Is the Abnormal Reaction of Lymphocytes of Patients with Atopic Bronchial Asthma to Adenosine a Component of Pathogenesis or a Compensatory Reaction?

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After a 30-min incubation in the presence of adenosine, the suppressor activity of peripheral blood lymphocytes from healthy subjects decreases as well as the count of $CD4^+$ cells, while the count of $CD8^+$ cells increases. The suppressor activity of peripheral blood lymphocytes from patients with atopic bronchial asthma and the count of $CD8^+$ cells increase after a 16-h incubation with adenosine, while a 30-min incubation enhances the expression of CD4 antigen.

Key Words: *lymphocytes; adenosine; surface phenotype; immunoregulatory activity*

It has been generally accepted that immune disorders contribute to the pathogenesis of atopic bronchial asthma (ABA). However, specific mechanisms underlying the development of ABA remain obscure. Presumably, impaired metabolism of biologically active compounds is one of these mechanisms. Adenosine (AD) and its derivatives: AD monophosphate, AD diphosphate, and AD triphosphate are of special interest.

This interest is motivated by the fact that the erythrocyte ATPase activity and concentrations of AD, AD mono- and diphosphates in erythrocytes [4,7] and blood plasma [11] as well as renal excretion of the adenine acid metabolites: hypoxanthine, xanthine, and uric acid increase in obstructive bronchopulmonary diseases [14]. Similar changes were revealed in experimentally sensitized animals and in patients with atopic dermatitis, systemic connective tissue diseases, rheumatism, etc. [3]. It can be suggested that intensification of the catabolism of macroergic purines in ABA is not caused by respiratory insufficiency, but reflects some processes common for these conditions. On the other hand, the ratio between the types of purine receptors on lymphocytes from patients with ABA is changed [2].

Taken together, these findings indicate that impaired purinergic regulation may play an important role in the pathogenesis of ABA. It is not clear whether these changes are compensatory or contribute to the development of this disease. Further investigations are required to clarify this issue. In the present study we examined the effects of AD on the expression of CD3, CD4, and CD8 antigens and on the immunoregulatory activity of peripheral blood lymphocytes from healthy subjects and ABA patients.

MATERIALS AND METHODS

Peripheral blood lymphocytes (PBL) from 40 donors and 42 patients with ABA were used in the study. The cells were isolated by centrifugation on a Ficoll-Verografin gradient as described elsewhere [10]. The effect of AD on CD3, CD4, and CD8 antigens was assessed after incubation of PBL in RPMI-1640

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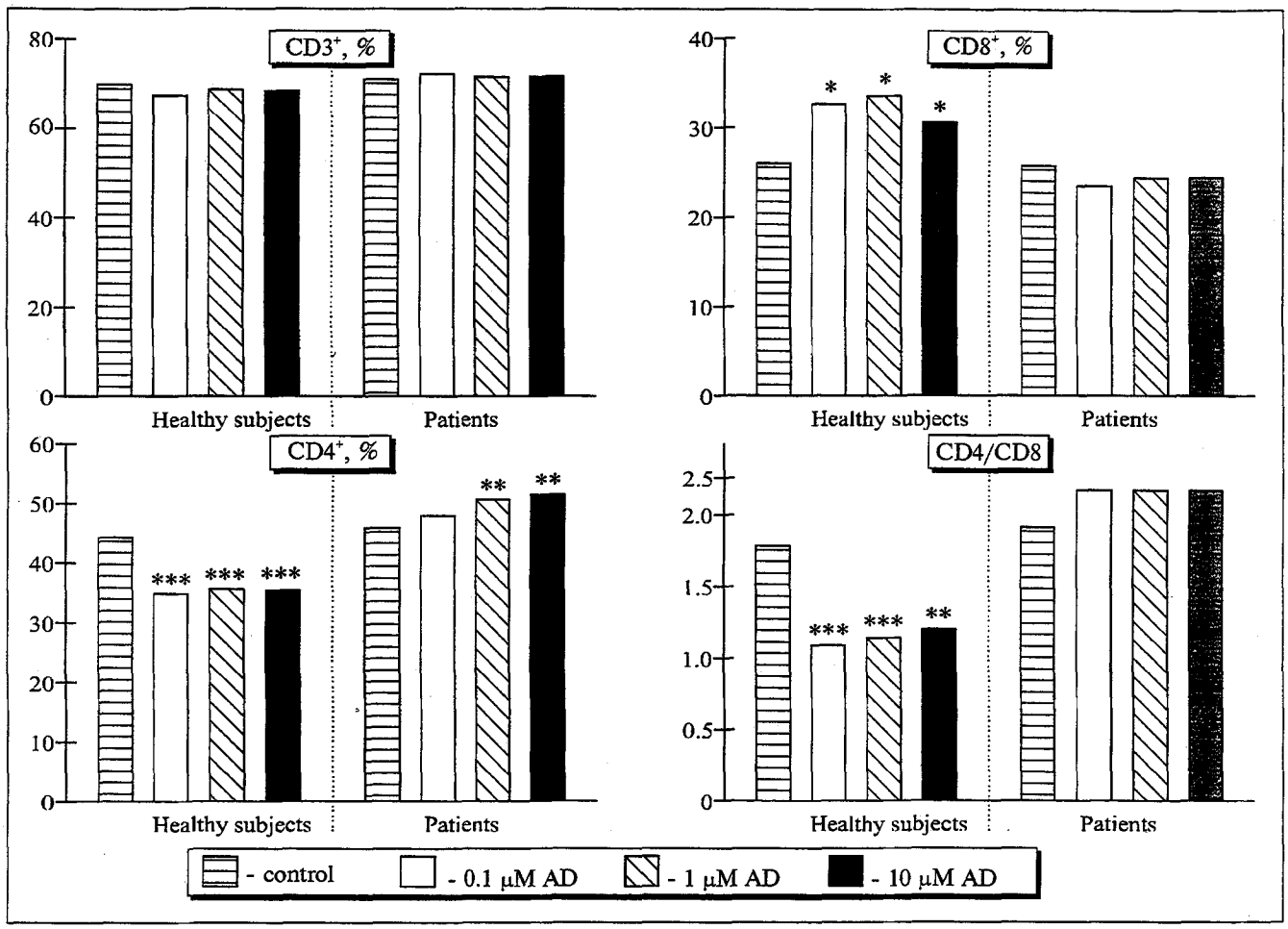

