Mediators of Inflammation, **9**, 45–48 (2000)

CD13 Ag and CD11a, CD11b, CD18 molecule expression on peripheral blood mononuclear cells (PBMC) were studied as these cells' adherent or transendothelial migration properties in three different multiple sclerosis (MS) patients groups (total 38): with clinically active MS (acute exacerbation of MS and primary chronic progressive MS (CP-MS)) and with MS remission. The control group consisted of patients, suffering from other non-inflammatory neurological diseases (OND). The results of our study suggest that CD11a/CD18 molecules expression on PB lymphocytes, although higher on these cells' surface in the course of MS as compared to OND, does not differentiate clinical forms of MS. CD11a molecule expression on monocytes did not differ significantly in all tested MS patient groups in comparison to OND. Although the expression of CD11b/CD18 molecules on monocytes' surface shows their activation in the course of MS, it does not differentiate them either. However, CD13 Ag of APN expression on PBMC surface may be an immunological marker of MS clinical form. CD13 Ag expression may also be a sensitive marker of these cells' transendothelial migration properties.

Key words: Multiple sclerosis, Lymphocytes, Monocytes, LFA-1, Mac-1, APN

Expression of aminopeptidase N (APN) on peripheral blood mononuclear cells' surface as a marker of these cells' transendothelial migration properties in the course of multiple sclerosis

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Introduction

Multiple sclerosis (MS) is a primary demyelinating disease in which the blood–brain barrier (BBB) damage and mononuclear cells' infiltration in active plaques within the central nervous system (CNS) represent characteristic pathologic features. Inflammatory processes in CNS may be initiated by activated peripheral blood mononuclear cells (PBMC).[1] They can migrate through BBB and enable development of MS itself and its clinical acute exacerbation. Such a disease evolution is based on the animal model of allergic encephalomyelitis (EAE). In the course of clinically active MS, the migration of PBMC through the BBB could be secondary to the local BBB disruption by inflammatory cytokines and extracellular matrix degrading proteases produced in plaques in CNS, and thus without the involvement of PBMC.[2] These create a totally different view on the disease immunopathology. The problem of whether PMNL can show adherent and transendothelial migration properties in the course of clinically active MS could be solved indirectly by evaluation of the β-integrin molecule expression and extracellular matrix degrading proteases production by these cells. The molecule expression on PBMC may also 'immunologically' differentiate clinical periods of MS.

Material and methods

There were three groups of patients (total 38) aged 21–56 years (mean 38.2 ± 10.1 years) with clinically definite MS and with Expanded Disability Status Scale scores of 5 or lower. Thirteen patients (seven women and six men) were examined during the course of acute MS exacerbation, 12 patients (seven women and five men) with primary chronic progressive MS (CP-MS) and 13 patients (six women and seven men) during MS remission. The control group (OND) consisted of 14 patients (seven women and seven men), aged 22–41 years (mean 31.3 ± 6.7 years), suffering from other neurological diseases, like vasomotor headache in 10 and epilepsy in four cases.

Immunostaining and FACS analysis

Blood samples were obtained in the morning between 8.00 and 9.00 a.m. by venipuncture. One hundred microlitres of blood was mixed and incubated at room temperature with appropriate quantities of monoclonal antibodies anti- CD11a, CD11b and CD18 provided by Dako (Denmark). A double-step staining procedure was used for the evaluation of CD13 Ag expression. Mouse immunoglobulin anti-

Table 1. The expression (mean±SD) of CD11a, CD11b, CD18 molecule and CD13 Ag on lymphocyte and monocyte surfaces in the course of acute exacerbation of MS (1), primary chronic progressive MS (CP-MS) (2), MS remission (3) and in the other neurological diseases (OND; 4)

	CD13 Ag expression on lymphocytes	CD11a molecule expression on lymphocytes	CD18 molecule expression on lymphocytes	CD13 Ag expression on monocytes	CD11a molecule expression on monocytes	CD11b molecule expression on monocytes	CD18 molecule expression on monocytes
1. MS acute exacerbation (n=13)	59±11 range: 47–71 1 vs 3 P<0.01 1 vs 4 P<0.01	120±11 range: 107–142 1 vs 4 P<0.01	140±20 range: 108–161 1 vs 4 P<0.01	256±58 range: 148–333 1 vs 3 P<0.05 2 vs 3 P<0.05	8±6 range: 4–17	253±59 range: 149–341 1 vs 3 P<0.05 1 vs 4 P<0.01	188±59 range: 132–298 1 vs 3 P<0.05 1 vs 4 P<0.05
2. CP-MS (n=12)	58±10 range: 44–70 2 vs 3 P<0.01 2 vs 4 P<0.01	122±15 range: 107–154 2 vs 4 P<0.01	143±28 range: 100–199 2 vs 4 P<0.01	276±69 range: 150–385 2 vs 3 P<0.05 2 vs 4 P<0.05	8±4 range: 3–13	287±49 range: 199–353 2 vs 3 P<0.01 2 vs 4 P<0.01	201±41 range: 160–315 2 vs 3 P<0.01 2 vs 4 P<0.01
3. MS remission (n=13)	28±3 range: 22–32	115±16 range: 94–148 3 vs 4 P<0.05	132±18 range: 105–154 3 vs 4 P<0.05	202±42 range: 124–263	9±5 range: 5–22	213±43 range: 164–290 3 vs 4 P<0.05	136±35 range: 90–188 3 vs 4 P<0.05
4. OND (n=14)	30±7 range: 22–47	109±12 range: 89–129	108±13 range: 94–121	186±35 range: 123–225	8±5 range: 4–16	169±52 range: 91–211	113±34 range: 73–181

