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Evaluation of the Expression of Early Activation Marker CD69 by CD8+ Lymphocytes in HIV-Infected Patients

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We studied the expression of CD69 antigen on CD8+ lymphocytes in response to nonspecific activator phytohemagglutinin in 202 patients with HIV infection. It was found that the number of CD8+CD69+ lymphocytes in HIV-infected patients increased; this parameter negatively correlated with relative and absolute content of CD4+ cells and positively correlated with IgG concentration.

Key Words: *HIV infection; immunophenotype; CD69 antigen*

According to WHO data, the number of HIV-infected individuals in the world is about 40 mln. New patients with HIV/AIDS are detected every year. It is beyond doubt that HIV infection is actual for Russia. The maximum number of HIV-infected individuals detected in Eastern Europe and Central Asia live in the Russian Federation [2].

Functional peculiarities of the immune system in HIV-infected patients play an important role in the evaluation of the disease and its progress. Of particular importance is evaluation of functional activity of lymphocytes in HIV-infected patients by the expression of membrane receptors CD69, CD38, *etc*. [6]. Expression of CD69 antigen, an early activation marker, by T cells in response to specific and unspecific stimuli reflects functional properties of immunocompetent cells [8]. The efficiency of using this marker in patients with type 1 diabetes mellitus and systemic lupus erythematous was demonstrated. For instance, the number of activated cells in these patients was lower than in healthy donors [9].

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Cytotoxic lymphocytes (CTL) play an important role in antiviral T-cell-mediated immune response. They carry surface membrane glycoprotein CD8 and recognize peptide antigens, including viral antigens, presented by major histocompatibility complex class 1 molecules [1].

A potent cellular response is initiated practically immediately after HIV infection, antiviral activity of this response is evaluated by CTL. This is achieved by direct killing of infected cells. At the same time, activation of CTL leads to synthesis of soluble antiviral factors, including chemokines that directly inhibit the development of the infection. Activation of CD8+ cells leads to the appearance of HIV-specific CTL which differentiate depending on their effector functions [3].

Here we studied functional state of CD8+ lymphocytes in HIV-infected patients by the expression of CD69 antigen in response to nonspecific activator.

MATERIALS AND METHODS

We examined 202 HIV-infected patients of Regional Center on Prophylaxis and Combating of AIDS

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and Infection Diseases (Krasnoyarsk). The diagnosis was verified by immunoblotting. The mean age of the examinees was 26 years. The duration of the disease varied from 6 months to 6 years. The study was performed before the start of specific antiviral therapy. The control group comprised 30 age-matched healthy volunteers. Informed consent was obtained from all patients.

Quantitative analysis of lymphocyte populations was performed by flow cytometry using three-color monoclonal antibody panel (CD3-FITC/CD4-PE/CD45-PerCP and CD3-FITC/CD8-PE/CD45-PerCP). The concentrations of IgA, IgM, and IgG were determined by radial immunodiffusion in gel [7]. The level of circulating immune complexes was determined in the reaction with polyethylene glycol [5].

For evaluation of the expression of CD69 antigen, whole heparinized blood obtained from the ulnar vein was incubated in a dry-air thermostate at 37°C for 4 h in the presence of 10 μg/ml phytohemagglutinin. The blood with activated T cells was stained with three-color antibodies CD8-FITC/CD69-PE/CD3-PerCP (BD). The number of $CD3^+$/$CD8^+$/$CD69^+$ cells was determined by three-color cytophotometry using CellQuest software on a FACS-Calibur flow cytometer (Becton Dickinson).

Significance of differences between two independent samples was evaluated using Mann—Whitney *U* test. The relations between two signs were analyzed using Spearman rank correlation coefficient (*r*). The results were presented as median and interquartile intervals *Me* (*LQ-UQ*), where *Me* is median and *LQ* and *UQ* are 25 and 75% percentiles.

RESULTS

Study of the immunophenotypic spectrum of peripheral blood lymphocytes showed that the percent of mature T cells ($CD3^+$) in HIV-infected patients was higher than in the control group (Table 1).

The absolute and relative contents of $CD4^+$ cells decreased and those of $CD8^+$ lymphocytes increased compared to the control. The $CD4^+/CD8^+$ ratio in the group of HIV-infected patients was disturbed, which led to a drastic decrease in the immunoregulatory index. The reaction of the humoral immunity was characterized by increased serum content of IgM and IgG compared to that in the control (Table 1).

The percentage of $CD8^+CD69^+$ cells in the group of HIV-infected patients was 12.26% (8.27-18.35%) *vs.* 7.92% in the control (7.12-8.98%; $p<0.001$). After nonspecific stimulation of peripheral blood cells, the expression of CD69 antigen on $CD8^+$ lymphocytes in HIV-infected patients increased compared to the control.

HIV-infected patients were divided into subgroups with low (<8.27%; $n=49$) and high (>18.35%; $n=53$) expression of CD69 antigen on $CD8^+$ cells (values exceeding the limits of the interquartile interval 25-75%).

