Hydrolase and Oxidoreductase Activities in Peripheral Blood Lymphocytes in Combined Exposure to Biological Allergens and Sulfur Dioxide

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Differences in the metabolic status of peripheral blood lymphocytes were observed after exposure of intact guinea pigs and animals sensitized with biological allergens to sulfur dioxide. When sensitization was complicated by chemical exposure, enzyme activities in lymphocytes depended on the type of allergen and degree of hypersensitivity.

Key Words: *lymphocytes; metabolism; cytochemistry; sensitization; sulfur dioxide*

Evaluation of the metabolic status of peripheral blood lymphocytes in functional disorders of homeostasis (primarily at the level of the immune system) is an interesting problem. Enzymatic processes in immunocompetent cells are differentiated, depending on the type of exposure, pathological and reactive states [1]. Metabolic parameters of lymphocytes are informative for disease diagnosis, prognosis, and evaluation of the treatment efficiency [4,9].

Changes in hydrolase and oxidoreductase activities in the peripheral blood lymphocytes under the effect of isolated exposure to biological allergens are now well studied. However, under real conditions exposure of this kind can be complicated by chemical factors, which can lead to progress of delayed-type and immediate hypersensitivity reactions even in cases, when the level of chemical exposure is in fact negligible [7,10,14,15]. Combined effects of biological allergens and low-dose chemicals on the metabolic status of peripheral blood lymphocytes were virtually never studied experimentally.

We evaluated changes in enzyme activities in immunocompetent cells of animals with different intensity of hypersensitivity reactions. Enzyme activities were measured before allergic tests; this gave information on the possibility of using intracellular metabolic parameters for prediction of the course of allergic process.

MATERIALS AND METHODS

Experiments were carried out on 80 guinea pigs (250-350 g) from Vektor Firm Breeding Center (Koltsovo, Novosibirsk region). The animals were divided into 8 groups, 10 per group. Group 1 comprised controls. Group 2 animals were exposed to sulfur dioxide (SO_2, a very prevalent atmospheric pollutant). Guinea pigs of other experimental groups were sensitized by single injection of 500 μg protein-containing powder (PCP) mixed with incomplete Freund's adjuvant under the hind paw aponeurosis. PCP was a ready biotechnological product produced by yeast-like fungi. Animals of groups 3-5 were injected with hydrolyzed protein (HP) obtained by microbiological synthesis using opportunistic *p. Trichosporon* fungi, guinea pigs of groups 6-8 received fodder protein (FP) synthesized by *p. Candida* yeast-like fungi. Animals of groups 3 and 6 were exposed to PCP alone (HP or FP, respectively), animals of groups 4, 5, 7, and 8 received combined exposure to biological and chemical factors.

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Inhalations of SO_2 (2-4 mg/m^3) were carried out for 10 days (4 h per day). In groups receiving combined exposure to factors of different nature SO_2 inhalations started on the day of PCP injection (groups 4 and 7) or 14 days after it (groups 5 and 8).

The metabolic status of lymphocytes in experimental animals was evaluated after inhalations of SO_2 were over (groups 2, 5, and 8) or 14 days after injection of PCP (groups 3, 4, 6, and 7). Activity of nonspecific esterase in peripheral blood smears was evaluated by the cytochemical method after Loeffler [5], acid phosphatase after Burston [13], SDH and lactate dehydrogenase (LDH) by Stuart and Simpson methods modified by Hayhoe and Quaglino [12]. The intensity of nonspecific esterase reaction was expressed in percent of positively reacting cells, SDH, LDH, and acid phosphatase activities were expressed in arbitrary units of the cytochemical index [5].

Immediate and delayed-type hypersensitivity reactions were evaluated in animals directly after blood collection. The level of skin sensitizing antibodies detected by passive skin anaphylaxis test, intensity of positive intracutaneous allergic tests, intensity of antigen-specific rosette formation, level of leukocyte migration inhibition with specific antigen [6] were evaluated. The results of allergic tests were expressed in cm by the size of stained skin area or edema and erythema (allergic skin tests) in percent of rosette-forming lymphocytes and in percent of positive reactions.

