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Predictive cytokine biomarkers of clinical response to glatiramer acetate therapy in multiple sclerosis☆

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ABSTRACT

A prospective study of 62 patients with relapsing-remitting multiple sclerosis (RRMS) treated with Glatiramer acetate (GA) was conducted to evaluate the value of baseline and treatment-modulated cytokines in predicting the clinical response to the drug after 2 years of therapy. There were 32 responders and 30 non-responders. GA upregulated Th2/regulatory cytokines and inhibited Th1 cytokines in sera or PBMC supernatants 3 and 6 months into treatment. We found two prognostic models with clinical utility. A model based on IL-18 at baseline, the change in TNFa from baseline to 3 months, the change in IL-4 from baseline to 6 months, and the change in the log of the ratio of TNFa/IL-4 from baseline to 6 months had an area under the curve (AUC) of 0.80. A high IL-18 level at baseline and a reduction of TNF-alpha over time are associated with a response to GA. Although the study identified predictive biomarkers of clinical response to GA, the results will need to be validated in other data sets.

1. Introduction

Glatiramer acetate (GA) is an approved drug for the treatment of relapsing-remitting (RR) multiple sclerosis (MS) and is effective in reducing relapse rate and disability accumulation (Martinelli et al., 2003; Mikol et al., 2008; Cadavid et al., 2009). GA is generally well tolerated; however, response to the drug is variable. The therapeutic effect of GA is supported by the results of magnetic resonance imaging (MRI) findings from various clinical trials (Comi et al., 2001; Zivadinov et al., 2015; Khan et al., 2012). However, to date there are no validated predictive biomarkers of response to GA treatment.

The exact mechanism of action of GA in MS is still unclear. Although we believe that mechanisms of action of GA do not seem to be antigen specific, or even multiple myelin antigen specific, we theorize that antigen-based therapy generating GA-specific immune responses seem to be a prerequisite for GA therapy. A possible initial mode of action of GA is associated with binding to MHC molecules and consequent competition with various myelin antigens for their presentation to T cells (Fridkis et al., 1999; Arnon et al., 1996). A further aspect of its mode of action is the induction of T regulatory cells (T-regs) that presumably can migrate to the brain and lead to in situ bystander suppression (Dhib-Jalbut et al., 2003). We and others have previously shown that GA treatment in MS results in the induction of GA-specific T cells with predominant Th2 phenotype both in response to GA and cross-reactive myelin antigens (Chen et al., 2001; Weber et al., 2007; Aharoni et al., 2003). Furthermore, the ability of GA-specific infiltrating cells to express anti-inflammatory cytokines such as IL-10 and transforming growth factor-beta (TGF-β) together with brain-derived neurotrophic factor (BDNF) seem to correlate with the therapeutic activity of GA in EAE (Sarchielli et al., 2007).

Initial studies by Balabanov et al. also demonstrated that GA-monocyte activation through urokinase plasminogen activator receptor (uPAR) could be a possible mechanism of GA in relapsing remitting MS (Balabanov et al., 2001; Stern et al., 2008). Subsequent studies indicated that GA induces immunomodulatory activity exerted by cells of monocytic lineage including antigen presenting cells (APC) through an increase in IL-10 and reduction in IL-12 and IL-1B.

In addition, most investigators currently believe that the immunomodulatory effect of GA is linked to its ability to alter T-cell differentiation, in particular promotion of Th2 polarized CD4 cells. These GA-induced cells are believed to mediate bystander immunosuppression through the induction of IL-10 producing T-regs (Stern et al., 2008).

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Current data also provide evidence that Tregs contribute to GA's therapeutic action in EAE and possibly MS. Recent reports indicate that the deficiency in CD4(+)CD25(+)FoxP3(+) regulatory T-cells observed in MS and EAE is restored by GA treatment (Jee et al., 2007; Hong et al., 2005). These findings represent a plausible explanation for GA-mediated T-cell immune modulation and may provide useful insight into the mechanism of action of GA in EAE and MS.

Since the induction of regulatory T-cells and clinical benefit are not universal among GA treated MS patients, it is important to determine if the immunological effects of GA treatment are predictive of the clinical response.

There is currently no practical in vitro assay for monitoring the immunological effects of GA. However, a triad of immune responses were proposed by Hohlfeld et al. that identify GA-treated from untreated patients: (1) a significant reduction of GA-induced PBMC proliferation; (2) a positive IL-4 ELISPOT response mediated predominantly by CD4 cells after stimulation with GA; and (3) an elevated IFN-gamma response partially mediated by CD8 cells after stimulation with high GA concentrations (Farina et al., 2001, 2002). In an earlier study, we demonstrated that lymphoproliferation to GA did not differentiate GA-responders (GA-R) from GA- non-responders (GA-NR). However, reduced IFN-gamma expression and stable IL-4 expression in peripheral blood mononuclear cells (PBMC) and an increased IL-4/IFN-gamma ratio were associated with a favorable clinical response (Valenzuela et al., 2007). More recently we identified an HLA-class II haplotype to be highly predictive of response to GA (DR15 + DQ6+; DR17-DQ2-) where as the DR15-DQ6-; DR17 + DQ2+ was predictive of a poor response (Dhib-Jalbut et al., 2013).