Comparative analysis revealed significant decreased in relative (22.88±1.13 *vs.* 31.49±1.05; $p<0.001$) and absolute (442.59±31.10 *vs.* 553.50±31.02; $p<0.005$) contents of CD4 cells in the group of HIV-infected patients with high content of $CD8^+CD69^+$ lymphocytes compared to patients with low content of activated CD8 lymphocytes.

TABLE 1. Immunological Parameters in HIV-Infected Patients

Parameter	Control group (n=30) *Me* (*LQ-UQ*)	HIV-infected (n=202) *Me* (*LQ-UQ*)
Lymphocytes, 10^9/liter	2199.0 (1770.6-2507.1)	1756.0 (1439.3-2236.5)*
$CD3^+$ cells, %	73.0 (64.5-78.0)	79.7 (75.5-84.2)**
$CD3^+$ cells, 10^9/liter	1391.5 (1265.0-1891.0)	1399.8 (1116.9-1777.0)
$CD4^+$ cells, %	41.0 (38.0-47.0)	28.5 (22.1-34.3)**
$CD4^+$ cells, 10^9/liter	797.0 (727.1-1060.8)	502.3 (340.1-631.8)**
$CD8^+$ cells, %	27.0 (21.0-29.0)	50.2 (44.6-56.2)**
$CD8^+$ cells, 10^9/liter	539.0 (442.0-639.0)	820.5 (670.4-944.2)**
$CD4^+/CD8^+$	1.65 (1.40-1.91)	0.57 (0.38-0.79)**
IgA, g/liter	1.62 (1.38-2.02)	1.85 (1.35-2.44)
IgM, g/liter	1.46 (1.17-1.75)	1.87 (1.46-2.34)*
IgG, g/liter	13.54 (10.64-15.48)	15.48 (12.41-19.15)*
Circulating immune complexes, arb. units	27 (20-38.5)	26 (20-40)%

Note. *$p<0.005$, **$p<0.001$ compared to the control.

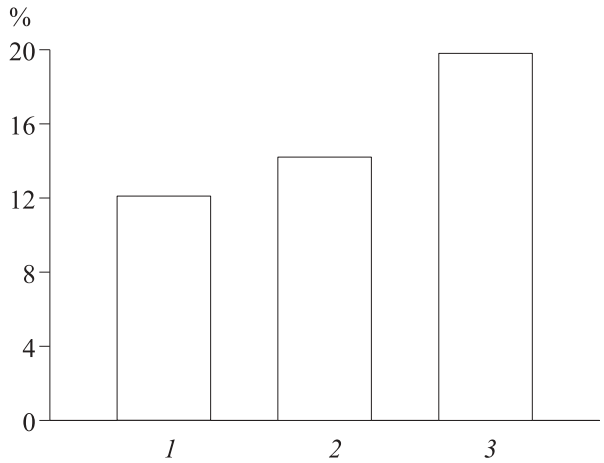

Fig. 1. Percent of $CD8^+CD69^+$ lymphocytes in HIV-infected patients with different content of $CD4^+$ cells: >500 (*1*), 200-499 (*2*), and <200 cells/ml (*3*).

Correlation analysis showed that the expression of $CD8^+CD69^+$ lymphocytes in HIV-infected patients negatively correlated with relative (r=-0.38) and absolute (r=-0.27) number of $CD4^+$ lymphocytes (p<0.001).

The initial group of HIV-infected patients was divided into 3 subgroups by the content of $CD4^+$ cells (according to classification of HIV infection, CDC (1994): $CD4^+$ lymphocyte count >500 cells/μl (subgroup 1, n=112), 200-499 cells/μl (subgroup 2, n=78), and <200 cells/μl (subgroup 3, n=12). It was found that the content of $CD8^+CD69^+$ lymphocytes in the compared subgroups of HIV-infected patients increased with decreasing the content of $CD4^+$ cells.

A significant positive correlation was found between the expression of CD69 antigen on $CD8^+$ lymphocytes and concentration of IgG (r=0.13; p<0.05). The role of humoral immunity factors in the progression of HIV infection is ambiguous [4]. Higher levels of total IgG and IgA were noted in HIV-infected patients with hemophilia and rapid progression of the disease. It should be noted that the degree of elevation of immunoglobulin concentration was a viremia-independent factor of the disease progression [10].

At the same time, we found no significant correlation between the level of viral load and relative content of $CD8^+CD69^+$ lymphocytes in the peripheral blood of HIV-infected patients.

Thus, functional changes in the response to unspecific mitogens manifesting in increased relative content of $CD8^+$ lymphocytes carrying CD69 antigen against the background of changes in phenotypic spectrum of circulating lymphocyte pool were observed in HIV-infected patients. Significant relationships between immunological markers of disease progression were found: the count of activated $CD8^+$ lymphocytes carrying CD69 antigen increased with decreasing the number of $CD4^+$ cells. In HIV-activated patients with high content of $CD8^+CD69^+$ cells, the level of IgG increased.

Our findings suggest that the expression of early activation marker $CD69^+$ by $CD8^+$ peripheral blood lymphocytes should be taken into account in complex evaluation of the immune status in patients with HIV infection.

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