurs in multiple sclerosis (MS).[1] In an attempt to confirm their findings and to see whether antiganglioside antibodies are associated with progressive disease, we measured circulating levels of IgG and IgM antibodies against gangliosides GM1, GM3, GD1a, GT1b, and GQ1b as well as sulphatide in 29 patients with relapsing-remitting or secondary progressive MS who underwent serial brain magnetic resonance imaging (MRI) and clinical examination over a 18-month period as part of a randomized controlled trial.[2]

Disease progression over 18 months was considered significant if there was a change in Expanded Disability Status Scale score (EDSS) of at least 1 point when the starting EDSS was less than 5.5, or at least 0.5 point if the starting EDSS was 5.5 or higher. The change in EDSS had to be confirmed on at least two occasions 3 months apart. Cerebral atrophy on MRI, which correlated with the development of clinical disability in these patients, was measured according to a previously described method.[3] In each patient, cerebral volume was calculated at baseline and month 18, and then the patients were dichotomized according to whether they exceeded or stayed within the 95% confidence limits for measurement variation of the technique by month 18.[3] Antiganglioside and antisulphatide antibodies were measured using standard enzyme-linked immunosorbent assays. Stored plasma samples from month 0 and month 18 were analyzed in duplicate. To eliminate interassay variability, samples from the same patients were analyzed on the same plate. The percentage change between the mean absorbance (492 nm) at 18 months and baseline was used to indicate a relative increase or decrease in antibody activity.

There were no differences in circulating antiganglioside levels between patients who progressed clinically or devel-

Table. Relative Change in Mean Absorbance (SD) as a Percentage between Baseline and Month 18

Ig	Ganglioside	No Disease Progression (n = 19)	Disease Progression (n = 10)	*p*	No Cerebral Atrophy (n = 13)	Cerebral Atrophy (n = 16)	*p*
IgG	GM1	115% (29)	109% (22)	0.63	106% (29)	119% (24)	0.20
	GM3	99% (20)	120% (54)	0.14	116% (43)	98% (28)	0.18
	GD1a	108% (44)	97% (33)	0.48	119% (44)	92% (33)	0.08
	GT1b	115% (56)	91% (37)	0.23	127% (61)	91% (35)	0.05
	GQ1b	110% (42)	93% (42)	0.29	118% (48)	93% (34)	0.12
	Sulphatide	99% (48)	165% (154)	0.09	148% (135)	100% (58)	0.21
IgM	GM1	110% (36)	133% (139)	0.51	107% (32)	127% (111)	0.53
	GM3	126% (82)	154% (126)	0.47	155% (124)	120% (70)	0.35
	GD1	109% (37)	154% (208)	0.35	106% (34)	140% (165)	0.47
	GT1	110% (38)	134% (139)	0.48	109% (35)	125% (112)	0.64
	GQ1	107% (41)	147% (159)	0.30	101% (34)	137% (128)	0.33
	Sulphatide	104% (30)	107% (38)	0.80	98% (29)	111% (35)	0.27

oped significant cerebral atrophy and those who did not during the 18 months of follow-up (Table). No differences were noted between patients with relapsing-remitting and those with secondary progressive disease, with frequent (two or more) or infrequent (none or one) relapses and with high or low gadolinium-enhancing MRI activity between the baseline and month 18 absorbance values (data not shown). In addition, none of the absorbance values were high enough to be considered positive using our standard cut-off for a positive assay. We could not confirm the finding that antiganglioside antibodies are raised in patients with progressive MS and found no increase in circulating levels after an 18-month follow-up. These results to not support a role for serum antiganglioside antibodies in the pathogenesis of disease progression in MS.

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Reply

B. Tayyebeh Sadatipour, MBBS, Judith M. Greer, PhD, and Michael P. Pender, MD, PhD

Giovannoni and colleagues conclude that circulating antiganglioside antibodies are not associated with disease progression in multiple sclerosis (MS). For several reasons it is difficult to compare their results with the results of our study.

