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Role of CD38 in Cell-Cell Interactions under Conditions of Endothelial Dysfunction

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We studied the role of receptor/ectoenzyme CD38 in the formation of endothelial damage and pathogenesis of endothelial dysfunction. CD38 was located in sites of protrusion of the outer cytoplasmic membrane. The majority of $CD38^+$ accumulation sites coincided with the protrusion pole, while in some cells expression of CD38 was spread along the entire cell membrane surface or located on the pole opposite to the protrusion. We hypothesized that these states reflect the processes of rafting and clustering of the receptor, which are essential for cell-cell interactions in the pathogenesis of endothelial dysfunction.

Key Words: *CD38; endothelial dysfunction; clustering*

Endothelial dysfunction is a complex of abnormal state of blood cells and endotheliocytes (EC) associated with modified cell-cell interactions mediated by both bioactive substances and expression of specific receptors.

Changes in the vascular wall reducing its elasticity, leading to the formation of atherosclerosis, and disturbing the production of vasodilators largely depend on the integrity of the endothelium and maintenance of its barrier functions.

The content of intercellular adhesion molecules increases in the pathogenesis of endothelial dysfunction (EDF), which increased endothelial permeability for immune cells. The latter plays a crucial role in vascular damage, which is determined by lipid imbalance followed by the formation of atherosclerotic plaque, hyalinosis of the vascular wall, metabolic changes, and damage to EC.

Among all glycoprotein receptors identified on immune system cells (lymphocytes, monocytes, leukocytes), CD38/NAD-glycohydrolase is of particular interest for the pathogenesis of EDF. This receptor/ectoenzyme is involved in various processes including regulation of hemopoiesis, maturation of immune system cells, cell activation and proliferation, due to metabolic conversion of NAD into compounds with pronounced regulatory activity. CD38 plays an essential role in adhesion of immune system cells to EC (CD38/CD31 interaction) and triggering of cytokine-mediated destruction of the contact cells [2,4-6].

These two events aggree with the theory of immune synapses [1], when receptor-ligand interactions formed by immune system cells are mediated by specific changes in cell membranes, receptor clustering, which facilitates cell contacts, information transfer, and triggering of the corresponding programs of cell death.

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Here we verified the hypothesis on the involvement of CD38 into the formation of immune synapses and damage to EC in patients with essential hypertension.

MATERIALS AND METHODS

The study included patients with complicated essential hypertension (ischemic stroke, group 1, n=110) and patients without cardiovascular catastrophes (group 2, n=50). All participants gave informed consent to participation in the study.

Peripheral venous blood (5 ml) was obtained from all patients. Lymphocyte fraction was isolated by centrifugation on Ficoll-verografin density gradient (ρ=0.077). The isolated cells were suspended in 50 μl phosphate-buffered saline (PBS) to a concentration of 10^6 cell/ml. Then, primary antibodies to human lymphocyte CD38 (3 μl, titer 1:50, Sorbent Ltd.) were added to the prepared lymphocyte suspension. After incubation, the lymphocytes were twice washed with PBS (150 μl), the supernatant was discarded. Secondary antibodies (FITC-labeled fragments of sheep antibodies to IgG, Sorbent Ltd, initial dilution of 1:100 in PBS with 1% sheep serum) were added to the cell pellet in a volume of 50 μl in dark. After incubation and washout with PBS, the cell suspension (20 μl) was transferred onto a slide and examined under a LUMAM luminescent microscope with a phase contrast attachment.

A total of 200 lymphocytes were analyzed (round cells sized 9-10 μ), $CD38^+$- and $CD38^-$ cells were identified by luminescent microscopy and by morphology of the cytoplasmic membrane (phase-contrast microscopy). Intact cells had visually unchanged plasma membrane and round and smooth surface. Cells at the initial and terminal stages of blebbing had small and large membrane vesicles ($^1/_3$ cell radius and $>^1/_3$ cell radius, respectively).

Identification and counting of desquamated EC (polygonal cells sized 20-50 μ) per 100 cells of the lymphocyte fraction were performed by microscopy of peripheral blood lymphocytes isolated on Ficoll-verografin density gradient as described previously [3] with our modifications: desquamated EC were isolated with lymphocyte fraction from the peripheral blood and were analyzed by phase-contrast microscopy.