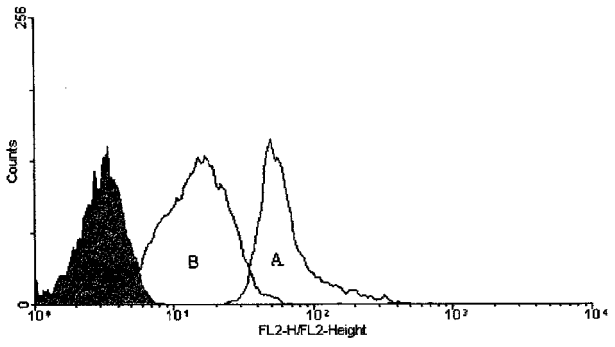

FIG. 1. An example of CD13 Ag expression on peripheral blood lymphocytes during the course of acute exacerbation of MS (A) in MS remission (B) and other neurological diseases (Control).

CD13 (Dako) was used as a first step. Rabbit anti-mouse polyclonal IgG stained with R-phycoerythrin was used as a secondary antibody. The rabbit IgG was also used as a negative control. Erythrocytes were eliminated by an addition of lysing solution (Becton Dickinson) into the blood samples. A FACscan flow cytometer with a 488-nm argon laser (Becton Dickinson) and Lysis II software were used. The results were expressed as the values of mean fluorescence intensity (MFI) of the labelled surface antigens.

An example of CD13 Ag expression on peripheral blood lymphocytes is shown on Fig. 1.

The obtained results were analysed, using Student's t-test and the Cochran–Cox rank test.

Results

The expression of CD11a/CD18 molecules of LFA-1 integrine was higher on lymphocytes in the course of clinically active MS (MS acute exacerbation and in CP-MS) as well as in MS remission comparing to the OND group (Table 1). Among MS patients the expression of LFA-1 molecules on lymphocytes surface did not differ significantly.

CD11a molecule expression on monocytes did not differ significantly in all the tested MS patient groups in comparison to the OND. The CD11b molecule of Mac-1 integrine expression was significantly higher on monocytes in the course of MS acute exacerbations and in CP-MS compared to MS remission and OND. CD11b expression was also higher in the course of MS remission compared to the OND group. Similarly to CD11b molecule expression on monocytes, the differences were noticed in molecule CD18 expression.

Conversely to the integrines molecule expression on PBMC, CD13 Ag expression on lymphocytes and on monocytes 'immunologically' differentiated clinical periods of MS. In the course of MS acute exacerbation and in CP-MS groups, it was significantly enhanced in comparison to MS remission and OND (Table 1).

Discussion

The mechanisms of PBMC migration through the BBB in the course of MS are not yet known. In a series of repeated tests, higher β1 and β2 integrin molecules' expression on $CD4^+$ T-cells in acute MS exacerbation compared to MS remission was not found.[3] Also, in our observations LFA-1 and Mac-1 integrine molecules' expression on PBMC did not 'immunologically' differentiate transendothelial migration properties of PBMC in the course of MS. However, CD11a/CD18 molecules' expression on PB lymphocytes, in each group of MS patients, was higher than in OND patients. In our opinion this could suggest an increased percentage of memory cells in the peripheral blood of all the MS patients. This was also shown in some other studies.[4] It indirectly suggests that circulating lymphocytes in MS are predisposed to cross endothelial barriers. Also CD11b/CD18 molecules of Mac-1 integrine expression were higher on monocytes' surface in the course of clinically active MS compared to MS remission. This can be an effect of non-specific stimulation of CD11b/CD18 molecules on these cells' surfaces by immunological complexes, or C3a and C5a. However Mac-1 has a much smaller effect on monocyte adherence to endothelium than LFA-1.[5] Thus, it cannot be a marker of monocyte transendothelial migration in the course of MS.

Conversely to integrine molecules, CD13 Ag expression on the PBMC surface can be such a marker. CD13 Ag assessment is widely applied in the evaluation of APN expression on PBMC surface.[6] APN is a zinc-dependent metalloproteinase. Similarly to 92 kDa gelatinase it hydrolyses the IV-type of collagen.[7] Therefore, it plays an important role in PBMC migration across the blood vessels. Several studies revealed a crucial role of 92 kDa gelatinase and dipeptydyl-peptidase-IV in these processes in the course of MS.[8,9] The results of our study suggest than APN may also participate in it.

We have shown the presence of acivated PBMC in the peripheral blood of MS patients. In the course of acute exacerbation and in CP-MS, these cells show a potentially greater ability to cross the BBB. The data obtained may suggest that PBMC might play an active role in BBB disruption in the course of MS.

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Received 15 November 99;
accepted 22 December 99