Journal of Neuroimmunology 276 (2014) 187–194

Contents lists available at ScienceDirect

Journal of Neuroimmunology

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

High CD6 and low chemokine receptor expression on peripheral blood lymphocytes correlates with MRI gadolinium enhancement in MS

Anna Jurewicz, Krzysztof Zaleski, Malgorzata Domowicz, Krzysztof Selmaj *

Department of Neurology, Medical University of Lodz, Lodz, Poland

ARTICLE INFO

Article history:
Received 26 May 2014
Received in revised form 17 August 2014
Accepted 19 August 2014

Keywords:
Multiple sclerosis (MS)
CD6
Peripheral mononuclear cells (PBMC)
MRI
Gadolinium enhancing lesion
Chemokine receptors

ABSTRACT

Correlation between gadolinium-enhancing [Gd(+)] lesions on MRI and expression of CD6 molecules and a group of chemokine receptors on peripheral blood (PB) and cerebrospinal fluid (CSF) immune cells was measured in multiple sclerosis (MS) patients. Twenty remitting–relapsing MS patients with ($n = 10$) and without ($n = 10$) Gd(+) lesions entered the study. mRNA and surface expression of CD6 and CCR1, CCR2, CCR3 and CCR5 was measured by immunostaining and flow cytometry. Expression of mRNA and surface staining for CD6 in PB T lymphocytes was increased in Gd(+) compared to Gd(−) patients ($p < 0.01$; $p < 0.05$, respectively). CD6 mRNA correlated with the number and size of Gd(+) lesions ($r = 0.67$, and $r = 0.65$ respectively). mRNA and surface expression for CCR1, CCR2, and CCR3 in PB cells was lower in Gd(+) compared to Gd(−) MS patients ($p < 0.05$, $p < 0.05$). The frequency of cells co-expressing CD6 with CCR1 and CCR5 was low in PB T lymphocytes and high in CSF ($p < 0.05$, $p < 0.05$). These results suggest that Gd(+) correlates with increased expression of CD6 and decreased expression of chemokine receptors on PB T lymphocytes. Co-expression of CD6 with CCR1 and CCR5 predisposes cells for transmigration into CSF.

1. Introduction

Gadolinium enhancement on T1-weighted images of MRI examination correlates with multiple sclerosis (MS) activity and according to current MS diagnostic criteria appearance of new gadolinium-enhancing lesion is equivalent to clinical relapse (Kilsdonk et al., 2011). Therefore, revealing the mechanisms leading to gadolinium enhancement should contribute significantly to our understanding of MS pathomechanisms. Gadolinium enhancement is associated with blood brain barrier breakdown during the inflammatory process of MS and thus the search for the mechanisms of gadolinium-enhancement has been focused on immune molecules involved in the process of immune cell homing to the CNS, blood brain barrier integrity and functionality.

CD6 is a surface receptor expressed on T lymphocytes correlated with T cell activation. CD6 mediates its activity by interacting with its receptor, activated leukocyte cell adhesion molecule (ALCAM, CD166) (Zimmerman et al., 2006). ALCAM is expressed on antigen presenting cells and engaged in forming immunological synapses (Zimmerman et al., 2006). It was recently shown that CD6 and ALCAM, expressed on the luminal surface of endothelium, are critical in T lymphocytes crossing the blood brain barrier (Cayrol et al., 2008). In addition in genome wide association analysis the CD6 gene was found to be one of three genes correlated with susceptibility to multiple sclerosis (De Jager et al., 2009). Lately it was shown that the CD6 susceptibility allele is associated with alterations in CD4+ T cell proliferation (Kofler et al., 2011) and it was suggested that CD6 is a co-stimulatory molecule which exerts its function in association with TCR/CD3 molecule (Gimferrer et al., 2004).

Chemokines were first identified by their ability to mediate leukocyte chemoattraction in vitro (Ubogu et al., 2006). Till now over 40 chemokines have been described and they interact with more than 20 chemokine receptors. Blood brain barrier endothelial cells produce, secrete and immobilize on their luminal vasculature surface several chemokines responsible for lymphocyte migration across the blood brain barrier in response to inflammatory signals (Holman et al., 2011).

