



## Immunological mechanisms underlying delayed-type hypersensitivity reactions to glatiramer acetate

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### ABSTRACT

**Background:** Delayed-type hypersensitivity to glatiramer acetate is rare, and the underlying immunological mechanisms are not completely understood.

**Objective:** To study the immunologic response in 2 patients with multiple sclerosis who developed maculopapular exanthema related with the administration of glatiramer acetate.

**Methods:** The allergologic study included general blood tests, viral serologic tests, and skin tests (patch and intradermal tests). The immunologic study was performed in skin biopsy specimens by immunohistochemistry and in the peripheral blood by flow cytometry and the lymphocyte transformation test.

**Results:** Skin test results were negative in both patients, and the diagnosis was confirmed by a drug provocation test. The evaluation of the acute phase showed an increase in the percentage of CD8 T lymphocytes (>50%) and the percentage of cells expressing skin-homing receptor (cutaneous lymphocyte-associated antigen) (>70%) and chemokine receptors (CCR4 and CXCR3) at T1. A positive proliferative response was observed in T lymphocytes (stimulation index [SI] = 3.5 in patient 1 and 3.59 in patient 2), especially the CD8<sup>+</sup> subpopulation (SI = 5.5 and 4.6 in patients 1 and 2, respectively), and NK lymphocytes (SI = 3.9 and 8.5 in patients 1 and 2, respectively) after glatiramer acetate stimulation.

**Conclusion:** This study demonstrates the important role of T<sub>H</sub>1 cells expressing skin-homing receptors in delayed-type hypersensitivity reactions to glatiramer acetate. A lymphocyte transformation test revealed a specific glatiramer acetate recognition by T lymphocytes and NK lymphocytes.

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### Introduction

Glatiramer acetate (Copaxone; Sanofi-Aventis, Barcelona, Spain) is a random mixture of synthetic polypeptides composed of L-glutamic acid, L-lysine, L-alanine, and L-tyrosine that is used for treating patients with relapsing-remitting multiple sclerosis (MS).<sup>1</sup> The therapeutic efficacy of glatiramer acetate has been attributed to the induction of an immune deviation from a T<sub>H</sub>1 to a T<sub>H</sub>2 response,<sup>2</sup> changes in antigen-presenting cells,<sup>3</sup> induction of CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells,<sup>4</sup> and expansion of CD8<sup>+</sup> T cells with regulatory capacity.<sup>5</sup>

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Administration of glatiramer acetate can induce adverse cutaneous events that are usually mild, such as injection site reactions, which appear in nearly 50% of patients undergoing treatment,<sup>6</sup> or systemic postinjection reactions, appearing in up to 10% of patients and involving symptoms similar to anaphylaxis, although they are not IgE mediated.<sup>7</sup> Immediate hypersensitivity reactions, such as anaphylaxis, with IgE involvement are rare.<sup>8,9</sup> These local and systemic reactions can hamper treatment, although desensitization has been used with good results.<sup>10</sup>

A few cases of delayed-type hypersensitivity (DTH) to glatiramer acetate have been described, usually concerning necrotizing lesions, erythema nodosum, vasculitis, or contact dermatitis,<sup>11–13</sup> although the immunologic mechanisms involved have not yet been sufficiently assessed. In the present study, we evaluated 2 patients with MS who developed a DTH after glatiramer acetate administration and in whom a T-cell-dependent mechanism has been demonstrated.

## Methods

### Patients

Two patients with confirmed nonimmediate hypersensitivity reactions to glatiramer acetate were included in the study. Evaluation of the acute phase of the reaction included blood samples collected for phenotypic analysis 24 hours (T1) and 5 days (T2) after the appearance of the symptoms and 30 days after the symptoms cleared (T3). A skin biopsy specimen of the lesion was obtained at T1.

The allergological workup was performed 3 months after the clearance of the reaction and included *in vivo* (skin testing and drug provocation test [DPT]) and *in vitro* (lymphocyte transformation test [LTT]) tests.

The results obtained in the 2 patients were compared with those obtained from 10 controls with MS treated with glatiramer acetate but presenting a good tolerance to this drug. The study was approved by the relevant institutional review boards, and written informed consent for the allergologic and laboratory procedures was obtained from the patients and controls.