The significance of results was evaluated using Wilcoxon—Mann—Whitney nonparametric test.

RESULTS

Significant changes in enzyme activities in peripheral blood lymphocytes in comparison with the control were observed in animals of all experimental groups (Table 1). The metabolic status of immunocompetent cells differed in experimental groups. The complex of changes in enzyme activities detected after isolated exposure to PCP and SO_2 was not observed after combined exposure to these factors.

Single sensitization with PCP (groups 3 and 6) led to similar metabolic shifts irrespective of the type of allergen (decrease of dehydrogenase levels and activation of acid phosphatase). However, a relationship between the lymphocyte metabolic status and the type of allergen was observed in animals, in whom sensitization was combined with SO_2 inhalations. SO_2 modified hydrolase activities in lymphocytes of animals sensitized by HP (Table 1). In guinea pigs receiving HP+SO_2 (groups 4 and 5) the number of nonspecific esterase-positive cells decreased and differed significantly from the control and from that in animals receiving HP alone. On the other hand, decreased intensity of nonspecific esterase reaction was not paralleled by activation of acid phosphatase, observed in all other experimental groups.

In contrast to hydrolytic enzymes, changes in dehydrogenase activities resulting from combined exposure to biological and chemical factors depended not only on biological characteristics of the allergen (HP or FP), but also on the time of SO_2 inhalations (simultaneously with PCP injection or 14 days after contact with the allergen). Stimulation of SDH caused by SO_2 in intact animals was not observed after SO_2 treatment of sensitized animals.

The intensity of specific hypersensitivity reactions to PCP varied in guinea pigs exposed to a combination of biological and chemical factors (Table 2). Immediate and delayed-type hypersensitivity reaction was most pronounced in groups 5 and 8 (SO_2 exposure 14 days after injection of the sensitizing factor, PCP).

TABLE 1. Changes in Enzyme Activities of Guinea Pig Peripheral Blood Lymphocytes after Isolated and Combined Exposure to PCP (HP and FP) and SO_2 ($M\pm m$)

Group	SDH, arb. units	LDH, arb. units	AP, arb. units	NE, %
1 (control)	0.60±0.05	0.75±0.05	0.56±0.04	10.2±0.9
2 (SO_2)	0.89±0.06*	0.55±0.05*	0.76±0.03*	12.2±2.5
3 (HP)	0.25±0.06*	0.20±0.03*	0.84±0.09*	11.4±1.8
4 (SO_2 from the day of HP injection)	0.18±0.04*	0.19±0.03*	0.58±0.04	4.1±0.4*
5 (SO_2 starting 14 days after HP injection)	0.30±0.03*	0.70±0.05	0.65±0.06	4.3±0.8*
6 (FP)	0.17±0.07*	0.38±0.05*	1.13±0.08*	13.2±1.4
7 (SO_2 from the day of FP injection)	0.65±0.03	0.82±0.03	1.05±0.06*	8.0±1.5
8 (SO_2 starting 14 days after FP injection)	0.34±0.03*	0.96±0.05*	0.90±0.05*	10.3±1.7

Note. *$p<0.01$ compared to the control.

