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Pecam-1 expression in patients with relapsing-remitting multiple sclerosis

Adam Niezgoda[1], Jacek Losy[1, 2]

[1]*Department of Clinical Neuroimmunology, University School of Medicine, Poznań, Poland*

[2]*Neuroimmunological Unit, Institute-Centre of Clinical and Experimental Medicine, Polish Academy of Sciences, Poznań, Poland*

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PECAM-1 is an adhesion molecule — a member of the immunoglobulin superfamily — involved in the transendothelial migration of leukocytes. PECAM-1 is expressed on lymphocytes, monocytes and granulocytes. It is also found on endothelial cells and platelets. We present data showing that the cell-bound form of PECAM-1 expression on monocytes is increased in MS patients, compared to controls. We also show that PECAM-1 is significantly over-expressed on lymphocytes in patients with active MRI lesions, when compared to those without gadolinium-enhancing lesions. Our results suggest that the cell-bound form of PECAM-1 can be regarded as a marker of MS activity.

key words: multiple sclerosis, PECAM-1, adhesion molecules

INTRODUCTION

Multiple sclerosis (MS) is the most frequent demyelinating disease of the human central nervous system (CNS) characterised by perivascular leukocyte infiltration and inflammation leading to destruction of the myelin sheaths. Adhesion molecules (AM) mediate many cellular processes — they participate in growth, spreading, homeostasis and wound repair, as well as leukocyte recruitment and extravasation to the CNS.

Selectins control the initial weak binding and rolling of leukocytes on endothelium [12], while vascular cell adhesion molecule 1 (VCAM-1, CD 106) and intercellular adhesion molecule 1 (ICAM-1, CD54), both members of the immunoglobulin superfamily, acting with integrins as their counterparts, the very late antigen-4 (VLA-4, CD49) and leukocyte function associated molecule-1 (LFA-1, CD11) respectively, participate in strong adhesion to endothelium before extravasation takes place [3]. Another member of the immunoglobulin superfamily, platelet-endothelial cell adhesion molecule-1 (PECAM-1, CD31), also seems to be involved in leukocyte transmigration across the blood-brain barrier (BBB). It is mainly seen within tight intercellular junctions of the endothelium [1]. While ICAM-1 and VCAM-1 have been studied in patients with demyelinating diseases, PECAM-1's role in pathophysiology remains unclear. Its concentration in body fluids exhibits a high level of constitutive expression and is upregulated less upon cytokine challenge than ICAM-1 or VCAM-1 [4]. Unlike ICAM-1 and VCAM-1, PECAM-1 is capable of binding to itself [7]. All of these molecules have two different forms — native cell–bound (cb), present on the surface of leukocytes, and soluble, found in blood and cerebrospinal fluid [8]. The latter are thought to participate in negative feedback, limiting competitively the adhesion of the cell-bound forms to their receptors.

Several authors have reported an increased expression of ICAM-1, LFA-1 and PECAM-1 on microvessel endothelium and on the surface of infiltrating

Address for correspondence: Adam Niezgoda MD, PhD, Department of Neurology, University School of Medicine, ul. Przybyszewskiego 49, 60–355 Poznań, Poland, tel: +48 61 869 15 83, fax: +48 61 869 15 83, e mail: adamniezgoda@wp.pl

mononuclear cells within demyelinating lesions in experimental allergic encephalitis (EAE), the animal model of MS [2, 6, 10].

Increased expressions of ICAM-1, VCAM-1 in MS patients [5] or elevated levels of soluble (s) PECAM-1 [13], sICAM-1 [9] and sVCAM-1 [14, 16, 17] have also been found.

The goal of this study was to compare the expression level of the cell-bound forms of PECAM-1 on white blood cells drawn from peripheral blood in patients with relapsing-remitting (RR) MS with and without gadolinium-enhancing lesions in MRI.

MATERIAL AND METHODS

14 MS patients with definite RR-MS (age 20–46 years; mean 27 ± 7 years; 10 females and 4 males) according to Poser's criteria [15] were studied. All patients had clearly defined disease relapses with full recovery or with sequelae and residual deficit upon recovery. Annual exacerbation rate for studied patients was 1.2.

The mean disease duration in all MS patients was 5.4 years (range: 5 months – 21 years). Disability was assessed using the expanded disability status scale (EDSS) according to Kurtzke [11]. The mean EDSS was 3.4 (range: 1.0–5.5).

