Journal of Neuroimmunology 298 (2016) 82–89

Contents lists available at ScienceDirect

Journal of Neuroimmunology

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

Dopaminergic receptors and adrenoceptors in circulating lymphocytes as putative biomarkers for the early onset and progression of multiple sclerosis

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ARTICLE INFO

Article history:
Received 30 May 2016
Received in revised form 24 June 2016
Accepted 7 July 2016

Keywords:
Clinically isolated syndrome
Multiple sclerosis
Dopaminergic receptors
Adrenoceptors
Peripheral blood mononuclear cells
CD4+ T effector lymphocytes
CD4+ T regulatory lymphocytes

ABSTRACT

Clinically isolated syndrome (CIS) is a first, usually recovering, episode of neurological disturbance(s) suggestive of multiple sclerosis (MS). CIS subjects might benefit from early disease-modifying drugs, provided that those at high risk of developing MS can be identified. Gene expression for dopaminergic receptors (DR) and adrenoceptors (AR) is dysregulated in lymphocytes of MS patients and is affected by treatment with interferon (IFN)-β. In particular, lymphocyte DR D_5 mRNA might be a marker of IFN-β response in MS patients. No information exists so far in CIS subjects. We investigated DR and AR gene expression in peripheral blood mononuclear cells (PBMC) and in CD4+ T effector (Teff) and regulatory (Treg) cells from CIS subjects, and assessed their relationship with MS progression after 12 months. Expression of several DR and AR are upregulated in PBMC, Teff and Treg from CIS subjects. DR D_3 and α_{2A}-AR mRNA in PBMC, and DR D_5 mRNA in Treg correlate with the risk of MS at 12 months. Results show the involvement of dopaminergic and adrenergic pathways in CIS as well as in MS pathogenesis, supporting the evaluation of dopaminergic and adrenergic agents in MS.

1. Introduction

In the majority of cases, clinical onset of multiple sclerosis (MS) is represented by acute or subacute neurologic disturbance(s), usually recovering, known as a clinically isolated syndrome (CIS). CIS may present with pure sensory or motor (long tract) symptoms and signs (46% of cases), optic neuritis (21%), brainstem syndrome (10%), or with multifocal abnormalities (23%) (Miller et al., 2012).

By definition, subjects with a CIS do not have MS, though they may be at a high risk to develop the disease. Indeed, between 19% and 61% of patients with CIS may have a second clinical attack and/or the appearance of new lesions at magnetic resonance imaging (MRI) within one year, leading to the diagnosis of clinically-definite MS (Miller et al., 2012; Alroughani et al., 2012; D'Alessandro et al., 2013). Diagnosis of MS requires evidence for dissemination of lesions in time and space. The 2001 diagnostic guidelines and subsequent revisions (reviewed in: Marziniak et al., 2016) integrated clinical and MRI criteria for diagnosis of MS and facilitated an early diagnosis of MS in patients presenting with a CIS. The potential benefit associated with early use of immunomodulators in subjects presenting a CIS has been suggested by several clinical trials (reviewed in: Miller et al., 2012; Brownlee and Miller, 2014; Leist et al., 2014). Thus, evidence from clinical research suggests that many subjects with CIS or early MS should be treated with disease-modifying drugs at an early stage, despite reimbursement limitations existing in most countries (Marziniak et al., 2016). Indeed, acute axonal damage starts very early in the course of the disease, possibly before the first clinical manifestation. This damage is irreversible and correlates with the level of inflammation, a feature that is predominant in the early stages of MS (Kuhlmann et al., 2002). The possibilities for an early treatment depend however on the ability to identify very early CIS subjects with a high risk of developing MS.

