Clinical Immunology 173 (2016) 133–146

Contents lists available at ScienceDirect

Clinical Immunology

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

Identification of a gene expression signature in peripheral blood of multiple sclerosis patients treated with disease-modifying therapies

Chiara Cordiglieri, Fulvio Baggi, Pia Bernasconi, Dimos Kapetis, Elisa Faggiani, Alessandra Consonni, Francesca Andreetta, Rita Frangiamore, Paolo Confalonieri, Carlo Antozzi, Renato Mantegazza *

Neurology IV – Neuroimmunology and Neuromuscular Diseases Unit, Neurological Institute "Carlo Besta", Milan, Italy

ARTICLE INFO

Article history:
Received 10 May 2016
Received in revised form 30 September 2016
accepted with revision 4 October 2016
Available online 5 October 2016

Keywords:
Multiple sclerosis
Therapy
Peripheral blood biomarkers

ABSTRACT

Multiple Sclerosis (MS) is an inflammatory disease with neurodegenerative alterations, ultimately progressing to neurological handicap. Therapies are effective in counteracting inflammation but not neurodegeneration. Biomarkers predicting disease course or treatment response are lacking. We investigated whether altered gene and protein expression profiles were detectable in the peripheral blood of 78 relapsing remitting MS (RR-MS) patients treated by disease-modifying therapies. A discovery/validation study on RR-MS responsive to glatiramer acetate identified 8 differentially expressed genes: ITGA2B, ITGB3, CD177, IGJ, IL5RA, MMP8, P2RY12, and S100β. A longitudinal study on glatiramer acetate, Interferon-β, or Fingolimod treated RR-MS patients confirmed that 7 out of 8 genes were downregulated with reference to the different therapies, whereas S100β was always upregulated. Thus, we identified a peripheral gene signature associated with positive response in RR-MS which may also explain drug immunomodulatory effects. The usefulness of this signature as a biomarker needs confirmation on larger series of patients.

1. Introduction

Multiple sclerosis (MS) is the most common chronic autoimmune disease of the central nervous system (CNS), affecting >2.5 million people worldwide [1,2]. MS mean age at onset is 30 years with variable disease prevalence according to geographical distribution and ethnicity [3–5]. Incidence of MS is increasing, particularly in women who are at increased risk of being affected; in recent decades the female:male ratio in MS has increased from 2.3 to 3.5:1, primarily in the relapsing-remitting form (RR-MS), and this is not restricted to developed countries only [1,2,4,6,7]. The increasing female:male ratio is thought to be linked to altered responsiveness to environmental factors or to epigenetic changes in females [8–10]. MS diagnostic criteria are currently based on clinical and radiological examinations [11–13], as no disease-specific laboratory tests are available [14–16]. A number of biomarkers have been proposed in MS but only few of them reached clinical value [16].

MS pathogenesis comprises genetic (i.e. HLA class I and II alleles, and >100 non-MHC regions) [17,18] and environmental factors (i.e. Epstein-Barr virus infection, vitamin D deficiency, cigarette smoking) [19], and many susceptibility genes are related to the adaptive and innate immune system [1,2]. In MS, immunopathological reactions preferentially occur in the CNS, but evidences of altered immune processes are also found in the peripheral blood of patients [20–23]. Most of the disease-modifying therapies (DMTs) available for MS patients are either immunosuppressive or immunomodulatory drugs, indicating that targeting the immune system is beneficial to patients with MS [24,25]. However, DMTs are not cell- or antigen-specific in their mechanism of actions, and the profile of molecules regulated by DMTs is still incomplete [24,25]. Analysis of interferon beta (IFNβ) treatment on gene expression in peripheral immunocompetent cells identified an 'IFNβ signature' [26], but the molecular mechanisms associated with IFNβ treatment are still unknown; similarly, little is known on immune cell gene expression profile in patients treated with other DMTs, such as glatiramer acetate (GA) [27]. Transcriptomic analysis of peripheral blood mononuclear cells (PBMCs) is potentially very informative for studies aiming to identify treatment-related biomarkers, and might be also useful for the evaluation of disease progression and treatment response.

In the present work, we performed a transcriptome analysis in blood RNA from relapsing-remitting MS patients (RR-MS), treated with different DMTs, to assess whether gene expression modifications occur. At first, we identified eight genes differentially expressed in GA responsive patients; then, the identified gene panel was evaluated in new cohorts of MS patients treated with GA, IFNβ or Fingolimod, followed-up for at least 12 months. For the first time, using a pragmatic approach, a gene

---

* Corresponding author at: Neurology IV – Neuroimmunology and Neuromuscular Diseases Unit, Neurological Institute "Carlo Besta", Via Celoria, 11, 20133 Milan, Italy.
E-mail address: renato.mantegazza@istituto-besta.it (R. Mantegazza).

http://dx.doi.org/10.1016/j.clim.2016.10.002
1521-6616/

Table 1
MS patients and healthy controls enrolled in the study.

	Healthy donors	MS patients	Age at onset	Disease duration	Annualized relapse rate
Total subjects	47	78			
Females	32	51	31 (±9)	7.5 (±7.2)	1.52 (±2.21)
Males	15	27	29 (±9)	6.6 (±9.2)	0.93 (±1.94)
Mean age at sampling (years ± SD)	35 (±12)	36 (±10)			

expression signature modulated by different DMTs was identified, which may be possibly used in clinical practice to evaluate drug effectiveness.

