Immunobiology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio

Research Paper

Decreased serum levels of sCD40L and IL-31 correlate in treated patients with Relapsing-Remitting Multiple Sclerosis

José de J. Guerrero-García[a,b], Argelia E. Rojas-Mayorquín[c], Yeminia Valle[a], Jorge R. Padilla-Gutiérrez[a], Víctor A. Castañeda-Moreno[a], Mario A. Mireles-Ramírez[d], José F. Muñoz-Valle[a], Daniel Ortuño-Sahagún[a,*]

[a] Instituto de Investigación en Ciencias Biomédicas (IICB), CUCS, Universidad de Guadalajara, Jalisco, Mexico
[b] Unidad Médica de Alta Especialidad (UMAE), Hospital de Pediatría (HP), Centro Médico Nacional de Occidente (CMNO), IMSS, Mexico
[c] Departamento de Ciencias Ambientales, Instituto de Neurociencias, CUCBA, Universidad de Guadalajara, Jalisco, Mexico
[d] Unidad Médica de Alta Especialidad (UMAE), Hospital de Especialidades (HE), Centro Médico Nacional de Occidente (CMNO), IMSS, Mexico

ARTICLE INFO

Keywords:
Multiple sclerosis
sCD40L
IL-31
Glatiramer acetate
IFN-β

ABSTRACT

The CD40/CD40L system is a binding key for co-stimulation of immune cells. Soluble form of CD40L has been widely studied as marker of inflammatory and autoimmune diseases. Here we analyze serum concentrations of sCD40L, as well as 14 cytokines, in patients with Multiple Sclerosis (MS) treated with Glatiramer acetate or Interferon beta. In the healthy control group, we found in serum a highly positive correlation between sCD40L and Interleukin (IL)-31, an anti-inflammatory Th2 cytokine. Additionally, an important reduction in IL-31 and sCD40L serum levels, as well as a significant reduction in CD40 mRNA expression and complete depletion of CD40L mRNA, detected from peripheral blood cells, was found in treated patients with MS. Therefore, sCD40L and IL-31 must be taken into account as possible prognostic markers when analyzing the disease progress of MS in order to provide more personalized treatment.

1. Introduction

Multiple Sclerosis (MS) is a demyelinating disease that is immune-mediated and that mainly affects the Central nervous system (CNS) (Bruck, 2005; Lassmann, 2014; Noseworthy et al., 2000) by destroying the myelin sheaths (Lassmann et al., 2001). More than 80% of patients express a Relapsing-Remitting form of MS (RRMS), characterized by exacerbations of partially or nearly completely reversible neurological disability (Steinman, 2014). MS causes physical disability in adults of working age, between 20 and 40 years old (Heydarpour et al., 2015; Swanton et al., 2014), which leads to an increase of care cost, associated with a loss in productivity, employment and quality of life of affected patients (Macias-Islas et al., 2013; Berg et al., 2006). Recently, several studies have reported an increase in feminine/masculine prevalence ratio (2:1 to 3:1) (Evans et al., 2013). However, the disease is less intense and less damaging in females (van den Broek et al., 2005).

Interferon beta (IFN-β) and Glatiramer acetate (GA) constitute first-line disease-modifying agents for treatment of MS. The mechanisms of action of IFN-β include decrease of antigen presentation, modulatory effects on co-stimulatory molecules, suppression of Th1 cell proliferation, increase of IL-10 production, and promotion of the shift of pro- to anti-inflammatory environment (Javed and Reder, 2006). Furthermore, it has recently been described that IFN-β treatment restores T regulatory cell (Treg) activity through the decrease of D1-like dopamine receptor expression, which inhibits dopamine-induced Treg suppression (Marino and Cosentino, 2016; Levite et al., 2017). On the other hand, GA is one of the agents most employed for the treatment of MS. The mechanism of action of GA has been the subject of numerous researches. At the cellular level, it acts as an altered peptide ligand, inhibiting the activation of myelin-basic, protein-specific T cells, resulting in an induction of protective regulatory cytokines, such as Interleukin (IL)-10, IL-4, and Transforming growth factor β (TGF-β) (Racke and Lovett-Racke, 2011; Lalive et al., 2011). However, analyses of Treg cells in patients with MS have provided controversial results; some reported a decrease in level (Huan et al., 2005; Borsellino et al., 2007) while others report no differences (Viglietta et al., 2004; Haas et al., 2005; Carbone et al., 2014), although they find a disturbance in the development or function of Treg subpopulations in patients with MS.

