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Modulatory Effects of Autologous Plasma on Functional Activity of Human Immunocompetent Cells

O. P. Ryabchikov, V. I. Kirillov, A. N. Tsygin, and I. I. Mishina

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Blast transformation of peripheral blood lymphocytes stimulated with phytohemagglutinin and concanavalin A was studied in children with pyelonephritis and glomerulonephritis. Activity of natural killer cells from children with pyelonephritis was estimated before and after treatment with 50% autologous plasma. The autologous plasma modulated blast transformation of lymphocytes and activity of natural killer cells, which depended on the stage of diseases.

Key Words: *immunocompetent cells; functional activity; autologous plasma*

Studies of the immune state in human and animals include evaluation of functional activity in immunocompetent cells [4]. Most previous experiments were performed with purified lymphocytes or other immunocompetent cells. However, this approach ignores the effects of various normal and pathological components of plasma and interstitial liquid. These compounds can affect various stages of the immune response [1,6,8-10,12-14], which makes the understanding of the pathogenesis of diseases difficult. The effects of specific microenvironment on the functional properties of immunocompetent cells are extensively studied [1,6,9]. Bearing in mind that immune reactions occur primarily in organs of immunogenesis and various somatic tissues (peripheral blood contains only 0.2% lymphocytes) [3], the effect of autologous plasma (AP) on functional activity of these cells closely contacting with AP are of considerable interest.

Here we studied the effects of AP on mitogenic stimulation of peripheral blood lymphocytes from children with pyelonephritis and glomerulonephritis and activity of natural killer (NK) cells from children with pyelonephritis.

Institute of Human Morphology, Russian Academy of Medical Sciences

MATERIALS AND METHODS

Peripheral venous blood was taken from children with glomerulonephritis (n=93) and pyelonephritis (n=34) 2-14 years. Lymphocytes were isolated on a Ficoll-Verografin density gradient [2]. RPMI-1640 medium containing 10% fetal bovine serum, 25 mM HEPES buffer (Flow), 2 mM L-glutamine (Sigma), and 40 μg/ml gentamicin was used as a complete nutrient medium. T-cell mitogens phytohemagglutinin M (PHA-M, Calbiochem) and concanavalin A (Con A, Flow) were used in optimum concentrations of 10 and 1 μg/ml, respectively. The reaction of lymphocyte blast transformation (RBTL) was performed routinely [2]. The index of lymphocyte stimulation (SI-1) was calculated as the ratio between experimental and control values (cpm).

Fresh AP (50%, v/v) was added to some wells containing cells alone or cells with mitogens. SI-2 was calculated as the ratio between experimental (mitogen+AP) and control parameters (mitogen-free culture+AP).

The index of cytotoxic activity of NK cells was estimated by routine methods using human erythroleukemia K-562 cells labeled with ^{51}Cr [7]. The cells were incubated at a 25:1 effector-target ratio for 14-16 h. AP (50%, v/v) was added to some wells (similarly

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to RBTL). To exclude cytotoxic effects of AP on lymphocytes, the number of dead karyocytes was estimated using acridine orange and ethidium bromide [2]. The results were processed by Student's *t* test and expressed as *M*±*m*.

RESULTS

In 35 samples from children with glomerulonephritis SI markedly decreased after treatment with AP ($p<0.001$, Table 1). In 22 samples this parameter decreased more than 3-fold. The number of dead cells in samples with and without AP did not exceed 15%. SI increased in 32 samples (by more than 3 times in 15 samples, $p<0.01$). The count of dead cells did not differ between the control and experimental samples (12%). AP modulated spontaneous proliferation of lymphocytes in 92 of 120 samples. This parameter increased by 300% in 54 samples and decreased by 50% in 38 samples. This AP-induced modulation of lymphocyte proliferation in children with glomerulonephritis is probably associated with the stage and type of the disease and massive drug therapy.

AP changed SI in children with pyelonephritis (Table 2). Proliferative activity of lymphocytes increased in the acute stage and, particularly, during convalescence (by 3 times, Table 2). It should be emphasized that AP potentiated mitogen-induced lymphocyte activity in healthy children by 2.4 times. These data indicate that in the acute stage of pyelonephritis and during convalescence the plasma from children contains factors that modulate lymphocyte stimulation, but do not cause cell death. This is consistent with published data [12].

AP considerably decreased cytotoxic activity of NK cells from children with acute pyelonephritis, but did not cause cell death (as differentiated from the convalescence period, Table 3). Washed NK cells possessed the same cytotoxicity in the acute stage and during convalescence.

Our findings and published data indicate that influence of microenvironmental factors (*e.g.*, AP) should be taken into consideration in evaluating functional activity of human immunocompetent cells under normal and pathological conditions. This method more precisely reflects *in vivo* processes and allows us to understand the pathogenesis of diseases and to correct the treatment, *e.g.* to use immunocorrection therapy.

TABLE 1. Proliferative Activity of Con A-Stimulated Lymphocytes in Children with Glomerulonephritis (*M*±*m*)

Effect of AP (number of children)	SI-1 (without AP)	SI-2 (with AP)
Decrease in SI (*n*=35)	6.5±0.8	1.5±0.2*
Increase in SI (*n*=32)	2.5±0.5	5.8±0.9**
No changes (*n*=26)	1.7±0.6	1.8±0.5

Note. *$p<0.001$ and **$p<0.01$ compared to SI-1.

TABLE 2. Proliferative Activity of PHA-Stimulated Lymphocytes in Children with Various Stages of Pyelonephritis (*M*±*m*, *n*=15)

Group	SI-1 (without AP)	SI-2 (with AP)
Healthy children	2.80±0.06	6.83±1.18*
Patients with pyelonephritis		
acute stage	0.66±0.13	1.06±0.05*
convalescence	1.32±0.27	3.89±0.31**

Note. *$p<0.001$ and **$p<0.05$ compared to SI-1.

TABLE 3. Index of Cytotoxic Activity of NK Cells in Children with Pyelonephritis (25:1 Effector-Target Ratio, *M*±*m*)

Effectors	Stage of pyelonephritis	
	acute (*n*=9)	convalescence (*n*=8)
Washed lymphocytes	43.9±3.0	36.0±5.8
+50% AP	26.7±2.2*	41.4±2.4

Note. *$p<0.001$ compared to cultures without AP.

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