2. Methods

2.1. Subjects and study design

This study was approved by the Ethic Committee of the Neurological Institute 'Carlo Besta' and conducted in accordance with the Declaration of Helsinki and its amendments. One hundred and twenty-five Caucasian (Italian) adult subjects were enrolled: 78 RR-MS patients, diagnosed according to the Revised McDonald's diagnostic criteria [13] and 47 age- and sex-matched healthy donors (HD) (Table 1; Fig. 1). All subjects gave their written consent to participate in the study.

The study was organized in sequential phases consisting in a discovery, a validation, and a longitudinal phase (Fig. 1). The gene expression profile was studied in the discovery phase on 27 RR-MS patients and 47 HD. Among the 27 RR-MS, 9 patients were not on treatment at time of bleeding (hereinafter referred as to 'NT') and 18 were on GA (Copaxone, TEVA) treatment. GA-treated patients were arbitrarily sub-grouped into: <12 months of treatment (n = 7, range 3–9 months, hereinafter referred to as 'GA3–9'), and >12 months of treatment (n = 11, range 15–24 months, hereinafter referred to as 'GA15–24') (discovery phase; Fig. 1). All GA-treated patients were drug-responder, according to the No Evidence of Disease Activity 3 criteria (NEDA-3, absence of relapses, no sustained progression in the Expanded Disability Status Scale – EDSS – score, no new or enlarging T2 or T1 gadolinium-enhancing lesions) [28].

Among the differentially expressed genes, eight genes were selected for subsequent validation by quantitative real-time PCR (RT-qPCR) in 8 NT, 7 GA3–9, and 8 GA15–24 MS patients, in which the 8 genes were differentially expressed in the discovery phase, and 7 HD subjects; 12 additional NT RR-MS patients were included (validation phase; Fig. 1).

The 8-gene panel was then analyzed in 39 newly enrolled RR-MS patients: 32 RR-MS patients started a first-line DMT (GA, n = 10; IFNβ, n = 22 – Avonex, Biogen Idec, or Rebif, EMD Serono-Pfizer) and 7 patients started Fingolimod as second-line DMT, being non-responder to a first-line DMT after 18–24 months of treatment. Blood samples were

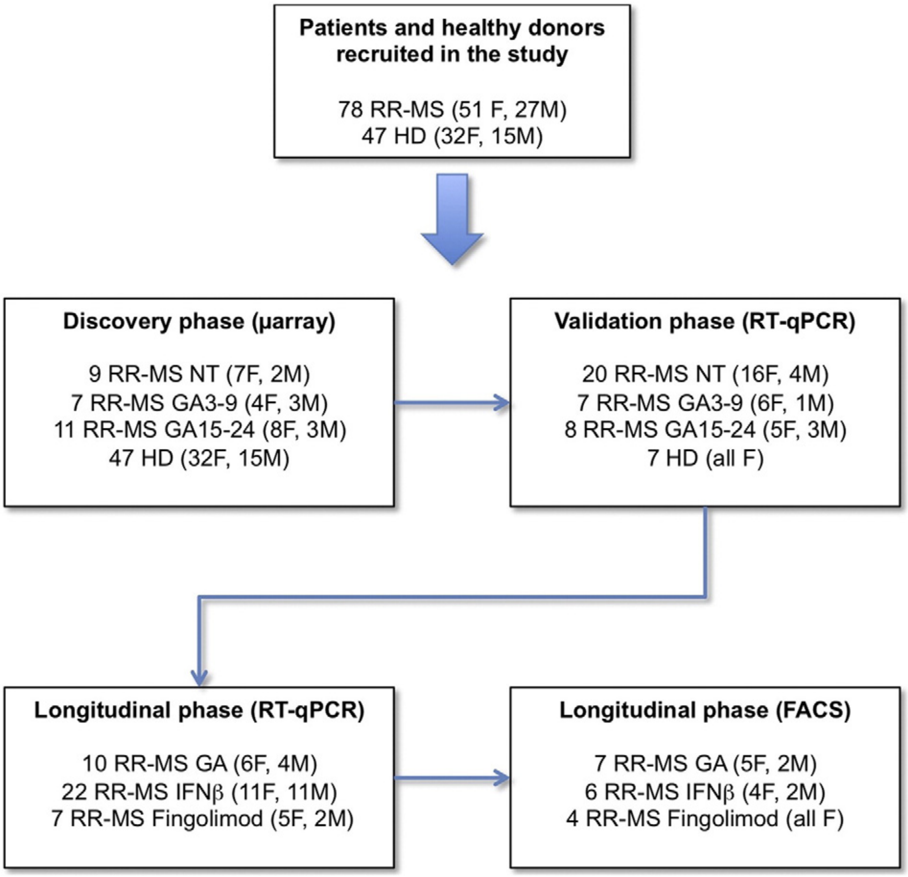

Fig. 1. Schematic representation of the study and patient distribution. Blood sampling in the longitudinal phase was done at T = 0 (baseline), T = 6 and T = 12 months of treatment. F, female; M, male; RR-MS, relapsing-remitting multiple sclerosis; HD, healthy donors; NT, not treated; GA3–9, glatiramer acetate 3–9 months of treatment; GA15–24, glatiramer acetate 15–24 months of treatment; IFNβ, interferon beta.