In this paper we have assessed the correlation between gadolinium enhancing lesions, Gd(+), and the expression of CD6 and CCR1, CCR2, CCR3 and CCR5 chemokine receptors on peripheral blood (PB) and cerebrospinal fluid (CSF) immune cells in MS patients.

2. Material and methods

2.1. Patients

20 patients with remitting–relapsing MS (MS RR) diagnosed according to the McDonald criteria (Polman et al., 2005) were included in this study. All patients were treatment naïve and in the past received only steroids for relapse management. Demographic, MRI and clinical data are presented in Table 1. The whole population of MS RR patients was divided into two cohorts, with gadolinium-enhancement Gd(+) and

* Corresponding author at: Department of Neurology, Medical University of Lodz, 22, Kopcinskiego Street, 90-153 Lodz, Poland.
E-mail address: kselmaj@afazja.am.lodz.pl (K. Selmaj).

http://dx.doi.org/10.1016/j.jneuroim.2014.08.620
0165-5728/

Table 1
Demographic data of MS patients with gadolinium-enhancing lesions.

Nr	Gender	Age	Gadolinum enhancement, Gd(+)	
			Quantity	Maximum diameter [mm]
1	M	26	14	12
2	M	28	15	9
3	F	20	3	11
4	F	21	2	8
5	F	45	2	10
6	F	34	5	4
7	M	30	7	13
8	F	20	2	6
9	M	39	2	11
10	M	46	4	27

without enhancement Gd(−) on MRI examination. The Gd(+) cohort was defined by the presence of at least one lesion on T1-weighted images showing enhancement after i.v. administration of gadolinium prior to MRI examination. There were 10 patients with Gd(+) and all of them showed more than one gadolinium-enhancing lesion (Table 1).

2.2. Magnetic resonance imaging

MRI of the brain with i.v. injection of gadolinium (Gd) was performed using 1.5 T Siemens Avanto Plus magnet. A fast spin echo (repetition time [TR] 2200–3000, echo time [TE] 15–50/80–120, echo train length 4–6, 3 mm slice thickness, and 44 contiguous axial slices) sequences were used to obtain proton density and T2-weighted images. Conventional spin echo T1-weighted images (TR 600–650, TE 10–20) with the same scan geometry were obtained 5 min after i.v. injection of 0.1 mmol/kg of Gd. Slices were positioned to run parallel to a line joining the most inferio-anterior and inferio-posterior parts of the corpus callosum.

The identification of Gd-enhanced, T2-hyperintese and T1-hypointense lesions was done and counted by consensus of two experienced observers.

2.3. Flow cytometry

Blood was drawn from each MS patient's cubital vein after they signed an informed consent, and with the approval of the Ethics Committee of Medical University of Lodz. The PBMC were separated by Histopaque (Sigma, Poznan, Poland) density gradient centrifugation of heparinized blood from the MS patients. The isolated cells were washed and pre-blocked with 10% FCS in PBS for 1 h at 4 °C. Then the PBMC were stained with the following antibodies: Alexa Fluor 647mouse anti-human CD195, Alexa Fluor 647 mouse anti-human CD193, Alexa Fluor 647 mouse anti-human CD192, Alexa Fluor 647 mouse anti-