In making the diagnosis, brain MRI, detection of oligoclonal bands and measurement of intrathecal IgG synthesis were utilised.

The patients had not received any immunosuppressive treatment for at least 1 year or any corticosteroids for at least 3 months before inclusion in the study. The control group consisted of 14 patients (9 females, 5 males) with diagnosis of tension type headache and neurasthenia.

Among the 14 studied MS patients, 7 had brain gadolinium-enhancing lesions apparent in T1-weighted spin echo images (MRI+), and 7 had multiple lesions that did not enhance (MRI–). All patients had oligoclonal bands in the CSF and an elevated IgG index (1.2 ± 0.4). Control group subjects did not show oligoclonal bands in the CSF, and had normal IgG indices (0.5 ± 0.1).

cbPECAM-1 measurement

cbPECAM-1 was measured in peripheral whole blood; blood was drawn from each patient on the day of admission; briefly blood was stained with the antibody (DAKO) directed against human PECAM-1 according to manufacturer's instructions; then red blood cells were lysed using FACS Lysing solution (BECTON DICKINSON); stained leukocytes were analysed on flow cytometer (Becton Dickinson). Expression of PECAM-1 was indicated as a percentage of leukocytes bearing this molecule.

MRI protocol

All patients had MRI performed using a Magneton Impact 1.0 T (Siemens). T1-weighted, proton-density, T2-weighted spin echo, as well as gadolinium-DTPA-enhanced T1 images after administration of 0.1 mmol/kg gadolinium-DTPA (Schering, Germany). T2-weighted spin echo images of the brain were done with an echo time (TE) of 80 ms, a repetition time (TR) of 2200 ms, a 256 × 256 acquisition matrix, and 5 mm contiguous interleaved slices. T1-weighted spin echo images were performed using a TE of 15 ms, a TR of 500 ms a 256 × 256 acquisition matrix and 5 mm contiguous interleaved slices.

Sample collection

Serum and cell free CSF samples were collected in order to determine IgG and albumin levels, as well as for oligoclonal bands detection.

Statistical methods

The Statistica statistical program was used. The Mann-Whitney U test was applied to determine the significance of differences between subject groups. The Spearman rank test was used to calculate correlation; p-values lower than 0.05 were considered significant.

RESULTS

PECAM-1 expression on monocytes was significantly higher in the MS group compared to healthy controls (Table 1). We also observed an increased level of PECAM-1 expression on lymphocytes in patients with gadolinium-enhancing lesions in MRI compared to those without active plaques (Table 2). Mean IgG

Table 1. PECAM-1 expression on monocytes in MS patients and control group

	RR-MS (n = 14)	Controls	P (MS patients vs. controls)
PECAM-1 on monocytes	95 ± 4%	81 ± 26%	0.04

Table 2. PECAM-1 expression on lymphocytes in MS patients with and without gadolinium enhancing lesions

	MRI+ (n = 7)	MRI– (n = 7)	P
PECAM-1 on lymphocytes	50 ± 3%	31 ± 3.5%	0.003

CSF level in MS group was 5.7 ± 3.1 mg% and in controls 2.1 ± 0.4. Mean IgG index in MS group was 1.2 ± 0.6 and in the control group 0.5 ± 0.1.

DISCUSSION

In the present study we found increased expression of PECAM-1 on monocytes in MS patients compared to controls. This finding suggests that immunological pathology in MS might not be restrained to lymphocyte population. Our finding is concordant with the observations of Shaw et al. [18], who found that blocking the monoclonal antibody to PECAM-1 significantly reduced monocyte transmigration through the blood-brain barrier, demonstrating that it retains a functional role even though its levels were reduced and redistributed away from junctions after incubation with interferon gamma or tumour necrosis factor. Neutrophil migration in the same study, however, was not affected by reduction of PECAM-1 density within intercellular junctions. This demonstrates that PECAM-1 might play a pivotal role not only for lymphocyte but also for monocyte transmigration across the blood-brain barrier.

This study is the first to show a significant increase in cbPECAM-1 on lymphocytes in MS patients with gadolinium-enhancing lesions. It is consistent with our previous report [13] emphasising the elevation of soluble PECAM-1 level in sera of patients with active disease. Our findings would require a subsequent PECAM mRNA expression analysis.

Since PECAM-1 expression on lymphocytes is augmented in subjects with gadolinium-enhancing lesions and in MS patients compared to normal controls, it should be expected that its soluble form concentration is also increased, which has been demonstrated. Thus, it might be considered a marker of disease activity.

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