TABLE 2. Effect of SO_2 on Allergic Test Values in Guinea Pigs Sensitized with PCP (HP and FP; *M*±*m*)

Exposure	Skin allergen tests, cm	Skin staining, cm	Antigen specific rosette formation test, %	Leukocyte migration inhibition test, %
Isolated exposure to HP (group 3)	0.66±0.04	0.15±0.01	16.0±2.4	62.5
Start of exposure to SO_2				
on the day of HP injection (group 4)	0.80±0.09	0.14±0.01	13.5±1.9	75
14 days after HP injection (group 5)	1.24±0.08**	0.26±0.05*	22.5±1.4*	100
Isolated injection with FP (group 6)	0.38±0.04	0.28±0.02	20.3±1.8	75
Start of exposure to SO_2				
on the day of FP injection (group 7)	0.64±0.13	0.30±0.03	23.7±2.2	70
14 days after FP injection (group 8)	0.97±0.15^{++}	0.41±0.06^{+}	26.6±1.4^{++}	100

Note. *p<0.05, **p<0.01 compared to group 3; ^{+}p<0.05, ^{++}p<0.01 compared to group 6.

Comparison of the results of allergic tests (Table 2) with the results of cytochemical studies of lymphocytes (Table 1) revealed a relationship between the type of changes in dehydrogenase (but not hydrolase) activities and the level of allergic restructuring of the body. When SO_2 exposure of sensitized guinea pigs was not paralleled by stimulation of hypersensitivity to PCP, activities of SDH and LDH were either reduced (group 4) or did not differ from the control (group 7). The effect of SO_2 leading to an increase in the incidence and intensity of allergic reactions was associated with opposite reactions of SDH and LDH, depending on the type of allergen. Opposite reactions were most pronounced after SO_2 exposure of group 8 guinea pigs, when the decrease in SDH activity was paralleled by elevation of LDH activity compared to the control level. Lymphocyte LDH activity in group 5 guinea pigs virtually did not differ from the control and was elevated only in comparison with groups 3 and 4 (p<0.01).

Changes in enzyme activities detected in lymphocytes of guinea pigs after isolated treatment with PCP (groups 3 and 6) in fact reflected a final phase of response to the antigen. On the other hand, metabolic features of these cells after combined exposure to factors of different nature can be associated with the development of allergic process. Decreased SDH activity paralleled by stimulation of LDH and acid phosphatase were regarded as a sign of the development of allergic restructuring of the organism [9]. On the other hand, increased level of acid phosphatase in allergic dermatoses of chemical origin is not obligatory, and activity of this enzyme in peripheral blood lymphocytes of guinea pigs can decrease [11]. It is assumed that low level of cytochemical reactions to acid phosphatase and nonspecific esterase is characteristic of less differentiated lymphocytes [2,3]. Moreover, opposite reactions of dehydrogenase activities observed in our experiments with increase of hypersensitivity to PCP can be due to the presence of immature lymphoid cells. A trend to an increase in the number of less differentiated lymphocytes and their precursors in the peripheral blood during the development of allergic process is clearly seen [1]. Based on the present findings, we distinguish different types of this cell population reaction (differing by specific enzyme status of lymphocytes), with consideration for the independent changes in hydrolases and dehydrogenase activities. Increased level of immature lymphocytes in the peripheral blood is suggested to be regarded as a sign indicating functional deficiency of normal immune response in allergic disorders [1]. However, it can be hypothesized that these changes in cell population structure, presumably differing by the type of metabolic manifestations, are functionally heterogeneous. Decreased intensity of reaction to acid phosphatase and esterase in peripheral blood lymphocytes is observed in vaccination; these shifts do not seem to be due to the development of immunopathological conditions [3]. When the shift towards immature lymphoid cells is paralleled by increased LDH activity, it can be paralleled by increased values of leukocyte migration inhibition test, indicating functional suppression of normal immune response, primarily at the level of its T-cell component [8].

Hence, changes in hydrolase and oxidoreductase activities in peripheral blood lymphocytes of guinea pigs with sensitization aggravated by SO_2 exposure depend on the type of allergen. The type of dehydrogenase response, in contrast to changes in hydrolytic enzyme activities, exhibits a certain

relationship with the level of hypersensitivity development, indicating liability to the progress of the allergic process. This indicates that intracellular metabolic parameters can be used in further studies aimed at the development of additional tests for evaluating the severity of allergic diseases in subjects exposed to combinations of biological and chemical factors.

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