### Phenotype immunofluorescence analysis

Phenotype immunofluorescence analysis was performed by flow cytometry in peripheral blood mononuclear cells isolated by Ficoll density gradient (Nycomed As, Oslo, Norway), as previously described,<sup>14</sup> using the following monoclonal antibodies: for different cell subsets (CD3, CD56, CD4, and CD8), cutaneous lymphocyte-associated antigen (CLA), and chemokine receptors (CXCR3 and CCR4) (all from Becton-Dickinson, San Jose, California). Cells were acquired on a FACScalibur flow cytometer and analyzed using Cell Quest software (both from Becton-Dickinson, San Jose, California). Results were expressed as the percentage of positive cells.

### Skin biopsy

Microtome sections (8 mm) were processed as previously described<sup>14</sup> for hematoxylin-eosin and immunohistochemical staining with the following monoclonal antibodies: CD3, CD4 (Novocastra Lab, Newcastle upon Tyne, United Kingdom), CD8, CD57, HLA-DR (Dako, Ely, Cambridgeshire, United Kingdom), CLA (BD Pharmingen, San Diego, California), and CXCR3 (Acris Antibodies, Hiddenhausen, Germany).

### Skin testing

Intradermal tests with 24- to 48-hour readings were performed in the volar forearm using glatiramer acetate at concentrations ranging from 0.2 to 20  $\mu\text{g/mL}$  as previously described.<sup>9,10</sup> Patch testing with undiluted glatiramer acetate was also performed. In the intradermal tests the wheal area was marked initially and at 20 minutes, 1 to 3 days after testing, and an erythematous induration at the skin test site in the delayed readings was considered a positive result. For patch tests, patches were removed and read at 48 hours, with an additional reading at 72 hours, and the result was considered positive according to the European Society of Contact Dermatitis.<sup>15</sup>

### Drug provocation test

A single-blind, placebo-controlled DPT was performed in those patients with a negative skin test result as described by the European Network for Drug Allergy group.<sup>16</sup> Glatiramer acetate at 20 mg/mL was administered subcutaneously at different doses at regular intervals. To control any adverse effect and to avoid severe systemic reactions, this was performed in 2 runs separated with sufficient time to detect reactions appearing later than 48 hours. In the first run, 0.1, 0.2, and 0.3 mL of glatiramer acetate were administered, and, if this was well tolerated, 1 week later glatiramer acetate was administered at 0.5 and 0.5 mL.

### Lymphocyte Transformation Test

The LTT was performed in the presence of glatiramer acetate at 5, 25, and 50  $\mu\text{g/mL}$  and tetanus toxoid and lipopolysaccharide as positive stimuli. Lymphocytes were stained with 0.5mM carboxy-fluorescein succinimidyl ester (CFSE; Cell Trace CFSE Cell Proliferation kit for flow cytometry, Molecular Probes Inc, Eugene, Oregon), cultured with the different stimuli for 7 days, and then stained with anti-CD3, CD4, CD8, and CD56 monoclonal antibodies and acquired on a FACScalibur cytometer. Proliferative cells were shown as the percentage of CFSE<sup>dim</sup> cells and results expressed as the stimulation index (SI), calculated as the ratio of the percentage of CFSE<sup>dim</sup> in glatiramer acetate-stimulated cells vs nonstimulated cells, and considered positive if greater than 3.0.

## Results

### Evaluation of the acute phase of the reaction

Patient 1 was a 48-year-old woman with MS who experienced generalized maculopapular exanthema 5 months after starting glatiramer acetate treatment and 30 hours after the last dose. Symptoms gradually disappeared in 6 days after stopping glatiramer acetate administration and initiating corticosteroid treatment.

Patient 2 was a 40-year-old woman with MS who developed an exanthema that affected the face and trunk 24 hours after the administration of the fourth course of glatiramer acetate that rapidly generalized during 2 days and disappeared in 9 days after stopping glatiramer acetate administration and initiating corticosteroid treatment.

In both patients, a viral infection caused by parvovirus B19, cytomegalovirus, Epstein-Barr virus, human herpesvirus 6, hepatitis B virus, hepatitis C virus, and human immunodeficiency virus was ruled out by viral serologic testing.

