Bystander Modulation of Chemokine Receptor Expression on Peripheral Blood T Lymphocytes Mediated by Glatiramer Therapy

Rameeza Allie, MS; Lina Hu, PhD; Katherine M. Mullen, BA; Suhayl Dhib-Jalbut, MD; Peter A. Calabresi, MD

Background: Glatiramer acetate therapy is thought to be effective for multiple sclerosis (MS) by promoting T$_{H}2$ cytokine deviation, possibly in the brain, but the exact mechanism and site of action are incompletely understood. Determining the site of action and effect of glatiramer on cell trafficking is of major importance in designing rational combination therapy clinical trials.

Objective: To determine whether glatiramer therapy will also act in the peripheral blood through bystander modulation of chemokine receptor (CKR) expression and cytokine production on T lymphocytes.

Design: Before-and-after trial.

Setting: A university MS specialty center.

Patients: Ten patients with relapsing-remitting MS.

Interventions: Treatment with glatiramer for 12 months and serial phlebotomy.

Main Outcome Measures: Cytokine production, CKR expression, and cell migration.

Results: The glatiramer-reactive T cells were T$_{H}2$ cytokine biased, consistent with previous studies. We found a significant reduction in the expression of the T$_{H}1$ inflammation associated with the CKRs CXCR3, CXCR6, and CCR5 on glatiramer- and myelin-reactive T cells generated from patients with MS receiving glatiramer therapy vs baseline. Conversely, expression of the lymph node-homing CKR, CCR7, was markedly enhanced on the glatiramer-reactive T cells derived from patients with MS undergoing glatiramer therapy. There was a reduction in the percentage of CD4$^+$ glatiramer-reactive T cells and an increase in the number of CD8$^+$ glatiramer-reactive T cells.

Conclusions: Glatiramer may suppress autoreactive CD4$^+$ effector memory T cells and enhance CD8$^+$ regulatory responses, and bystander modulation of CKRs may occur in the periphery.

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Herein, we sought to determine whether glatiramer-reactive T cells derived from patients with MS receiving this therapy have a significant reduction in chemokine receptors (CKRs) associated with effector cell homing to sites of inflammation but enhancement of a more naive lymph node-homing phenotype. This would support the possibility that glatiramer may also act peripherally in the lymph nodes through bystander suppression.
METHODS

Human peripheral blood mononuclear cells were obtained from 10 patients with MS before and at various times after therapy with glatiramer after obtaining informed consent according to an institutional review board–approved protocol at the University of Maryland, Baltimore. Patients had not been previously treated with any immune-modulating drugs. Antigen-specific T-cell lines (TCLs) were generated from peripheral blood mononuclear cells using a split-well assay and were then studied using 4-color fluorescent-activated cell sorter analysis on day 7 after restimulation (21 total days in culture), as reported previously.9 Supernatants were collected for cytokine measurements after antigen restimulation and were frozen until assayed. Interferon γ (IFN-γ) and interleukin 5 levels were measured as markers of TH1 and TH2 phenotypes using a sandwich enzyme-linked immunosorbent assay according to the manufacturer's recommendations (Biosource International, Camarillo, Calif). Cell migration in response to chemokines was measured using 24-well chambers separated by a polycarbonate filter with a 5-µm pore size (Transwell; Corning Costar, Cambridge, Mass). The cells that transmigrated into the lower compartment were counted in a hemacytometer. Results are expressed as a migration index that is the ratio between the numbers of migrating cells in the chemokine-containing chamber and in the medium control chamber, which is normalized to a value of 1. All migration conditions were run in triplicate.

Statistical analyses were performed using a software program (Prism; GraphPad Software, San Diego, Calif). Comparisons between times or culture conditions were made using either a 2-tailed t test (parametric data) or a Mann-Whitney U test (nonparametric data). In serial analyses, significance was determined using 1-way analysis of variance with the Dunnett multiple comparison test. Significance was defined as P<.05.