Before using parametric methods of statistical analysis, the normality of distribution for the compared samples was verified using Kolmogorov—Smirnov test with Lilliefors correction. Student—

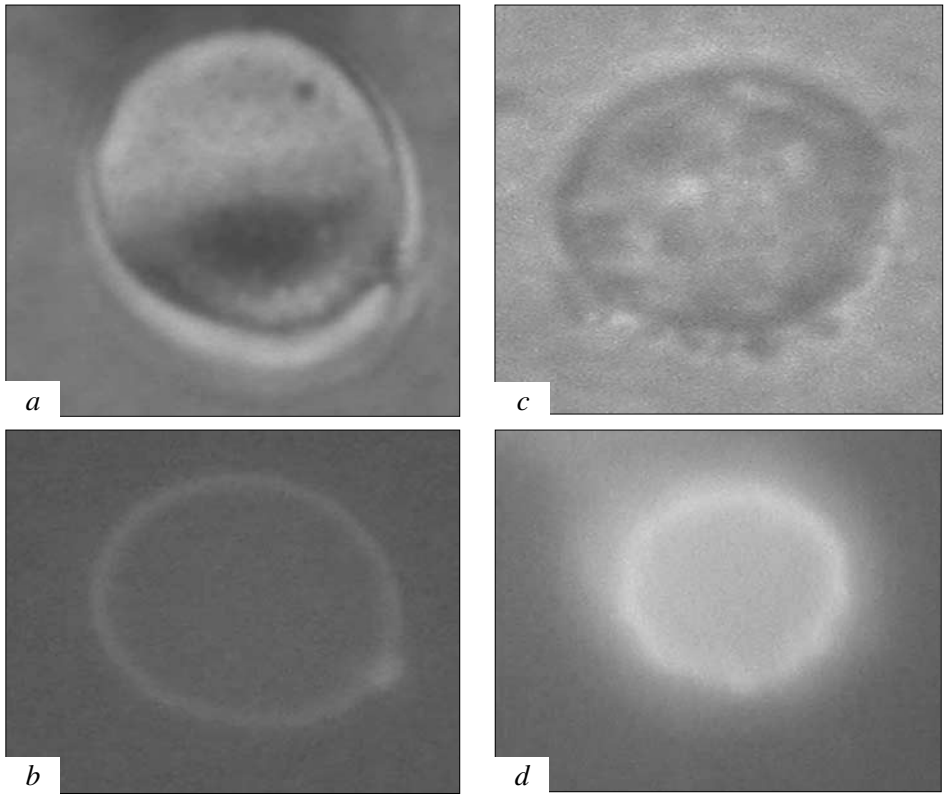

Fig. 1. Blebbing of outer cytoplasmic membrane and expression of $CD38^+$ on lymphocytes, ×900. *a*, *c*) phase-contrast microscopy; *b*, *d*) luminescent microscopy; *b*) expression of CD38 coincides with protrusion of the outer cytoplasmic membrane at 16.00 (*a*); *d*) CD38 is expressed along the entire cell surface, maximum accumulation coincides with the site of intensive blebbing at the low pole of the cell (*c*).

Fisher *t* test was used for comparison of samples conforming the normal distribution. Otherwise the samples were compared using Wilcoxon *W* test and Mann—Whitney *T* test for nonparametric values.

RESULTS

The maximum number of lymphocytes expressing CD38 was observed in patients with progressing EDF comprising group 1 (17.85±0.75% from all lymphocytes); in group 2 with stable course of EDF, only 2.32±0.18% lymphocytes expressed CD38 ($p<0.01$).

The sites of CD38 expression coincided with protrusions of the outer lymphocyte plasma membrane in 93% cases. The most part of $CD38^+$-lymphocytes were in a state of intensive blebbing (Fig. 1).

No considerable differences in CD38 externalization and localization of protrusions of the outer plasma membrane between the groups were found. Therefore we present pooled data: the projection of $CD38^+$-sites coincided with the pole of cytoplasmic membrane protrusion in 65%, while in 28% lymphocytes $CD38^+$-sites and blebbing sites were spread along the entire surface. In 7% lymphocytes, $CD38^+$-sites were also detected on the pole opposite to bubble protrusion (Fig. 2).

For evaluation of the effect of CD38 expression on EC we analyzed the content of desquamated EC in the peripheral blood of patients. On day 1 after hospitalization, the content of desquamated EC was 15.54±0.49 in group 1 patients and 1.54±0.15 in group 2 patients.