Currently, MRI represent the best available prognostic marker to predict conversion of CIS to clinically definite MS. Conversely, efficient biological markers are not available in this respect (Bielekova and Martin, 2004). Potentially promising biomarkers in CIS include: down-regulated TOB1 (a critical regulator of cell proliferation), CSF and serum neuron specific enolase, CSF-tau and neurofilaments, CSF and serum anti-MBP and anti-MOG IgG, CSF IgG or IgM oligoclonal bands,

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http://dx.doi.org/10.1016/j.jneuroim.2016.07.008
0165-5728/

blood CD8 + CD26 + CD69 + T cells, blood CD26 + CD4 T cells, VLA-4, LFA-1alpha and MMP-9. Unfortunately, no one of these biomarkers is specific and predictive of subsequent conversion to MS.

The catecholamines dopamine, noradrenaline and adrenaline are neurotransmitters and neurohormones in the CNS and in the autonomic nervous system, which nonetheless play key roles as transmitters connecting the nervous and the immune system, as well as immune cells and peripheral tissues (Basu and Dasgupta, 2000; Elenkov et al., 2000; Sarkar et al., 2010; Marino and Cosentino, 2013; Levite, 2012; Scanzano and Cosentino, 2015). Over the last decade, several groups, including us, have documented the occurrence during MS of extensive dysregulation of dopaminergic receptors (DR)- and adrenoceptors (AR)-operated pathways in T lymphocytes, and in particular in CD4 + T cells (reviewed in Cosentino and Marino, 2013). In summary, untreated MS patients have reduced expression and activity of D_1-like DR (but possibly not of D_2-like DR) and of β_2-AR on circulating PBMC and on CD4 + T effector lymphocytes (Teff), which likely explains the unresponsiveness of MS patients to the antiinflammatory effects of dopaminergic and adrenergic agents. On the contrary, D_1-like DR are overexpressed on CD4 + $CD25^{high}$ T regulatory lymphocytes (Treg), a specialized subset of lymphocytes which maintain the immune homeostasis through inhibition of T effector cells, and which are peculiarly sensitive to dopamine, which results in their functional suppression (Cosentino et al., 2007). Functional dysregulation of Treg may indeed contribute to disease pathogenesis and activity in both experimental autoimmune encephalomyelitis (EAE), the animal model of MS, as well as in patients with MS (reviewed in: Venken et al., 2010; Zozulya and Wiendl, 2008). Remarkably, during treatment with interferon (IFN)-β, the functional responsiveness of D_1-like DR and β_2-AR on CD4 + Teff is restored (Zaffaroni et al., 2008), while the sensitivity of Treg to dopamine is suppressed (Cosentino et al., 2012), thus setting the conditions to use dopaminergic and adrenergic agents as add-ons to conventional immunomodulatory treatments in MS (Khoury et al., 2010; Marino and Cosentino, 2016).

Despite the extensive evidence supporting dysregulation of DR- and AR-operated pathways as major contributors in MS pathogenesis and response to immunomodulatory treatments, no information so far exists about DR and AR pathways in CIS. Moreover, some of us recently reported that before treatment circulating lymphocytes of MS patients who will thereafter respond to IFN-β have higher mRNA levels for the D_1-like DR D_5 in comparison to cells from patients who will not respond, leading to the suggestion that DR D_5 mRNA levels in circulating lymphocytes might represent an early marker of response to IFN-β in MS patients (Cosentino et al., 2014). The main aim of this study was therefore to investigate the possible occurrence of dysregulation of gene expression in DR and/or AR pathways in circulating lymphocytes from subjects with CIS and to assess their possible relationship with subsequent progression to clinically established MS.

2. Material and methods

2.1. Subjects

We performed a one-year longitudinal study at the Centre for research on Multiple Sclerosis, Ospedale S. Antonio Abate of Gallarate (VA) (Investigator in charge: Mauro Zaffaroni), at the Neurology Unit of the "Ospedale di Circolo e Fondazione Macchi", University of Insubria - School of Medicine of Varese (Investigator in charge: Giorgio Bono), and at the Neurological Department, Valduce Hospital, Como (Investigator in charge: Mario Guidotti). Eligible subjects were those who had a first, isolated, well-defined neurologic event consistent with demyelination involving the optic nerve (unilateral optic neuritis), spinal cord (incomplete transverse myelitis), or brain stem or cerebellum (brain stem or cerebellar syndrome) and which was confirmed on ophthalmologic or neurologic examination.