A skin biopsy specimen was obtained from both patients at the acute phase of the reaction (Fig 1). Hematoxylin-eosin staining revealed a perivascular mononuclear cell infiltrate, predominantly in the dermis, with no presence of eosinophils. The immunohistochemical study revealed a T-cell infiltrate with both CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes, although there was a predominance of the latter in patient 1 (Fig 1A). Infiltration of natural killer (NK) cells (CD57<sup>+</sup>) was found in both patients but was higher in patient 2 (Fig 1B). Similar levels of activation (HLA-DR), skin homing (CLA), and T<sub>H</sub>1 chemokine receptor (CXCR3) were found in the 2 skin biopsy specimens.

The phenotype of peripheral blood mononuclear cells was determined by immunofluorescence analysis, revealing no differences in the percentage of CD4<sup>+</sup>, CD8<sup>+</sup>, or NK cells (CD56<sup>+</sup>) in sequential samples from the 2 patients. Notably, the normal CD4:CD8 ratio (2:1) was altered because of the high percentage (>50% at T1) of CD8<sup>+</sup> T lymphocytes, whereas the levels of CD4<sup>+</sup> T lymphocytes were similar (Fig 2A). In addition, there was an important increase in the expression of the skin-homing marker CLA in CD3<sup>+</sup> T lymphocytes at the early stage of the reaction: 70.54% in patient 1 and 76.9% in patient 2, decreasing gradually 5 and 30 days later. This increase appeared in the CD4<sup>+</sup> (54% and 51.24%, respectively) and CD8<sup>+</sup> (43.1% and 45.77%, respectively) subpopulations, indicating the migration of these cells to the skin (Fig 2B). Concerning the skin-homing chemokine receptor CCR4, changes were mainly detected in the CD8<sup>+</sup> subpopulation, from 19% to 6% in patient 1 and from 19.3% to 10.6% in patient 2 (Fig 2C). High expression of the T<sub>H</sub>1 chemokine receptor CXCR3 was found in the CD4<sup>+</sup> and CD8<sup>+</sup> subpopulations, indicating a recruitment of T<sub>H</sub>1 cells to the skin (Fig 2D). No changes in all these markers were detected in the control group during glatiramer acetate treatment.

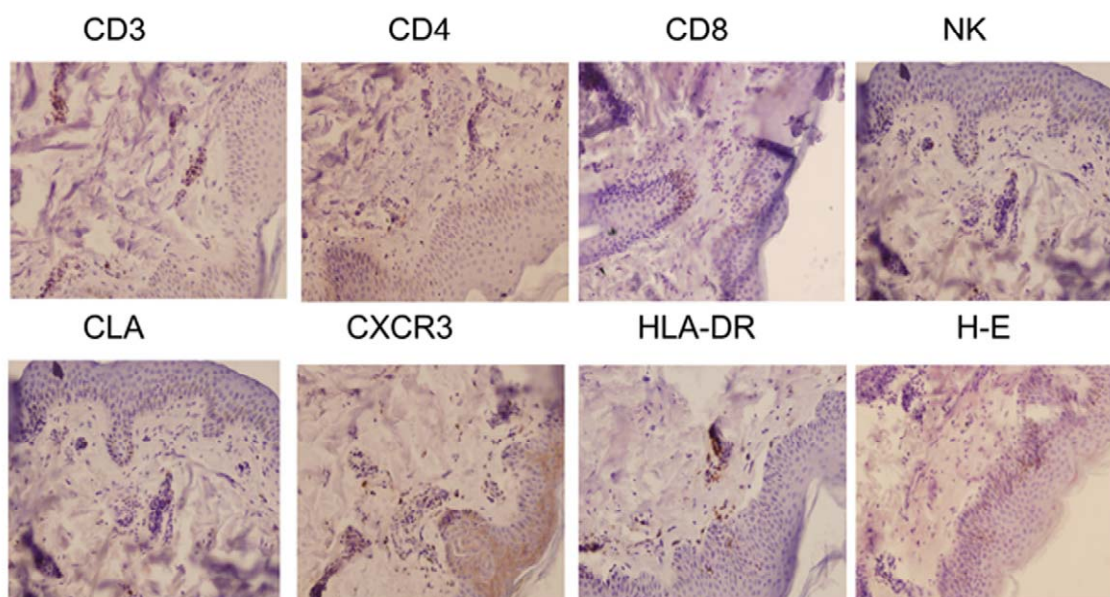
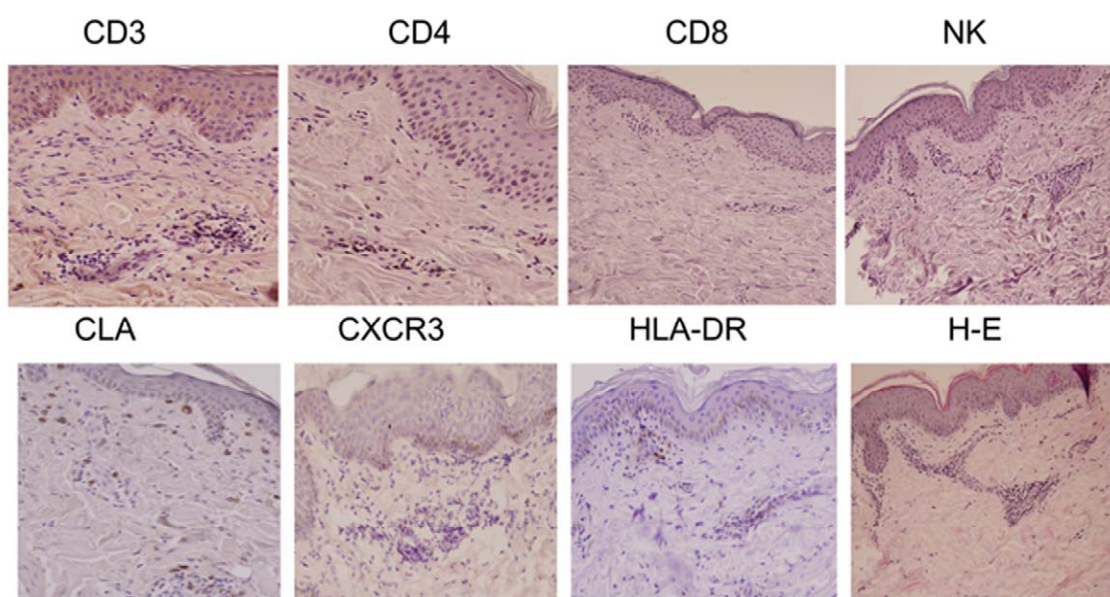
**Patient 1****Patient 2**