This attests to a clear-cut relationship between enhanced expression of CD38, intensive blebbing

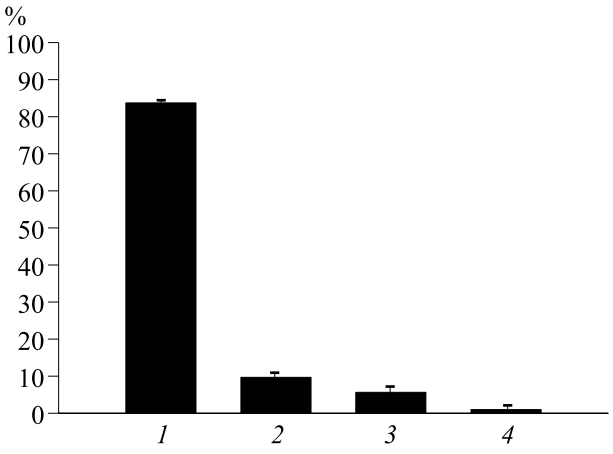

Fig. 2. Content of lymphocytes of identified categories in the peripheral blood of group 2 patients. *1*) lymphocytes with intact membrane; *2*) lymphocytes in the state of initial blebbing; *3*) lymphocytes in the state of terminal blebbing; *4*) necrotic lymphocytes.

of peripheral blood lymphocytes, increased content of desquamated EC, and EDF progression.

Our findings suggest that expression of CD38 in sites of intensive blebbing and coincidence of $CD38^+$ expression with sites of initial blebbing and spreading along the entire cell surface can be explained by rafting and clustering of the receptor, when the density of the receptor apparatus at the cell pole increases due to translocation of membrane sites, which improves the efficiency of cell-cell interaction and contact and amplified the signal from the receptor. Moreover, enhanced expression of CD38 and increased content of desquamated EC ($r=0.87$, $p<0.05$) attest to initiation of apoptosis, in particular, mediated by CD38, which is determined

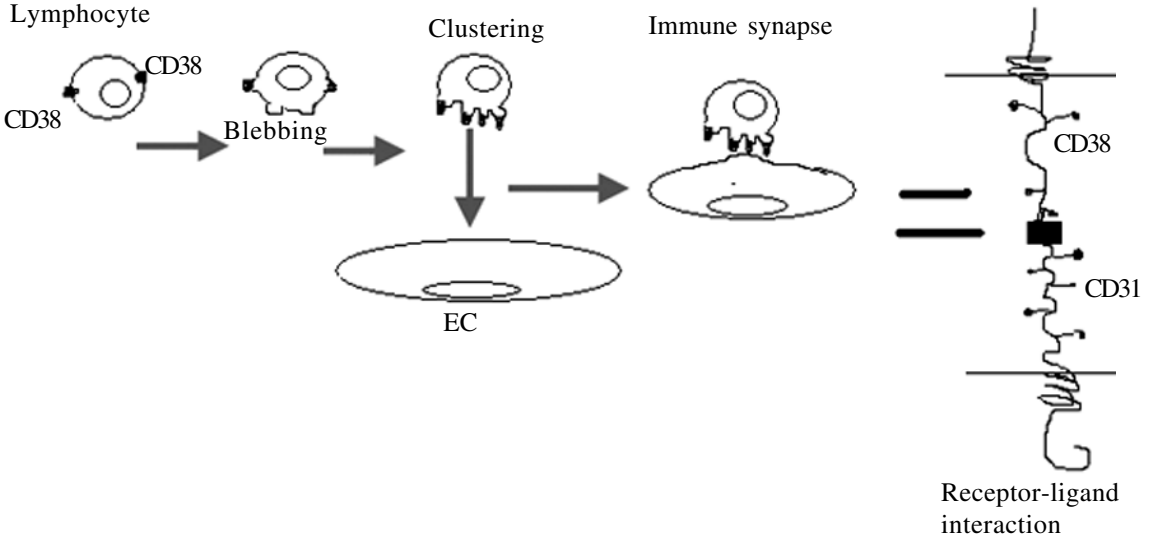

Fig. 3. Scheme of CD38 clustering and formation of immune synapse in the pathogenesis of EDF.

by accumulation of intracellular Ca^{2+} (the effect typical of CD38/31) and cytokine activation (Fig. 3).

The influence of lymphocyte on EC induced mobilization of Ca^{2+}, activation of nuclear factors, synthesis and activation of cytokines, *etc*.

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