First, the patient populations are substantially different. It is unclear exactly how many of the patients in the study of Giovannoni and colleagues had relapsing-remitting MS and how many had secondary progressive MS, although 10 patients showed some disease progression over the 18-month course of the study. We reported that relapsing-remitting MS patients had levels of antiganglioside antibodies comparable to those of healthy controls, and we would therefore not be surprised if Giovannoni and colleagues also found that patients in this category did not have high concentrations of antibodies. In addition, we reported that patients with primary progressive MS had the highest levels of circulating antiganglioside antibodies, and a similar finding has been reported by Acarin and co-workers;[1] however, no patients with this diagnosis were included in the study by Giovannoni and colleagues.

Second, Giovannoni and colleagues state that they could not detect a single positive response to any of the gangliosides tested using their standard cut-off for a positive result. This raises the question of whether the assay used was sensitive enough to detect antiganglioside antibodies, as there are several reports in addition to ours showing that antiganglioside antibodies can be detected in the sera and cerebrospinal fluid of MS patients (see references 5–12 in our article). It would be important to know whether positive control sera were used to ensure that the assay conditions were appropriate for the detection of antiganglioside antibodies. Finally, there is no healthy control group in the study of Giovannoni and colleagues, which also makes the interpretation of their results difficult.

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Effect of Glatiramer Acetate (Copaxone) Given Orally in Human Patients: Interleukin-10 Production During a Phase 1 Trial

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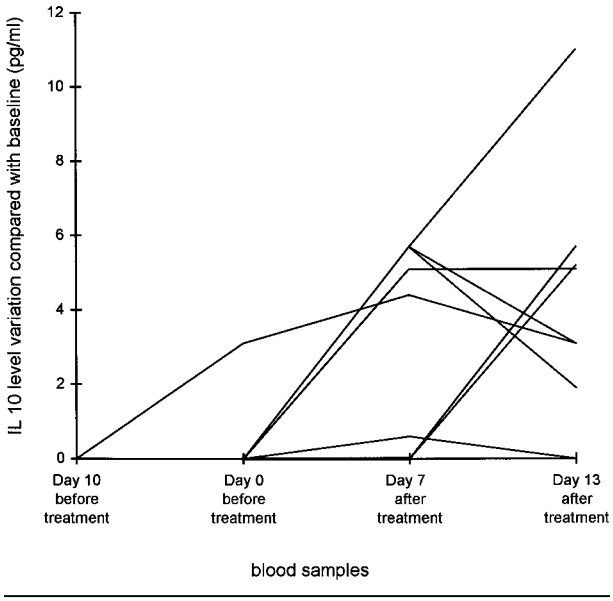
Glatiramer acetate (GA; formerly known as copolymer-1 [Copaxone]) is active in suppressing experimental autoimmune encephalomyelitis (EAE) and in the treatment of multiple sclerosis (MS) patients when injected parenterally.[1,2] Recently, GA has been demonstrated to be active when administered orally in an EAE model.[3] The aim of our study was to determine whether oral administration of GA can modify the cytokine profile in MS patients.

As part of a phase 1 trial testing an oral formulation of GA, we evaluated cytokine profiles longitudinally in 15 MS patients. MS was defined according to the criteria of Poser and his colleagues.[4] The treatment was administered orally in a randomized double-blind fashion daily for 10 days. Four patients received 20 mg/day, 4 patients received 100 mg/day, 4 patients received 300 mg/day, and 3 patients were given a placebo. The oral GA and placebo were safe and well tolerated in all patients. Blood samples were obtained for each patient twice before the treatment and then at days 7 and 13. Plasma was collected immediately and stored at −20°C. Cytokine profile was determined by using specific enzyme-linked immunosorbent kits (Immunotech; Coulter-Beckman, Mergency, France) for tumor necrosis factor-α, interferon-γ, interleukin (IL)-2, IL-4, IL-6, and IL-10.

We observed no substantial variation in levels of tumor necrosis factor-α, interferon-γ, IL-4, or IL-6. Conversely, IL-10 levels were dramatically increased in 7 of the 12 patients treated with GA (Fig.) but in none of the patients given a placebo. IL-2 levels fell in 4 of the GA-treated patients, although it remained stable in the others. There was no difference in the observed changes based on the dose of GA (20, 100, or 300 mg).