RESULTS

We first determined the IFN-γ and interleukin 5 cytokine profiles of 372 antigen-specific (122 glatiramer, 122 myelin basic protein [MBP], and 128 tetanus toxoid [TT]) TCLs at visit 1 (baseline, before initiation of therapy) and at visit 2 (after 12 months of treatment with glatiramer). In vivo treatment of patients with MS taking glatiramer resulted in a significant TH2 bias in cytokine production not only from glatiramer-reactive T cells but also from a dampening of the MBP- and TT-reactive TH1 cell cytokine release (Figure 1).

Next we analyzed the CKR profiles of these TCLs (Figure 2). At baseline, glatiramer-reactive TCLs expressed high levels of the TH1-associated CKRs CXCR3, CXCR6, and CCR5. This was not significantly different from the levels of expression of the CKRs on MBP- or TT-reactive TCLs. However, after 12 months of therapy with glatiramer, the ex vivo–generated glatiramer-reactive T cells had significant reductions in the expression of all of these T12-biased T cells and had significant reduction in the expression of all of these T12-biased T cells. Conversely, there was markedly elevated expression of the lymph node–homing CKR CCR7 (Figure 2B). We also examined the expression of 1 CKR, CCR4, that has been linked to T2 effector cells, and found that it too was reduced after glatiramer therapy (Figure 2E). A critical finding was that the reduction in CKR expression was not specific for glatiramer-reactive T cells.
because MBP- and, to some extent, even TT-reactive TCLs also had decreased expression of effector CKRs (Figure 2F and Table). This effect was most notable in CD4 subsets, but in some circumstances, CD8 cells had significant and concomitant shifts in CKR expression (Figure 2 and Table).

We next performed a serial examination of CKR expression across time to determine the kinetics of this response and whether the changes were sustained. The reduction in CXCR3 expression could be seen at 6 months, but the increase in CCR7 expression was not seen until 1 year, suggesting that these switches may be dissociated (Figure 3).

The functional relevance of the observed shifts in CKR expression was confirmed using migration assays (Transwell) (Figure 4). At baseline, glatiramer-reactive TCLs migrated best to the chemokine CXCL-10 (CXCR3 ligand), consistent with their high expression

![Figure 2](image-url)
Table. Chemokine Receptor Expression on Antigen-Specific T-Cell Lines Before (V1) and After 12 Months of (V2) Glatiramer Therapy*

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<td><strong>Glatiramer acetate</strong></td>
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<td>V1</td>
<td>74.4 ± 1.9</td>
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<td><strong>P value†</strong></td>
<td>.001</td>
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<td>V1</td>
<td>78.5 ± 2.9</td>
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<td>V1</td>
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<td>37.3 ± 4.5</td>
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<td>38.4 ± 2.6</td>
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<td>V2</td>
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<td><strong>P value†</strong></td>
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Abbreviations: MBP, myelin basic protein; TT, tetanus toxoid; V1, visit 1; V2, visit 2.
*Data are given as mean ± SEM percentages. CXCR3, CXC6, CCR5, and CCR7 are chemokine receptors.
†For V1 vs V2.

Figure 3. Serial analysis of mean CXCR3 (A) and CCR7 (B) (chemokine receptors) expression on CD4+ glatiramer acetate–specific T-cell lines at baseline (month 0) and at months 6, 12, and 16 after initiation of glatiramer therapy. Significant differences were determined using 1-way analysis of variance (ANOVA), and posttest analysis of change from baseline was made using the Dunnett multiple comparison test. Error bars represent SEM.