Healthy controls (HC) were usually spouses and caregivers of enrolled patients. The Ethics Committee of the Ospedale S. Antonio Abate of Gallarate (VA) approved the protocol and all the participants signed a written informed consent before enrollment.

At the time of enrollment, brain MRI and (if indicated by clinical presentation) spinal cord scan, cerebrospinal fluid (CSF) analysis and Visual Evoked Potential (VEP) tests was done as part of the pre-study evaluation. Results were assessed using McDonald criteria (Polman et al., 2005). All patients with a CIS were eligible to enter the study. Patients under corticosteroids given to treat symptoms related to CIS were included 1 month after drug withdrawal.

Exclusion criteria were: patients with a prior neurologic or visual event consistent with the occurrence of demyelination that lasted longer than 48 h; any previous immunomodulatory treatment or intake of sympathoadrenergic agents during at least the prior 3 months; other serious intercurrent systemic illnesses or psychiatric disorders; inability to give an informed consent for any cause.

Enrolled CIS subjects were submitted to a complete neurological examination, quantification of disability was assessed according to Kurtzke (1983), and a venous blood sample was obtained after a fasting night, between 8:00 and 9:00 AM, by use of heparinized vacuum tubes.

According to the revised McDonald criteria (Polman et al., 2005), conversion of CIS to MS (CISc) was defined as dissemination of disease in space or time, the occurrence of a relapse with new symptoms and clinical signs indicating the involvement of a new CNS area, and/or the appearance of a single new lesion in T2-weighted images or of a new gadolinium-enhancing lesion on T1-weighted images. When such conditions did not occur within 12 months since the first neurologic event, CIS subjects were defined as non converted to MS (CISnc).

2.2. Cell preparation

PBMC, Teff and Treg were prepared as described in detail in Cosentino et al. (submitted for publication). Briefly, PBMC were isolated from whole blood by Ficoll-Paque Plus density gradient centrifugation, washed twice in phosphate buffer saline (PBS), and 1×10^6 cells/ml were finally resuspended in RPMI 1640/10% heath-inactivated fetal bovine serum (FBS), with added 2 mM glutamine and 100 U/ml penicillin/streptomycin, and cultured at 37 °C in a moist atmosphere of 5% CO_2. PBMC were cultured for 48 h, alone or in the presence of PHA 10 μg/ml. PBMC were finally harvested and assayed for DR, AR and TH mRNA expression by means of real-time PCR. Immunomagnetic sorting of Treg and Teff from freshly isolated PBMC was performed by Dynal CD4 + CD25 + Treg Kit (Dynal, Oslo, Norway), according to the manufacturer's instructions. Treg and Teff were directly assayed for DR, AR and TH mRNA expression by means of real-time PCR.

2.3. Real-time PCR assays

Total RNA was extracted by PerfectPure™ RNA Cell & Tissue kit (5Prime, Milano, Italy) and reverse-transcribed using a random primer, high-capacity cDNA RT kit (Applied Biosystems, Life Technologies Corporation, USA). Amplification of cDNA was performed by *Taq*Man® Universal PCR Master Mix (Applied Biosystems), using the *Taq*Man Gene Expression Assay. Amplified cDNA was assayed on an ABI PRISM® 7000 System (Applied Biosystems). Gene expression levels in a given sample were represented as $2^{-\Delta Ct}$ where ΔCt = [Ct (sample) − Ct (housekeeping gene)], and relative expression was determined by normalization to 18S cDNA. Interrogated gene sequences together with further details about real-Time PCR conditions are shown in Cosentino et al. (submitted for publication). Levels of mRNA were finally expressed as $2^{-\Delta Ct} \times 10^7$.