Effects of Tissue Injury Mediators on the *In Vitro* Expression of Surface Antigens by Human Lymphocytes

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A short-term incubation of healthy donor lymphocytes with adenosine or adenosine diphosphoric acid was shown to reduce the number of cells expressing CD4 antigen and to increase the number of $CD8^+$ lymphocytes. The hydra peptide morphogen shifts the balance of immunoregulatory lymphocytes toward a predominance of $CD4^+$ cells. A hypothesis of a two-level system for the regulation of surface antigen expression by lymphocytes during exposure to extreme factors is proposed.

Key Words: *lymphocytes; surface phenotype; adenosine; adenosine diphosphoric acid; hydra peptide morphogen*

Immunologic restriction by antigens of the major histocompatibility complex is one of the main functions of membrane glycoprotein molecules denoted as CD4 or CD8 antigens. This function is closely connected with the predominant presentation of CD4 antigen on cells with helper function and of CD8 molecules on suppressor cytotoxic lymphocytes [10].

Traditionally the alteration of CD4 and CD8 antigen expression is considered in the context of thymic cell differentiation [13,14]. However, many authorities have demonstrated the possibility of rapidly changing the surface phenotype of differentiated lymphocytes under appropriate conditions.

Phorbolmyristatacetate has been shown to induce calcium-dependent rapid phosphorylation and internalization of the CD4 molecule [5,8]. Moreover, similar treatment with phorbolmyristatacetate leads to a reduced expression of CD8 molecules presented as α/β heterodimer but not α/α homodimer [15]. Different effects of cell treatment with phorbolmyristatacetate on the expression of membrane CD4 and CD8 proteins have been demonstrated [7].

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The data on the capacity of other compounds to influence the expression of lymphocyte surface antigens are less numerous. Suramin is known to have a negative regulatory impact on CD4 expression [4]. Short-term treatment with isoproterenol leads to an increase of the number of $CD8^+$ cells [6]. Purine compounds are characterized by the capacity to modulate the expression of CD4 and CD8 antigens [6]. And, finally, circadian fluctuations have been revealed in the number of cells expressing CD4 but not CD8 antigen in the peripheral blood.

All the aforesaid explains the current interest in the mechanism of the rapidly unfolding changes in the antigen profile of the lymphocyte membrane which, we believe, may represent a mechanism of emergency immunoregulation under conditions of extraordinary external factors. We thus attempted to study the phenomenon of phenotypic change of the peripheral blood lymphocytes in healthy subjects under the influence of adenine compounds regarded as local immunomodulators which are released in tremendous amounts in the intercellular space during injury to or the death of virtually any cell, during shock, intensive muscular work, or clot formation [3,11]. One of the acute phase response factors, hydra peptide mor-

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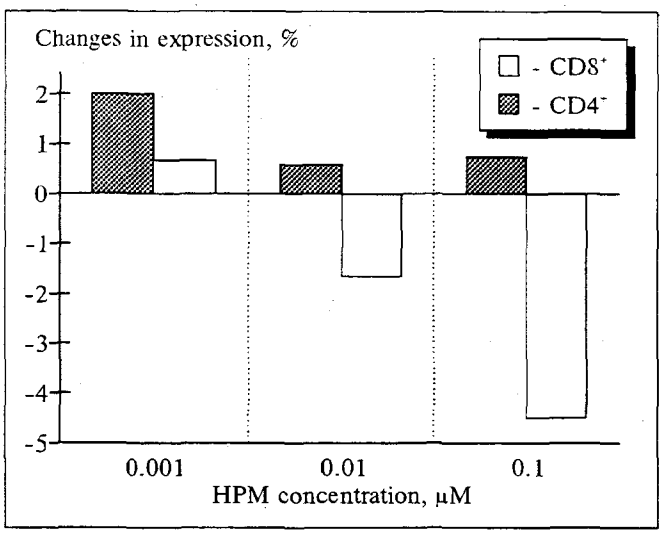

Fig. 1. Effect of HPM on the expression of surface antigens by normal human lymphocytes.

phogen (HPM, a peptide with the amino acid structure pGlu-Pro-Pro-Glu-Glu-Ser-Lys-Val-Ile-Leu-Phe) was selected as a mediator of long-range impairment; it is secreted by mammalian hypothalamic neurons and is capable of activating the processes of protein synthesis and regeneration under conditions of experimental trauma.

MATERIALS AND METHODS

Mononuclear cells were isolated from the peripheral venous blood of 18 healthy donors (7 men and 11 women) by centrifugation in a Ficoll-Verograffin one-step density gradient. Cells collected from the interphase ring were washed three times in Hanks solution without Ca^{2+} and Mg^{2+}, after which a cell suspension was prepared containing 2.5×10^6 cells per ml of medium 199. Then mononuclear cells were incubated in adenosine (0.01, 0.1, 1, and 10 μM) or adenosine diphosphate (ADP - 0.1, 1, and 10 μM) solutions for 30 min at 37°C. The cells were incubated with HPM in concentrations of 0.001, 0.01, and 0.1 μM for 1 h in a thermostat. After incubation the cells were washed twice in buffered normal saline at 4°C.

Cells expressing membrane antigens CD3, CD4, and CD8 were counted using indirect immunofluorescence with murine monoclonal IKO antibodies and Fab fragments of goat immunoglobulines to mouse antibodies labeled with fluorescein isothiocyanate. The microvariant of the test was employed [1]. The preparation was examined under a Lyumam-I3 fluorescence microscope in a water immersion system. The presence and specificity of fluorescence were assessed in at least 200 cells. Cells with monocyte morphology were disregarded. For every experiment cell viability control with trypan blue (at least 98%) and nonspecific binding of labeled serum (no more than 4%) were carried out.