Fig. 1. Effect of a 30-min incubation with adenosine (AD) on the expression of surface antigens by lymphocytes of healthy subjects and patients with atopic bronchial asthma. Here and in Figs. 2 and 3: *$p<0.05$, **$p<0.01$, and ***$p<0.001$ *vs.* the control.

with AD for 30 min or 16 h at 37°C. Cells expressing these antigens were counted under a LYuMAM-I3 fluorescent microscope using an aqueous immersion system. A microvariant of the indirect immunofluorescence method with ICO murine monoclonal antibodies was employed [1]. At least 200 cells were analyzed for the presence and specificity of fluorescence. Monocytes were not included in the analysis. In each experiment, cell viability was >98% (Trypan Blue exclusion test) and nonspecific binding of labeled serum control was <4%.

In order to study immunoregulatory activity, peripheral blood mononuclear cells were incubated for 48 h at 37°C with or without 1 μM AD, then with mitomycin C (40 μg/ml) for 40 min at 37°C, after which a test culture of freshly isolated lymphocytes from healthy donors was added (1:1). The test cultures served as the control. Proliferation was as-

TABLE 1. Effect of a 16-Hour Incubation with Adenosine (AD, 1 μM) on the Expression of Surface Antigens by Lymphocytes of Patients with Atopic Bronchial Asthma (ABA) and Donors

Experimental conditions		CD3+	CD4+	CD8+
Healthy subjects	Control, 0 h	65.24±4.65	45.09±3.69	25.19±3.28
	Control, 16 h	66.14±4.01	47.06±3.76	26.42±2.71
	AD, 16 h	64.94±4.48	44.44±2.62	24.81±2.12
Patients with ABA	Control, 0 h	69.07±1.43	45.36±2.43	26.30±2.38
	Control, 16 h	70.17±0.67	46.54±1.69	26.01±1.76
	AD, 16 h	69.97±0.43	45.95±1.96	31.57±1.77*

Note. *$p<0.05$.

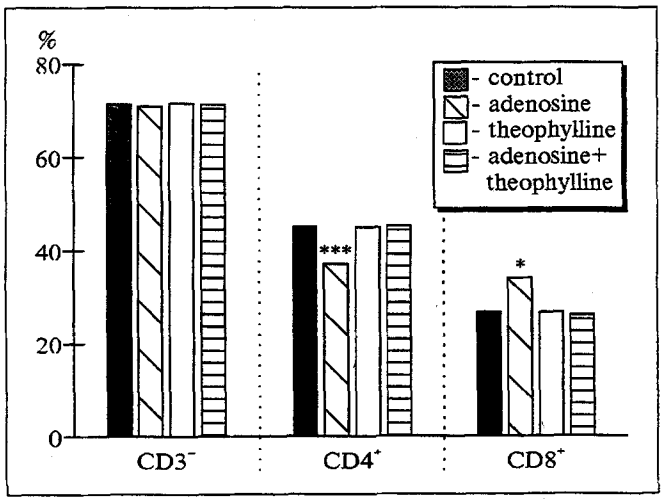

Fig. 2. Theophylline abolishes the effect of 1 μM adenosine on the expression of surface antigens by donors' peripheral blood lymphocytes.

sessed by ^{3}H-thymidine incorporation after incubation of lymphocytes for 72 h at 37°C in the presence of 2 μg/ml phytohemagglutinin. The immunoregulation index (IRI) was calculated from the following formula: $(C\text{-}E)/C$, where K and E are cpm in control and experimental samples, respectively. If the proliferation rate in experimental cultures was lower than that in control cultures, IRI was positive and was referred to as the index of suppression; otherwise IRI was negative and was referred to as the index of activation. The data were analyzed using Wilcoxon—Mann—Whitney's nonparametric test.