The evidence indicating that metabolism controls T-cell activation and loss of immune tolerance is increasing. Recently, Carrieri et al. performs a longitudinal assessment of immune-metabolic parameters in patients with MS during GA treatment (Carrieri et al., 2015). They

* Corresponding author at: Instituto de Investigación en Ciencias Biomédicas (IICB), CUCS, Universidad de Guadalajara, P.O. Box 52-126, 45020, Guadalajara, Jalisco, Mexico.
E-mail address: dortuno@cucs.udg.mx (D. Ortuño-Sahagún).

http://dx.doi.org/10.1016/j.imbio.2017.10.001
Received 19 May 2017; Received in revised form 9 August 2017; Accepted 3 October 2017

concluded that the clinical outcome of GA treatment is associated with changes in immune cell subpopulations and modulation of specific immune factors.

One of these immune factor is CD40 ligand (CD40L), a marker of increased plaque burden, which was initially identified as T-cell molecule for ligation of the co-stimulatory receptor CD40. Since then, CD40-CD40L interaction has been considered a key event for effective adaptative immune response (Noelle, 1996; van Kooten and Banchereau, 2000), playing a major role in the induction of the Th2 response (MacDonald et al., 2002). CD40L may be cleaved from the platelets by matrix metalloproteinase-2 (MMP-2) (Reinboldt et al., 2009) and by a disintegrin and metalloproteinase domain-containing protein (ADAM)-10 and −17 from T cells (Yacoub et al., 2013). The soluble form of CD40L (sCD40L), also known as CD154, has cytokine-like properties (Wagner, 2009) and binds to the same CD40 receptor (Aloui et al., 2014). sCD40L has been studied in inflammation and autoimmune diseases (van Kooten and Banchereau, 2000; Aloui et al., 2014). Increasing serum levels of sCD40L has been involved in angiogenesis (Melter et al., 2000) and tumor progression in the pathogenesis of cancer (Sabel et al., 2000). Also, it has been reported as a risk factor for acute coronary syndromes (Varo et al., 2003) and in patients with type 1 diabetes, as a risk factor for the development of microvascular complications (Harding et al., 2004; El-Asrar et al., 2012). CD40L is transiently expressed in T cells and other non-immune cells under inflammatory conditions, and their interaction with CD40 participates in the initiation and progression of cellular and humoral adaptive immunity (Elgueta et al., 2009), recently considered a common link in the pathogenesis of autoimmune diseases (Sokolova et al., 2013). On this regard, Zhong et al., reported recently that CD40L is elevated in MS patients without treatment (Zhong et al., 2016), and Chen et al., reported a significantly reduced in CD40-mediated P65 phosphorylation in naive B cells from RRMS patients treated with GA (Chen et al., 2016). On the other hand, CD40L participates in protection from demyelination and in the development of spontaneous remyelination in the mouse model of Theiler's Murine Encephalomyelitis Virus (TMEV) (Drescher et al., 2000). Taken together, these results support a relevant role for CD40L in the pathogenesis of MS, however this role is only start to being explored and remains highly unclear.

On this basis, and to further understand the immune modulating effect of treatment during RRMS, we hypothesize that sCD40L has an important role in the expression of Th1/Th2/Th17 cytokine profile in patients with MS treated with GA or IFN-β. To test this hypothesis, we analyze serum concentrations of sCD40L, as well as 14 cytokines, in patients with RRMS treated with GA or IFN-β. sCD40L levels were further correlated with 14 cytokines in an effort to provide information regarding the clinical significance of sCD40L in the modulatory network response to treatment in these patients with MS.