COMMENT

We demonstrated that in vivo therapy with glatiramer causes a statistically significant reduction in the expression of CKRs associated with T<sub>H1</sub> cell homing to tissue sites of inflammation. Specifically, CXCR3 and CCR5, which have been demonstrated on pathogenic infiltrating T cells in MS brain tissue, and CXCR6, which is expressed on T<sub>H1</sub> cells in the periphery, were reduced on not only the glatiramer TCL but also the MBP TCL, suggesting that bystander modulation of CKRs occurs in the peripheral blood compartment of glatiramer-treated patients with MS. Alternatively, the lymph node–homing CKR, CCR7, was significantly elevated after 1 year of therapy. The observed switch in CKR expression is reminiscent of the recently described paradigm of effector memory T cells being CD45RA<sup>−</sup>/CCR7<sup>+</sup> and central memory T cells being CD45RA<sup>−</sup>/CCR7<sup>+</sup>. Our results are consistent with the concept that glatiramer may bias against effector memory T cells through continued weak affinity interactions with cross-reactive T-cell receptors such as MBP, as has been previously demonstrated. Because glatiramer is a large copolymer and does not access the CNS itself, it has been postulated that glatiramer-reactive cells mediate bystander suppression in the CNS through cross-reactivity with myelin antigens being presented by microglial cells; indeed, this has been shown to the alternative strong T<sub>H1</sub>–associated chemokine RANTES (regulated upon activation, normal T-cell expressed and secreted) (CCR5 ligand) at either time.

Finally, we observed a significant reduction in glatiramer-reactive CD4<sup>+</sup> cells at visit 1 vs visit 2 (mean±SEM: 79%±2% vs 62%±3%; P<.001) and an increase in CDS<sup>+</sup> cells after initiation of glatiramer therapy (mean±SEM [visit 1 vs visit 2]: 20%±2% vs 35%±3%; P<.001) (Figure 5). Although there was a trend toward decreased proliferation as measured by the stimulation index, this was not significant (mean±SEM [visit 1 vs visit 2]: 7%±1% vs 5%±1%; P=.15).
in experimental autoimmune encephalomyelitis.4 Our data provide an explanation for how glatiramer itself could suppress the number of myelin-specific effector memory T cells migrating into the CNS through bystander modulation in the peripheral lymph nodes. The timing of the observed CKR conversion is also consistent with a delayed immunologic mechanism of action and a radiologic response, as seen in the serial magnetic resonance imaging–based clinical trial.11 Long-term follow-up12 of glatiramer–treated patients with MS suggests that an unusually high number of patients exhibit sustained disease quiescence after years of therapy. The data herein support the possibility that glatiramer could induce tolerance to pathogenic myelin-reactive T cells that results in sustained remissions in some patients. Tolerance may occur through bystander modulation by T1,2-secreting CD4+ T cells, regulatory CD8+ T cells, or both, as was recently demonstrated.13 It is also possible that either the T1,2 milieu or even a direct effect of glatiramer on antigen-presenting cells could promote a T1,2 bias of naive-responding T cells with cognate T-cell receptor for myelin antigens, as has been seen by other investigators.6,14

Several recent studies15-17 have focused on ways of enhancing T1,2 immune deviation with agents that are known to act systemically, including IFN-γ, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins), and albuterol. Furthermore, a large double-masked, placebo-controlled trial of combination therapy with IFN-β and glatiramer acetate is planned. Our data provide further rationale for combination therapy with IFN-β or other agents for 2 reasons.18 A peripheral mechanism of action characterized by selective inhibition of T1,1 effector memory T cells would be complementary to the effects of IFN-β, which acts systemically and at the blood-brain barrier. In addition, in cases in which IFN-β does not completely eliminate gadolinium-enhancing lesions and blood-brain barrier permeability, it would be preferable to have T1,2 influx with brain-derived neurotrophic factor–secreting cells that may diminish the likelihood of T1 black hole formation, as opposed to the case with IFN-β, in which T1 black hole formation seems to be unaffected when an enhancing lesion forms.19

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Author Contributions: Study concept and design: Calabresi. Acquisition of data: Allie, Hu, and Mullen. Analysis and interpretation of data: Allie, Dhib-Jalbut, and Calabresi. Drafting of the manuscript: Allie and Calabresi. Critical revision of the manuscript for important intellectual content: Hu, Mullen, Dhib-Jalbut, and Calabresi. Statistical analysis: Calabresi. Obtained funding: Calabresi. Administrative, technical, and material support: Allie, Hu, Mullen, and Dhib-Jalbut. Study supervision: Calabresi.

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REFERENCES


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