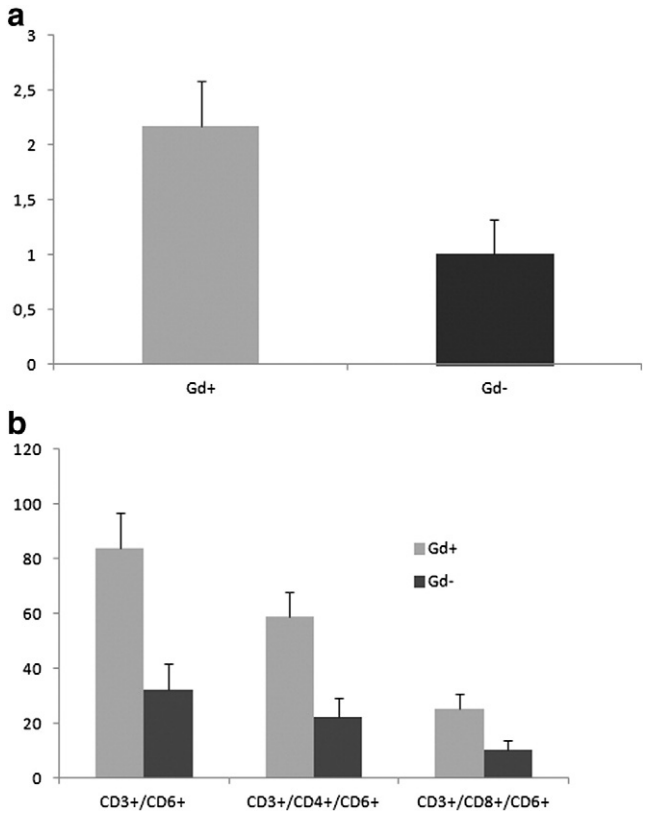

Fig. 1. Expression of mRNA for CD6 in PBMC in Gd(+) and Gd (−) MS patients assessed by real time PCR. Bars represent relative expression of mRNA for CD6 ± SD (a). Expression of surface CD6 on CD3+, CD4+ and CD8+ T lymphocytes in Gd(+) and Gd(−) MS patients assessed by immunostaining and flow cytometry. The bars represent the percentage of positive cells ± SD (b). Correlation coefficient between MFI (mean fluorescence intensity) and mRNA and for CD6 was 0.64 ($p < 0.05$).

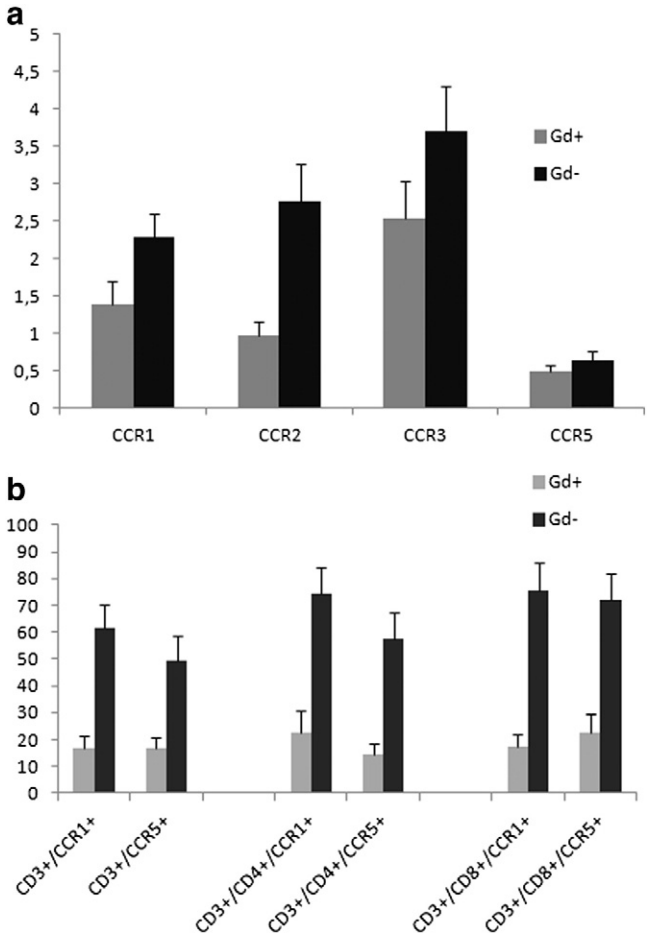

Fig. 2. Expression of mRNA for CCR1, CCR2, CCR3, CCR5 in PBMC of Gd (+) and Gd (−) MS patients assessed by real time PCR. Bars represent relative expression of mRNA for CCR1, CCR2, CCR3 and CCR5 ± SD (a). Surface expression of chemokine receptors, CCR1 and CCR5, on CD3+, CD4+ and CD8+ T lymphocytes of MS patients with and without Gd-enhanced lesions in MRI assessed by immunostaining and flow cytometry. The bars represent the percentage of positive cells ± SD (b). The level of CD3+ cells in Gd (+) patient was not decreased and remained in normal range. Correlation coefficient between MFI (mean fluorescence intensity) and mRNA and for CCR1 was 0.72, for CCR2 0.46, for CCR3 0.58, and for CCR5 0.61 ($p < 0.05$).