**Fig. 1.** Immunohistochemical analysis of different markers (CD3, CD4, CD8, natural killer [NK], cutaneous lymphocyte-associated antigen [CLA], CXCR3, HLA-DR, and hematoxylin-eosin [H-E]) in a skin biopsy specimen obtained from the acute reaction in patients 1 and 2.

*Allergological workup*

Both patients had negative intradermal test results at the immediate and delayed readings. Patch test results were also negative in both patients. Both patients had positive DPT results. Patient 1 developed a generalized maculopapular exanthema 16 hours after glatiramer acetate administration (0.6 mL), which persisted for 48 hours, and patient 2 had a maculopapular exanthema that affected the face and trunk 24 hours after glatiramer acetate administration (1 mL).

The LTT after stimulation with glatiramer acetate at different concentrations (5, 25, and 50  $\mu\text{g/mL}$ ) revealed a positive proliferative response of T lymphocytes ( $\text{CD3}^+$ ) in both patients ( $\text{SI} = 3.5$  in patient 1 and 3.59 in patient 2). The highest response in patient 1 was detected in  $\text{CD8}^+$  cells ( $\text{SI} = 5.5$ ) followed by NK cells ( $\text{SI} = 3.9$ ),

whereas the highest proliferative response in patient 2 was detected with NK lymphocytes ( $\text{SI} = 8.5$ ) followed by  $\text{CD8}^+$  T lymphocytes ( $\text{SI} = 4.6$ ). In both patients the lowest response was detected with  $\text{CD4}^+$  cells, although it was still positive in patient 1 ( $\text{SI} = 3.3$ ). In the controls ( $n = 10$ ), no proliferation was detected after glatiramer acetate stimulation at any of the concentrations assayed in any of the subpopulations analyzed (Fig 3).

**Discussion**

We describe 2 patients who developed maculopapular exanthema related to the administration of glatiramer acetate. Both patients were skin test negative, as has been previously described in 1 patient with contact dermatitis.<sup>13</sup> However, in low-molecular-weight compound-induced allergy, as occurs with glatiramer ace-