2. Methods

2.1. Patients

A total of 73 patients (43 females and 30 males) diagnosed with RRMS (38 in clinical relapse and 35 in clinical remission), who had received IFN-β (n = 27) and GA (n = 46), were recruited at the Neurology Service of the Western National Medical Center's Specialty Hospital of the Mexican Social Security Mexican Institute (IMSS), and from the Neurology Service of Guadalajara Civil Hospital, in Jalisco State, Mexico. Patients with RRMS who were included fulfilled the following criteria: 1) were diagnosed with RRMS according to the revised McDonald diagnostic criteria (2005) (Polman et al., 2005); 2) were aged between 20 and 60 years, and 3) had been under treatment for RRMS with IFN-β or GA for at least 3 months. Clinical disability was evaluated employing the Kurtzke Expanded Disability Status Scale (EDSS) (Kurtzke, 1983), and disease severity was evaluated utilizing the Multiple Sclerosis Severity Score (MSSS) (Roxburgh et al., 2005). The RRMS clinical form was determined according to the classification of Lublin and Reingold (1996 and 2013 revisions) (Lublin and Reingold, 1996; Lublin et al., 2014). Both, patients with RRMS in clinical relapse or clinical remission were included. Clinical relapse is defined as an episode of acute worsening of neurological function, and clinical remission, as the period of variable degree of recovery with no relapse episodes within the 3 months prior to the time of enrollment in this study. Untreated patients with RRMS were not included. Patients with the following conditions were excluded: 1) diagnosed with Secondary Progressive MS (SPMS) or Primary Progressive MS (PPMS); 2) under treatment with corticosteroids in the previous 3 months; 3) with a clinical history of another autoimmune or inflammatory disease; and 4) diagnosed with any other chronic-degenerative disease of the CNS. All patients gave their written informed consent for study inclusion. The control group consisted of 30 age- and gender-matched healthy individuals (20 females and 10 males) who were selected from among the general population in the same geographical areas as the patients. Samples were collected at the same time in order to diminish bias associated with circadian-cycle cytokines. This study was conducted in accordance with the ethical guidelines of the 2013 Declaration of Helsinki and was approved by the Ethical Committee of the Mexican Institute of Social Security (IMSS) (R-2015-1301-125) in Mexico.

2.2. Blood sample collection

Peripheral blood was collected in BD Vacutainer® tubes without anticoagulant to obtain serum by centrifugation and aliquots were immediately stored at −80 °C until assayed. For total RNA extraction, peripheral blood was collected in BD Vacutainer® tubes with ethylendiaminetetraacetic acid (EDTA) and cells were obtained by density gradient centrifugation.

2.3. Cytokine measurements

Serum cytokine levels for sCD40L, IL-31, IL-1β, IL-4, IL-6, IL-10, IL-17A, IL-17F, IL-21, IL-22, IL-23, IL-25, IL-33, IFN-γ and TNF-α, were measured with a bead-based multiplex assay utilizing a commercial kit (Bio-Plex® Multiplex System) according to the manufacturer's instructions. Results were expressed in picograms/milliLiters (pg/mL). Cytokine specificity and sensitivity was defined according to the manufacturer's instructions as follows: Specificity: the manufacture reported non-cross reactivity in sCD40L and IL-31 cytokine measurement, and sensitivity: minimal detectable concentrations were sCD40L = 2.4 pg/mL and IL-31 = 3.9 pg/mL.

2.4. mRNA obtaining and qPCR determinations

For CD40L and CD40 mRNA expression, total RNA was obtained from peripheral blood cells of RRMS patients by using the modified Chomczynski and Sacchi method (Chomczynski and Sacchi, 1987). Briefly, peripheral blood cells were homogenized in Trizol reagent. After chloroform addition, aqueous phase was separated and total RNA was precipitated with isopropanol at 4 °C. Total RNA was reconstituted in RNase-free water and quantified. Total RNA was reverse transcribed to obtain cDNA using iScript™ DNA Synthesis Kit (BIO-RAD). Subsequently, quantitative Polymerase Chain Reaction (qPCR) was performed on cDNA in 96-well plate with Applied Byosistems StepOnePlus™ and Taqman® Gene Expression Assays. Gen relative expression was analyzed using GAPDH and β-Actin as constitutive controls for normalization.

2.5. Statistical analysis

A database was created using the Microsoft Office Excel program and statistical analysis was performed using GraphPad Prism ver. 5.0. Data analysis of cytokine levels were performed by calculating Means