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Original research article

Lymphocyte populations and their change during five-year glatiramer acetate treatment

Zbyšek Pavelek [a,*], Oldřich Vyšata [a], Lukáš Sobíšek [b], Blanka Klímová [a], Ctirad Andrýs [c], Doris Vokurková [c], Radka Mazurová [a], Pavel Štourač [d], Martin Vališ [a]

[a] Department of Neurology, Faculty of Medicine and University Hospital Hradec Králové, Charles University in Prague, Hradec Králové, Sokolská 581, 500 05, Czech Republic
[b] Department of Statistics and Probability, University of Economics in Prague, Prague, Czech Republic
[c] Department of Clinical Immunology and Allergology, University Hospital Hradec Králové, Hradec Králové, Sokolská 581, 500 05, Czech Republic
[d] Department of Neurology, Masaryk University Brno, Jihlavská 20, 625 00, Czech Republic

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ABSTRACT

Background: The goal of this study was to determine the characteristics that are affected in patients treated with glatiramer acetate (GA).
Methods: A total of 113 patients were included in this study. Patients were treated with glatiramer acetate (subcutaneous injection, 20 mg, each day). Peripheral blood samples were obtained just prior to treatment as well as 5 years after GA treatment. All the calculations were performed with the statistical system R (r-project.org).
Results: After 5 years of treatment, a significant decrease was found in the absolute and relative CD3+/CD69+ counts, the absolute and relative CD69 counts, the relative CD8+/CD38+ count and the relative CD38 count. A significant increase was found in the absolute and relative CD5+/CD45RA+ counts and the absolute CD5+/CD45RO+ count after 5 years of treatment.
Conclusion: This study presents some parameters that were affected by long-term GA treatment.

1. Introduction

Multiple sclerosis (MS) is a disease characterized by both relapses and insidious progression and is notably heterogeneous in clinical course, symptomatology, and severity [1]. Despite considerable progress in the diagnosis and treatment of this serious disease, the aetiology of MS remains unknown. Genetic and environmental factors are assumed to play a role in the pathogenesis of the disease [2]. Environmental influences include vitamin D deficiency, insufficient exposure to sunlight, viral infections, stress, smoking and intestinal microbiota [3–7].

* Corresponding author.
E-mail address: zbysekpavelek@email.cz (Z. Pavelek).
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Immune responses caused by genetic dispositions are likely to have a primary role in the pathogenesis of MS. A ''genome-wide association study'' clearly proved that there is a genetically determined dysfunction of T and B cells [8,9]. The HLA (human leucocyte antigen) system demonstrably influences the development of MS. The carriers of HLA-DRB5*0101, HLA-DRB1*1501, HLA-DQA1*0102 and HLA-DQB1*0602 alleles are at increased risk [10]. In an individual with genetic predisposition, the influence of external factors causes the development of inflammatory responses leading to demyelination and axonal and neuronal loss [11,12].

MS is one of the autoimmune diseases with a well-explored pathogenesis. The reasons for such research are the extraordinary clinical seriousness of the disease and the experimental animal model for MS that has existed for several decades. The experimental animal model is called experimental autoimmune encephalomyelitis (EAE). According to classic conceptions of immune system functions, the onset and development of the autoimmune immunopathological reaction are caused by disruption of the recognition and self-tolerating mechanisms. Although T-lymphocytes are considered the key component of autoimmune disease pathogenesis (and thus MS), the role of humoral immunity components in damaging inflammatory reactions cannot be ignored.