To determine whether GA-induced immunological changes in vivo can predict the clinical response to GA therapy, we conducted a prospective 2-year study in which cytokine levels (Th1, Th2, and Th17) from GA-treated MS patients were correlated with the clinical response to the drug at the end of at least 2 years of therapy. The laboratory personnel were blinded as to whether the patients were clinical responders or non-responders.

2. Methodology

2.1. Subjects

Seventy-one patients diagnosed with definite RRMS according to the 2010 revised McDonald criteria (Polman et al., 2011) were enrolled in the study. Patients were treated with GA, 20 mg subcutaneous daily. Subjects were followed up at four MS centers: 15 from the University of Medicine and Dentistry New Jersey (UMDNJ)-Robert Wood Johnson Medical School MS Center, 31 from the University of Maryland Center for MS in Baltimore Maryland, 8 from the Gimble MS Center in Teaneck, New Jersey and 17 from the Carolinas Medical Center-MS Center, Charlotte, North Carolina. The study was approved by the Institutional Review Board for Human Subjects at each Center. Enrollment criteria included RRMS with at least one clinical relapse in the year preceding enrollment. Multiple sclerosis relapse was defined as new or worsening neurological deficit lasting 24 h or more in the absence of fever or infection (Poser et al., 1983). Patients with relapse were typically seen within 1–2 weeks, and followed closely every three months thereafter. Relapses were determined by an MS specialist at each of the MS center. Relapses and progression were measured by Expanded Disability Status Scale (EDSS) recorded by two independent evaluators. Patients were examined and an expanded disability status scale (EDSS) was determined at base line. Annualized relapse rate (ARR) at baseline was determined historically based on chart review of at least 2 years preceding enrolment. ARR and EDSS were also determined at the end of 2 years. Sixty-two patients completed treatment with GA for at least 2 years. Nine patients either did not complete treatment or were lost to follow-up. This is shown in Supplementary Table 1 (Table 1S). Although there are recommendations on follow-up imaging while on disease modifying therapy, the practice of performing imaging varies significantly among MS centers. Since this was a multicenter trial, we had no control over neuro-imaging time points. Therefore, we did not include MRI scans in this study to determine "no evidence of disease activity" (NEDA).

2.2. Classification of clinical responders and non-responders

After at least 2 years on GA therapy, patients were classified as GA-R (n = 32) or GA-NR (n = 30) based on a clinical criteria more stringent than those recently reported in the literature (Rio et al., 2006). A responder (R) is a patient with no relapses and no evidence of disease progression as measured by EDSS (expanded disability status scale) at the end of two years of treatment with GA. A non-responder (NR) is a patient with one or more relapses or with progression in the EDSS of at least 1 point sustained for 6 months.

2.2.1. Blood samples

Approximately 60 cm^3 of heparinized blood and 10 cm^3 of blood in serum separation tubes were obtained by venipuncture from each MS patient pre-treatment and at 3 and 6 months during treatment. PBMC were purified using Ficoll-Hypaque gradients as described in the supplier's protocol (ICN Biomedicals Inc. Ohio, USA). Samples from collaborating centers were sent via overnight delivery at room temperature and processed immediately upon arrival. The cells and sera from baseline, 3 months, and 6 months were saved frozen and then run simultaneously. The same is true for samples taken at 9 and 12 months.

2.3. Immune markers assays

All cytokine levels were assayed in sera except for IL-17 which was measured in supernatants of GA-stimulated PBMC (Two × 10^5 PBMC/well were seeded in 96 well U-bottom micro titer plate in the absence of antigen (unstimulated condition) (US) or the presence of GA at 40 and 100 μg/ml (Teva Pharmaceutical Industries, Ltd., Israel). Delta IL-17 (difference between US and GA-stimulated supernatants levels) was used in the analysis. Interleukin-18 and Caspase-1 levels were detected by human ELISA kits (Sandwich ELISA, Bender Medsystems , USA). The sensitivity of the ELISA was 9.2 and 3.3 pg/ml for IL-18 and Caspase-1 respectively. TGF-β, TNF-α, IL-4, IFN-γ, IL-17 and IL-10 levels were detected by human ELISA kits from ebioscience (San Diego CA, 92,121 USA). The sensitivity of the ebioscience kits were 60 pg/ml, 4 pg/ml, 2 pg/ml, 4 pg/ml, 4 pg/ml and 2 pg/ml respectively. According to assay instructions, the limit of detection of the cytokines was defined as the analyte concentration resulting in an absorption significantly higher than that of the dilution medium (calculated as the mean plus 2 standard deviations).

2.4. Statistical analysis

The R statistical environment was used for statistical analysis (R Development Core Team, 2011). Because 76% of patients were missing at least one measurement, the R package Amelia was used to impute 10 complete datasets (Honaker et al., 2011). The multiple imputation procedure replaced missing values with values randomly drawn from the inferred distribution of that variable. Results of statistical procedures were combined across the imputations. "Rubin's rules" were used to correctly compute standard errors taking into account both the observed variability and the additional uncertainty due to the imputation process (Rubin, 1987).

The prognostic performance of each marker individually was summarized using the AUC (area under the receiver operating characteristic, or ROC, curve). Because we wished to investigate the use of binary predictors, we also dichotomized each variable, using as a cutoff the value minimizing the distance between the ROC plot and the upper left corner of the unit square. For each dichotomized variable, the