Journal of Neuroimmunology 288 (2015) 98–101

Contents lists available at ScienceDirect

Journal of Neuroimmunology

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

Short communication

Peripheral blood non-MAIT CD8 + CD161hi cells are decreased in relapsing-remitting multiple sclerosis patients treated with interferon beta

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ARTICLE INFO

Article history:
Received 23 July 2015
Received in revised form 14 September 2015
Accepted 22 September 2015

Keywords:
Multiple sclerosis
CD8 + CD161hi
Tc17
CD161
Mucosal-associated invariant T cells
Interleukin-17
Immunomodulatory treatments

ABSTRACT

CD8 + CD161hi cells, comprising MAIT and non-MAIT cells, have been involved in multiple sclerosis (MS) pathogenesis. Here, we investigated the frequency of CD8 + CD161hi, MAIT and non-MAIT cells by flow cytometry in peripheral blood samples from 41 untreated MS patients, 48 patients receiving disease modifying therapies, and 17 healthy controls (HC). IFNβ treatment was associated with a decrease in the frequency of Tc17 cells compared to untreated patients ($p = 0.019$). No significant differences were observed between untreated MS patients and HC for any of the study cell populations. These results suggest previously unknown mechanisms of action of IFNβ.

1. Introduction

Th1 and Th17 cells are key players in the immunopathogenesis of multiple sclerosis (MS) (Loleit et al., 2014). Recently, it has been shown that interleukin-17 (IL-17) production is not restricted to Th17 cells and CD8+ T cells can also contribute (Annibali et al., 2011). CD161 has been proposed as a marker of IL-17-producing lymphocytes (Maggi et al., 2010). It is important to emphasize that CD8 + CD161hi cells comprise both mucosal-associated invariant T cells (MAIT) and non-MAIT cells, which can only be differentiated by assessing the expression of the semi-invariant T cell receptor (iTCR) Vα7.2-J33 (Dusseaux et al., 2011). MAIT cells include mainly CD8+, but also double negative (DN) and CD4+ cells. Studies of CD8 + CD161hi cells in MS have resulted in controversial results. Whereas CD8 + IL-17 + lymphocytes were observed in brain MS lesions (Tzartos et al., 2008), and CD8 + CD161hi lymphocytes were increased in peripheral blood and brain lesions of MS patients (Annibali et al., 2011), other studies found a decrease of MAIT cells in peripheral blood of MS patients (Willing et al., 2014; Miyazaki et al., 2011). However, these studies did not evaluate a potential differential behavior of MAIT and non-MAIT cells in MS. Furthermore, beyond one study showing almost undetectable MAIT cells in peripheral blood of MS patients following non-myeloablative autologous hematopoietic stem cell transplant (AHSCT) (Abrahamsson et al., 2013), the potential modification of CD8 + CD161hi cells by current disease modifying therapies (DMT) remains largely unknown.

Based on these observations, in the present study we aimed to assess the frequency of CD8 + CD161hi, CD3 + CD161hiVα7.2 + (total MAIT) and CD8 + CD161hiVα7.2- (non-MAIT) lymphocytes in peripheral blood of untreated MS patients with different clinical forms of the disease and healthy controls (HC), and to investigate the effect of DMT on the frequency of these cell populations.

2. Materials and methods

2.1. Patients

Peripheral venous blood was collected from 41 untreated patients (23 with relapsing-remitting MS — RRMS; 8 with secondary progressive MS — SPMS; and 10 with primary progressive MS — PPMS), 48 RRMS patients treated with DMT (16 with interferon-beta — IFNβ; 10 with glatiramer acetate — GA; 10 with fingolimod — FTY; and 12 with natalizumab — NTZ), and 17 healthy controls (HC). All MS patients fulfilled the 2010 revised McDonald criteria. Untreated patients had not received DMT for at least 1 year previous to blood collection and had

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http://dx.doi.org/10.1016/j.jneuroim.2015.09.007
0165-5728/

never received immunosuppressive treatment. Patients with concurrent autoimmune diseases or cancers were excluded. Blood was collected during remission. In treated MS patients, IFNβ/GA or FTY/NTZ treatment was started at least 1 year or 6 months previous to blood collection, respectively. The study was approved by the Hospital Ethics Committee and written informed consent was signed by the participants. Demographic and clinical data of MS patients and HC is shown in Table 1.

2.2. Flow cytometry

Surface staining of fresh whole blood (100 μL) was performed using CD3 (clone SK7, BD Bioscience), CD161 (clone 191B8, Beckman Coulter), CD8 (clone SK1, eBioscience), and Vα7.2 (clone 3C10, BioLegend) fluorochrome-conjugated antibodies and their corresponding isotype controls. Vα7.2 expression was used to stratify CD8 + CD161hi cells into CD8 + CD161hiVα7.2- (non-MAIT) and CD8 + CD161hiVα7.2 + (CD8 + MAIT) cells. Briefly, after 25 min of incubation at room temperature, 2 ml of diluted (1:10) BD Pharm Lyse (BD Bioscience) buffer was added at 37 °C and incubated at room temperature for 10 min (previously established optimal conditions). Cells were then washed using 2 ml of PBA-azide (1% BSA and 0.1% sodium azide in PBS) and analyzed using a FACSCanto cytometer (Beckton Dickinson). Analysis was performed using the BD FACSDiva software v.5.3. The percentage of CD8 + CD161hi, MAIT and non-MAIT cells was expressed as percentage of total CD8 + cells, while the percentage of total MAIT cells was expressed as percentage of CD3 + cells.