One of the basic medications for treating clinically isolated syndrome (CIS) or relapsing-remitting MS is glatiramer acetate (GA). GA, originally named Copolymer 1, was developed in the 1960s in the Weizmann Institute of Science in the Israeli town of Rechovot. GA is a mixture of synthetic polypeptides containing L-amino acids, glutamic acid, alanine, lysine and tyrosine. Paradoxically, GA was originally synthesised with intentions to induce EAE. In 1971, Teitelbaum et al. showed that GA suppressed the induction of acute EAE. The mechanism of action of GA is not precisely understood. The effect of GA is immunomodulatory [13]. GA relieves inflammation and has neuroprotective properties. GA causes rearrangement from activity of the Th1 subset of T-lymphocytes to activity of the Th2 subset of T-lymphocytes [14]. The Th1 subset is responsible for the development of cytotoxic reactions. Th1 T-lymphocytes produce proinflammatory cytokines such as interferon γ (INFγ), tumour necrosis factor β (TNFβ) and interleukin 2 (IL-2). In contrast, the Th2 subset of T-lymphocytes acts antagonistically to the Th1 subset and produces anti-inflammatory cytokines such as IL-4 and IL-13. With antigen-presenting cells such as monocytes and dendritic cells, it changes the function of CD4+ and CD8+ T-lymphocytes. It binds with high affinity to MHC (major histocompatibility complex) class II molecules on cells presenting MBP (myelin basic protein) and thus prevents the advancement of antigen fragments derived from MBP. In other words, it competes with MBP immunogen fragments for receptors on autoreactive T-lymphocytes and presumably energises them or causes their apoptosis [15]. GA-reactive T cells can deliver brain-derived neurotrophic factor (BDNF) to neurons, which upregulates the corresponding full-length signalling receptor tyrosine kinase gp145 trkB in multiple sclerosis lesions [16,17].

In this study, individual populations of lymphocytes in MS patients (CIS and relapsing-remitting MS) were examined. The goal was to determine the populations that are affected in patients treated with GA.

2. Material and methods

2.1. Population sample

All the subjects (aged from 17 to 55, mean age 35 ± 9) were of Caucasian origin and fulfilled the McDonald criteria or revised McDonald criteria for RR MS [18–20]. Between 2008–2017, a total of 113 patients (25 men and 88 women) were included in the study. The patients were treated by GA (subcutaneous injection, 20 mg per day). Seventy-two patients completed the study (12 men and 60 women), and 41 patients dropped out prematurely because of treatment ineffectiveness or intolerance (13 men and 28 women).

All the participants were recruited during their hospital visit, during which all relevant MS information (Expanded Disability Status Scale (EDDS), disease duration, MS treatment history) was obtained. Clinical evaluations were performed by an attending neurologist. Peripheral blood samples were obtained just prior to treatment and 5 years after GA treatment.

All the participants gave written informed consent.

2.2. Sample collection and sample processing

Blood samples were collected from the antecubital fossa vein. Relative numbers of clusters of differentiated CD3+, CD4+, CD8+, CD19+, CD3-/CD16+56+, CD3+CD69+, CD3+CD25+, CD4+/CD45RA+, CD4+/CD45RO+, CD8+/CD38+, CD19+/CD5+, CD40 and CD40L lymphocytes were analysed by two-colour flow cytometry. For surface staining, 100 l of blood was added to tubes containing 10 l of a cocktail of fluorochrome-labelled mAbs. mAbs used included fluorescein isothiocyanate (FITC)-conjugated anti-CD3 (clone UCHT1), anti-CD4 (clone 13B8.2), anti-CD45RA (clone ALB11), anti-CD8 (clone B9.11) and anti-CD19 (clone J3-119) and phycoerythrin (PE)-conjugated anti-CD25 (clone B1.49.9), anti-CD69 (clone TP1.55.3), anti-CD4 (13B8.2), anti-CD45RO (clone UCHL1), anti-CD38 (clone LS198-4-3), anti-CD5 (clone BL1a), anti-CD40 (clone MAB89) and anti-CD40L (clone TRAP-1) all supplied by Beckman Coulter (Miami, FL, USA). Class-matched isotype immunoglobulin FITC- and PE-conjugated negative control monoclonal antibodies were added simultaneously to separate tubes for all the samples to detect nonspecific binding.

Subsequently, 100 l of heparinised peripheral blood was mixed with the monoclonal antibody cocktail and was incubated for 15 min at room temperature. After the incubation, lysing solution (OptiLyse C, Beckman Coulter) was added, and the mixture was incubated for a further 10 min. Flow cytometric analysis was performed using Cytomics FC 500 cytometer (Beckman Coulter) equipped with a 15-mW air-cooled 488-nm argon laser and a 625-nm neon diode laser, and the data were analysed using the CXP Analysis Software (Beckman Coulter). Data on at least 10,000 events were acquired for each staining and stored as list mode.

2.3. Statistical analysis

We compared paired values of parameters of peripheral blood samples at baseline and the end of the follow-up (after five years

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