Results were statistically processed using the nonparametric Wilcoxon-Mann-Whitney and parametric Student tests to assess the mean difference between samplings with paired variants.

RESULTS

The experiments demonstrated that adenosine in concentrations of 0.1 μM and higher had a manifest modulating effect on the expression of membrane CD4 and CD8 antigens. As is seen in Table 1, treatment of the cell suspension with adenosine solutions in concentrations of 0.1, 1, and 10 μM led to an appreciable (not dose-dependent) reduction of the number of cells carrying CD4 antigen. On the other hand, the number of $CD8^+$ cells increased by 6%, on average, after treatment with adenosine in the same doses. The said shifts in expression of differentiation antigens by lymphocytes led to a statistically reliable change of the immunoregulatory index (IRI-CD4/CD8 ratio), which is a highly sensitive indicator of the type of immunoregulatory activity of lymphocytes. IRI decreased from 1.78 in the control to 1.09 after lymphocyte treatment with adenosine in a concentration of 0.1 μM, this indicating an increase of the suppressor potential of the cells in question.

The absence of an effect of adenosine (Table 1) on the expression of CD3 antigen associated with T-cell antigen-specific receptor also confirms

TABLE 1. Effect of Adenosine on *in Vitro* Expression of Surface Antigens by Normal Human Lymphocytes, % ($M \pm m$)

Antigen	Control	Adenosine concentration, μM			
		0.01	0.1	1	10
$CD3^+$	69.71±1.15	71.02±2.06	67.43±1.60	68.66±1.48	68.33±1.59
$CD4^+$	44.24±0.73	43.05±1.63	34.91±1.49**	35.57±1.55**	35.41±1.85**
$CD8^+$	25.95±1.32	25.53±2.67	32.65±1.83*	33.53±2.23*	30.55±1.51*
IRI	1.78±0.10	1.79±0.22	1.09±0.08**	1.14±0.12**	1.20±0.10**

Note. Here and in Table 2: one asterisk denotes $p<0.05$, two asterisks $p<0.01$ *vs.* the control.

TABLE 2. Effect of ADP on *in Vitro* Expression of Surface Antigens by Normal Human Lymphocytes, % ($M \pm m$)

Antigen	Control	ADP concentration, μM		
		0.1	1	10
$CD3^+$	69.71±1.15	68.03±1.11	68.99±1.10	68.20±1.09
$CD4^+$	44.24±0.73	44.13±1.01	40.13±1.54*	36.93±1.38**
$CD8^+$	25.95±1.32	29.47±2.36	31.72±2.00*	30.79±1.65*
IRI	1.78±0.10	1.60±0.13	1.43±0.10*	1.37±0.12*

the idea that the studied immunoregulatory mechanism does not involve the capacity of a cell to receive an antigenic stimulus, but just determines the trend of the immune response to it.

It is noteworthy that the lowest of the studied adenosine concentrations (0.01 μM) had no effect on the surface phenotype of lymphocytes. This finding correlates with a previous report [9], according to which adenosine induced cAMP accumulation in lymphocytes only in concentrations surpassing 0.01 μM. On the other hand, in concentrations of 0.1 to 100 μM adenosine was shown to stimulate the Ra purine receptor presented on lymphocytes. This eventually leads to activation of adenylate cyclase [12]. Hence, comparison of these data points to a possible contribution of cAMP accumulation in the realization of the studied adenosine effect.

ADP enters the extracellular space from the same sources as adenosine. Moreover, it is a metabolic precursor of adenosine in the reaction catalyzed by lymphocytic 5'-nucleosidase [2,11]. With this in mind, we attempted to study the effects of different concentrations of ADP on the expression of differentiation antigens by lymphocytes. As is seen in Table 2, the modulating effect of ADP on the surface phenotype of peripheral blood lymphocytes is similar to that of adenosine, but the adenine nucleotide concentration needed to achieve this effect was one order of magnitude higher. For example, in a concentration of 0.1 μM ADP virtually did not influence the expression of CD4 molecules and demonstrated just a tendency to induce an increase of the number of $CD8^+$ cells. Higher ADP concentrations exerted an effect similar to that of adenosine. This finding, as well as the lack of published data on the presence of specific binding sites for ADP in T lymphocytes, suggest that the effect of ADP on the expression of CD4 and CD8 molecules is mediated by its preliminary dephosphorylation to adenosine.

A study of the effect of HPM on the counts of $CD4^+$ and $CD8^+$ cells revealed that in all the concentrations studied this compound induced changes of the helper-suppressor balance toward the predominance of cells with an immunoactivating function. Figure 1 demonstrates a clear-cut dose-dependent type of this effect (the mean values of changes in the number of cells expressing a particular antigen is presented).

Comparison of the results of studies of the effects of purine compounds and HPM permits us to hypothesize a bilevel mechanism of regulation of immunological functions under extreme conditions. On the one hand, this involves increased secretion of neuropeptide stimulating the immune system, besides exerting the usual anabolic effects, while on the other hand, a local increase of the adenosine concentration at the site of cell injury may serve as the mechanism preventing lymphocyte activation, this primarily referring to autoreactive clones which are capable of reacting to changed autoantigens or to autoantigens immunologically available under the influence of the injurious factor. Finally, the trend of the immune response may be determined in each individual case by the ratio of the central and local immunoregulatory mechanisms.

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