2.3. Statistical analysis

Statistical analysis was performed using IBM SPSS v.22. A Kolmogorov–Smirnov test was used to assess the distribution of the variables followed by Mann–Whitney tests or Student's t-tests as appropriate. Parametric and non-parametric correlations were used to assess possible linear association between cell populations and demographic and clinical variables.

3. Results

In untreated MS patients, no significant differences were observed in the frequency of CD8 + CD161hi, non-MAIT, CD8 + MAIT and total MAIT cells between patients with different clinical forms of MS and between MS patients and HC (Fig. 1A).

In treated RRMS patients, IFNβ was associated with a significant decrease in the frequency of non-MAIT cells compared to untreated patients ($p = 0.019$: Fig. 1B). No significant differences were observed in the frequency of CD8 + CD161hi, CD8 + MAIT and total MAIT cells between untreated and IFNβ-treated patients. Treatment with GA, FTY or NTZ was not associated with significant changes in the frequency of the study cell populations (Fig. 1B). There were no significant correlations between the different cell populations evaluated with clinical and demographical data in any of the groups.

4. Discussion

Although MAIT cells represent over 90% of CD8 + CD161hi cells, the differential behavior of non-MAIT and MAIT cells in MS has not been fully established and the study of CD8 + CD161hi cells in MS resulted in controversial results. Some authors found an increase in their frequency in peripheral blood of MS patients and suggested a pathogenic role for these cell populations in MS (Annibali et al., 2011), while others found a decrease of MAIT cells in peripheral blood of MS patients (Willing et al., 2014; Miyazaki et al., 2011). In this context, Miyazaki et al. showed that MAIT cells possess immunomodulatory properties, inhibiting the production of Th1 pro-inflammatory cytokines in a contact-dependent manner (Miyazaki et al., 2011). Willing, et al., which also observed a decrease of MAIT cells in peripheral blood of MS patients, found MAIT cells in MS brain lesions thus attributing the peripheral decrease to a preferential tissue migration (Willing et al., 2014).

There is scarce evidence regarding the modulation of these cell populations with DMT. In one small study, IFNβ and GA showed no effect on IL-17 + CD8 + cells in MS patients (Peelen et al., 2013). Another study showed a decrease in IL-17/IFNγ-producing CD161hiCD8 + cells after 1 month of treatment with FTY (Serpero et al., 2013). In addition, CD8 + CD161hi cells were found to be almost undetectable up to 2 years following AHSCT (Abrahamsson et al., 2013).

In our study the frequency of CD8 + CD161hi cells was similar among untreated MS patients (including RRMS and progressive MS patients) and between MS patients and HC. However, non-MAIT cells, but not MAIT cells, were significantly decreased by IFNβ treatment. Although there are some limitations, mainly the small sample size, the imperfect matching of controls and the heterogeneity of the patients included, as well as the high variability of these cell populations, which must be taken into account when analyzing the data, our results suggest a differential role of DMT on these cell populations, as well as a differential behavior of MAIT and non-MAIT cells. These results also point towards a previously unknown modulation of non-MAIT cells by IFNβ,

Table 1
Demographic and clinical characteristics at the time of blood collection.

	HC (n = 17)	RRMSu (n = 23)	SPMS (n = 8)	PPMS (n = 10)	RRMS IFNb (n = 16)	RRMS GA (n = 10)	RRMS FTY (n = 10)	RRMS NTZ (n = 12)
Female sex	70.6%	52.2%	50.0%	50.0%	87.5%	60.0%	60.0%	66.7%
Age[a]	37.4 (10.2)	41.9 (8.9)	51.1 (7.4)	47.2 (8.5)	39.4 (4.6)	39.0 (7.5)	42.3 (6.2)	42.2 (6.7)
Disease duration,yrs.[b]	_	2.0 (0.2, 22.8)	16.4 (13.7, 29.5)	7.7 (3.6, 28.7)	10.0 (2.2, 26.5)	9.1 (3.0, 23.7)	10.7 (4.2, 27.6)	14.4 (3.8, 21.8)
Progressive disease duration, yrs.[b]	_	_	10.7 (3.5, 17.8)	7.7 (3.6, 28.7)	_	_	_	_
Treatment duration, yrs[b]	_	_	_	_	6.6 (1.0, 17.7)	3.0 (1.1, 7.7)	1.2 (0.8, 6.8)	4.2 (2.7, 6.2)
EDSS[b]	_	2.0 (0.0, 4.0)	5.5 (3.5, 8.5)	4.0 (2.0, 8.5)	1.5 (0.0, 3.5)	1.5 (0.0, 2.5)	2.8 (1.5, 6.0)	4.0 (3.0, 6.0)
Relapses in the 2 previous yrs[a]	_	1.1 (0.8)	0.4 (1.0)	0.0 (0.0)	0.3 (0.6)	0.1 (0.3)	1.8 (1.5)	0.1 (0.3)
Relapses since treatment onset[a]	_	_	_	_	0.6 (0.9)	0.2 (0.4)	0.4 (0.5)	0.6 (0.8)

Abbreviations: EDSS: expanded disability status scale; FTY: fingolimod; GA: glatiramer acetate; HC: healthy controls; IFNb: interferon beta; MS: multiple sclerosis; NTZ: natalizumab; PPMS: primary progressive MS; RRMSu: untreated relapsing-remitting MS; SPMS: secondary progressive MS; yrs.: years.

[a] Data is expressed as mean (SD).

[b] Data is expressed as median (min., max.).