Fig. Interleukin-10 profile in glatiramer acetate–treated multiple sclerosis patients.



The predominant mechanism underlying the immunomodulatory activities of Copaxone resides in the cross-reactivity of GA with myelin basic protein (MBP) and its competition with MBP for major histocompatibility complex class II presentation. This may result in the inhibition of MBP-specific T-cell activation.[3] Oral tolerance is a well-recognized mechanism of antigen-specific peripheral immune tolerance; however, up to now, there was no evidence for an immunomodulatory effect of any orally administered treatment in MS. We found no difference in cytokine changes relative to the dose of GA, although in EAE, model disease suppression was better at low than at high doses of oral GA.[3]

A role of IL-10 has been demonstrated in peptide-induced T-cell regulation of EAE.[5] In this model, repeated peptide intranasal administration resulted in downregulation of the capacity of antigen-specific CD4+ T cells to proliferate and produce IL-2, interferon-γ, and IL-4, but it caused the increased production of IL-10. Considering the immunosuppressive properties of IL-10 and the protection from EAE induced with MBP,[6] our study showing increased IL-10 production in 7 of 12 GA-treated MS patients indicates for the first time an effect of the oral administration of GA in human beings that might be of therapeutic potential in MS patients.

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No Association between the NOS3 Codon 298 Polymorphism and Alzheimer's Disease in a Sample from the United States

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The expression and activity of endothelial NOS (eNOS) have been observed to differ between normal and aging or neurodegenerating brains.[1,2] These observations and the potential role of eNOS in LTP[3,4] have suggested that it is contributory to the Alzheimer's disease (AD) process. The eNOS gene (NOS3) has recently been reported to be associated with late-onset AD in a clinic-based sample.[5] Examining the codon 298 Glu⇒Asp polymorphism, Dahiyat and colleagues[5] found that possession of the Glu/Glu genotype conferred almost a two-fold increased risk of AD, and interaction with the apolipoprotein E (APOE) gene was also observed. We have investigated this polymorphism in 287 clinic-based late-onset probable AD cases compared with 120 population-based controls (Mini-Mental State Examination score $>$ 27) and find no evidence for an association with AD or interaction between NOS3 and APOE on the risk for AD.

In comparing the AD and control samples, the χ^2 statistic revealed no significant differences in terms of gender, age of onset/age, or ethnicity (33%/42% male; 73.0/73.3 years; 100% caucasian, 78% caucasian, 22% Hispanic) in cases and controls, respectively. As expected, significant associations with AD diagnosis were found when analyzing by APOE genotype or by ε4 carrier status ($p < 0.001$ for both). We analyzed the codon 298 polymorphism in relation to AD diagnosis by allele, genotype, possession of the Glu allele (data not shown), and Glu/Glu homozygosity as per Dahiyat and co-workers.[5] We found no evidence for an association between the NOS3 codon 298 polymorphism and AD in any of these analyses (Table). Logistic regression analysis revealed a significant contribution of APOE $\varepsilon 4^+$ genotype (one or two copies; $p < 0.0001$, $r^2 = 0.207$), no contribution of NOS3 codon 298 genotype ($p = 0.529$, $r^2 = 0.004$), and no interaction between the two ($p = 0.793$, cumulative $r^2 = 0.208$) on prediction of AD.

Therefore, in contrast to Dahiyat and colleagues,[5] we find no evidence for an association between the NOS3 gene and risk for AD in a clinic-based sample and no interaction with the APOE gene. A comparison of our case and control groups with those of the Dahiyat study shows that they are well matched in terms of gender, age/age of onset, and ethnicity. The reason for the failure to replicate this association is thus not immediately apparent, but the discrepancy may suggest undetected genetic differences between the US and UK populations that influence the NOS3 genotype distribution.