RESULTS

A 30-min incubation with 0.1 μM AD and higher modified the expression of CD4 and CD8 antigens on PBL from healthy subjects. After incubation of PBL suspension with 0.1, 1, or 10 μM AD, the number of CD4$^+$ decreased by 9% in a dose-independent manner (Fig. 1). Under the same experimental conditions the number of CD8$^+$ increased by 6%. By contrast, after incubation of PBL from ABA patients with 1 μM AD, the number of CD4$^+$ cells increased to 50.6% *vs.* 45.8% in the control, while the number of CD8$^+$ cells remained virtually unchanged. Adenosine had no effect on the expression of CD3 antigen on PBL from normal subjects and ABA patients. Since the responses of lymphocytes to 0.1 and 10 μM AD were similar, in subsequent experiments AD was applied in a concentration of 1 μM.

We hypothesized that the above-mentioned effects of AD are associated with stimulation of purine receptors on PBL. In order to check this hypothesis P_1 receptors were blocked by a 5-min preincubation of PBL with 16 μg/ml theophylline. This prevented the AD-induced changes in the expression of surface antigens by PBL from healthy donors (Fig. 2).

We then examined the effect of a 16-h incubation with AD on the expression of surface antigens by PBL from healthy subjects and ABA patients (Table 1). Prolonged incubation of PBL from healthy subjects and patients with ABA did not affect the expression of CD3, CD4, and CD8 antigens. Incubation with AD had no appreciable effect on the expression of these antigens by PBL of healthy subjects. However, AD significantly increased the share of CD8$^+$ cells (from 26 to 31%, $p<0.05$) in cultures of PBL from ABA patients. This was paralleled by a decrease (from 1.89 to 1.65) in the CD4/CD8 ratio reflecting the pattern of the immunoregulatory activity of lymphocytes.

Thus, our findings demonstrate that short- and long-term incubation of PBL with AD induces opposite changes in the expression of surface antigens. The functional significance of this phenomenon is unclear. On the one hand, variations of the CD4$^+$/CD8$^+$ ratio reflects functional activity of the regulatory lymphocyte population [5]. On the other hand, activation of these cells with lectins [12], phorbol 12-myristate 13-acetate [8], or antibodies to various membrane proteins [13] leads to a sharp decrease in the expression of CD3, CD4, and, consequently, to a decrease in the proportion of the corresponding lymphocyte subset. Finally, the presence of CD4 or CD8 antigen determines only the process of immunological recognition restricted by the histocompatibility antigens. The type of immunoregulatory activity may disagree with the generally recognized relationship between lymphocyte function and surface antigen expression [9].

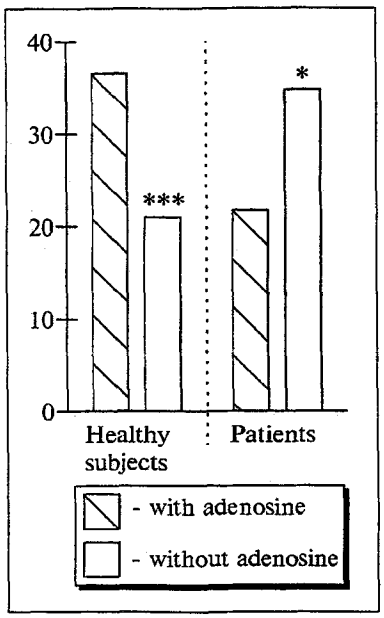

Fig. 3. Effect of 1 μM adenosine on the immunoregulatory activity of peripheral blood mononuclear cells of healthy subjects and patients with atopic bronchial asthma.

In an attempt to solve this problem, we examined the effect of AD on the immunoregulatory activity of lymphocytes. Lymphocytes of healthy subjects and ABA patients reduced the incorporation of ^{3}H-thymidine by test cells by 36.5 and 21.7%, respectively (Fig. 3). The addition of AD lowered the immunosuppressive activity of PBL from healthy subjects by 27.8% of the baseline activity ($p<0.001$). By contrast, AD increased the suppressor activity of PBL from ABA patients by 16.9% ($p<0.05$).

Our results suggest that a rapid decrease in the count of $CD4^+$ cell caused by incubation of healthy donors' PBL in the presence of adenosine reflects the early activation events in the immunocompetent cells. An increase in the count of $CD8^+$ cells after prolonged incubation of PBL from ABA patients with AD indicates the real increase in the suppressor potential of these cells. Presumably, these responses and the higher rate of purine catabolism are the mechanisms operating in atopic and autoimmune diseases to compensate for suppressor insufficiency [6]. On the other hand, an increase in the suppressor activity of AD-treated PBL from ABA patients may be a component of the antisuppressive activity of these cells toward the antigen-specific T suppressor cells.

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