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Table. Allele and Genotype Frequencies for the NOS3 Codon 298 Polymorphism in Alzheimer's Disease Cases Compared with Controls

NOS 298	Controls	Alzheimer's Disease Cases	Odds Ratio (95% Confidence Interval)
Glu	171 (71.3%)	392 (68.3%)	1
Asp	69 (28.8%)	182 (31.7%)	1.15 (0.827–1.60)
	(N = 240)	(N = 574)	(total = 814)
		$\chi^2 = 0.694$, $df = 1$, $p = 0.405$	
Glu/Glu	61 (50.8%)	129 (44.9%)	1
Glu/Asp	49 (40.8%)	134 (46.7%)	1.29 (0.827–2.022)
Asp/Asp	10 (8.3%)	24 (8.4%)	1.13 (0.511–2.52)
	(N = 120)	(N = 287)	(total = 407)
		$\chi^2 = 1.273$, $df = 2$, $p = 0.529$	
Glu/Glu	61 (50.8%)	129 (44.9%)	1
Glu/Asp + Asp/Asp	59 (49.2%)	158 (55.1%)	1.27 (0.826–1.94)
	(N = 120)	(N = 287)	(total = 407)
		$\chi^2 = 1.176$, $df = 1$, $p = 0.278$	

Mutations in the Neuroserpin Gene Are Rare in Familial Dementia

French Alzheimer's Disease and Fronto-Temporal Dementia Genetics Study Groups*

Recently, Davis and co-workers[1] showed that two point mutations causing the Ser49Pro, Ser52Arg amino acid substitutions in neuroserpin are responsible for familial dementia in 2 kindreds. In these families, presenile dementia is associated with specific eosinophilic neuronal inclusion bodies, which are composed mainly of neuroserpin polymers. As the clinical presentation in neuroserpin families is that of nonspecific dementia with early onset and autosomal dominant transmission, we tested the potential implication of the neuroserpin gene in the two major forms of early-onset dementia with autosomal dominant inheritance: Alzheimer's disease (AD) and frontotemporal dementia (FTD). In these disorders, mutations in known genes such as those encoding the amyloid precursor protein or presenilin-1 and presenilin-2 in AD and the tau protein in FTD, respectively, account for approximately 50% and less than 20% of the families analyzed.

Seventeen index patients fulfilled the NINCDS-ADRDA criteria for probable AD,[2] and 21 fulfilled those for FTD.[3] All families were of French origin. Affected individuals in at least two successive generations suggested autosomal dominant inheritance. Mean ages at onset of the 17 early-onset AD and 21 FTD index cases were 46 $\pm$ 5 years (range, 34–60 years) and 60 $\pm$ 8 years (range, 45–77 years), respectively. The eight coding exons and the corresponding exon-intron boundaries of the neuroserpin gene were sequenced in 17 AD index patients in whom mutations in the amyloid precursor protein gene (exons 16 and 17) and presenilin-1 and presenilin-2 genes[4] were excluded and in 21 index patients with FTD in whom no mutations in the tau gene[5] were found. Neither the previously described Ser49Pro or Ser52Arg nor new causative mutations were detected. Several polymorphisms were identified, however, including two that resulted in amino acid substitutions: 21C→G (Phe7Leu) in exon 2 was found in 5 AD and 5 FTD index cases, and 838G→A (Ala280Thr) in exon 5 was seen in a single FTD index case. A silent polymorphism 51 A→G (Thr17) was detected in exon 2 in 4 FTD patients. A 32delA change in intron 6 was seen in 4 AD index cases. The silent 576G→A (Ser192) change in exon 4 was in complete linkage disequilibrium with the 21C→G variant. The two base changes resulting in amino acid substitution, Phe7Leu and Ala280Thr, were found with similar frequency in patients and 26 unaffected controls from the same population, suggesting that they do not represent genetic susceptibility factors for dementia in these families. The analysis of this large series of patients shows that variants in the neuroserpin gene do not account for a significant proportion of either early-onset AD or FTD with autosomal dominant inheritance. It would be interesting to determine if the two variants with amino acid substitutions identified in this study modify the function of the neuroserpin protein and if they might represent genetic susceptibility factors for other dementias.

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