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SPONTANEOUS AND MITOGEN-INDUCED PROLIFERATIVE ACTIVITY OF MONONUCLEAR CELLS IN PATIENTS WITH POLLINOSIS

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The increased number of B lymphocytes [6] and changes in representation of membrane immunoglobulins in B cells depending on the stage of an allergic disease [17] suggest changes in functional activity of individual B-cell subpopulations. If it is recalled also that an increased number of "null" cells [2] is found in atopic patients and, according to some workers [15], the "null" cells include precursors of mature B lymphocytes, the need to study proliferative activity of B cells in atopic patients will be evident.

In the activation described below induction of the proliferative response of B cells by lipopolysaccharide (LPS) and the proliferative activity of T lymphocytes during stimulation by phytohemagglutinin (PHA) in patients with pollinosis, with hypersensitivity to timothy-grass pollen, were studied.

EXPERIMENTAL METHOD

Peripheral blood mononuclear cells (MNC) from 17 patients with pollinosis aged from 16 to 40 years, were used. The diagnosis of pollinosis was based on the allergologic history and the results of skin tests and inhalation provocation tests with the possible allergen. The duration of the disease did not exceed 5-7 years. Patients with pollinosis were studied while free from seasonal exacerbations and accompanying diseases, and who had not previously been subjected to specific hyposensitization or pharmacotherapy, were chosen. Patients with a moderately severe and severe degree of clinical manifestations of pollinosis with positive skin tests to timothy grass pollen allergen were investigated. The control consisted of 13 healthy blood donors. In a separate series of investigations the paired control method was used. For this purpose clinically healthy persons were selected individually to correspond to sex, age, and race to a patient chosen for investigation, and with subsequent parallel conduct of experiments.

Lymphocytes (MNC) were isolated from heparinized blood by centrifugation on a one-step Ficoll–Verografin gradient with density of 1.080 g/cm^3 at 400g for 40 min, followed by washing with medium 199 at 200g three times, for 5 min each time. MNC were resuspended in medium 199 containing 10% embryonic calf serum, HEPES (5 mM), glutamine (20 mM), and monomycin (100 U/ml), and cultured in flat-bottomed microplates at the rate of 3.6×10^5 cells per well in volume of 200 µl of medium for 7 days at 37°C at an atmosphere of 5% CO_2.

LPS from *E. coli* serotype 026:B6 (Sigma, USA) in final concentrations of 2, 20, and 100 µg/ml, was used as B-cell activator. The available information on the use of LPS as B-cell mitogen for human peripheral blood lymphocytes is highly contradictory. In particular, during culture for 3 days with LPS the proliferative response of MNC was negligible [3] or absent

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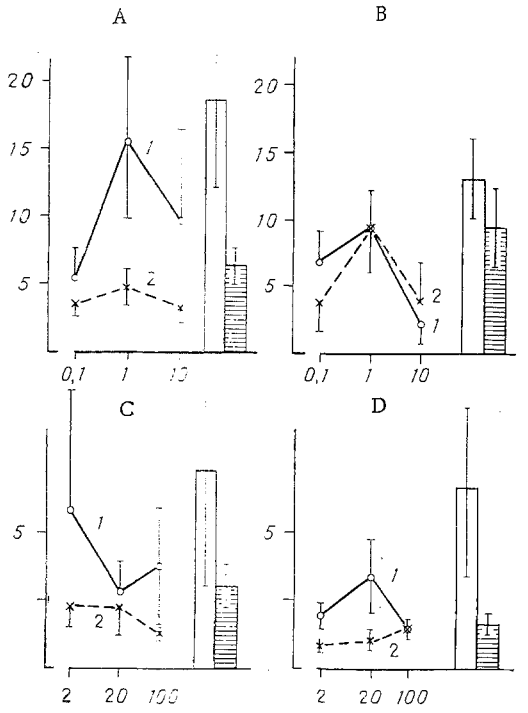

Fig. 1. Mitogen-induced proliferative response of MNC of patients with pollinosis. Abscissa: A, B) doses of PHA (in μg/ml), C, D) doses of LPS (in μg/ml); ordinate, SI of proliferative response of MNC. A) On stimulation by PHA, B) on stimulation by PHA, using paired control method, C) on stimulation by LPS, D) on stimulation by LPS using paired control method. 1) Healthy subjects, 2) patients with pollinosis. Unshaded columns — data of maximal responses for SI of healthy subjects, shaded columns — the same for patients with pollinosis. Results presented in the form of M ± m.

[13], whereas on culture for 7 days the LPS-induced proliferative response of MNC was manifested more clearly [11, 12]. According to data in the literature [9], manifestation of a response to LPS requires preliminary culture of the lymphocytes for 20 h without mitogen, which, according to the authors cited, enables short-living suppressor cells to be removed. All that can be said is that, by culturing MNC with LPS for 7 days we were able to obtain a proliferative response of human peripheral blood B cells, in agreement with results published elsewhere [5, 11, 12]. To induce a proliferative response of T lymphocytes in MNC culture, PHA P (Difco, USA) was used in final concentrations of 0.1 and 10 μg/ml. [^{3}H]Thymidine in a dose of 1 μCi and in a volume of 20 μl per well was added 18 h before the end of the culture time. After incubation the cells were transferred to glass wool filters by means of an automatic CM Harvester. Incorporated radioactivity was counted in a Mark III liquid scintillation β-counter (Tracor Northern). The results were assessed as the stimulation index (SI), the ratio of the stimulated to the unstimulated response, and also the maximal value of SI. To confirm the diagnosis and for comparison with the level of B-cell proliferation, the content of allergen-specific antibodies (IgE) and the total IgE level were determined in seven patients with pollinosis by the use of standard Phadezym RAST and Phadezym IgE PRIST kits (from Pharmacia Diagnostica AB, Sweden). The keeping time of the blood serum at −20°C did not exceed 4 months by the time of investigation. The results of this part of the work were obtained and generously presented by V. G. Chitaeva. Statistical analysis of the results was carried out by Student's t test. To discover correlation between the level of MNC proliferation and total IgE level, correlation analysis was used.

EXPERIMENTAL RESULTS

On determination of increased sensitivity to timothy green allergen in the patients with pollinosis the level of the skin tests with the allergen varied from 3+ to 4+, the content of allergen-specific IgE-antibodies corresponded to a reaction of the 2nd–4th degree, and the serum IgE level varied from 40 to 2925 kU/liter. In the control investigation, skin tests with allergen on clincially healthy subjects were negative. Hypersensitivity to timothy grass pollen in the patients was thus confirmed both by positive skin tests to the allergen and by the presence of allergen-specific IgE antibodies.

The results of assessment of the proliferative response of MNC showed, first, a higher level of spontaneous proliferation in the patients than in normal subjects of the control group ($n = 12$, 3059 ± 529 cpm, and $n = 8$, 1716 ± 258 cpm; $P < 0.05$), thus confirming previous findings [4].

No significant differences could be found between dose–response curves or values of maximal responses to PHA reflected in SI, during investigation of lymphocytes from patients and normal subjects. The study of the effect of LPS on lymphocytes from patients with pollinosis and healthy subjects likewise revealed no significant differences between the corresponding parameters (Fig. 1A and C).

In the next series of experiments the paired control method was used to exclude random differences in the conditions of the determinations and to reduce to a minimum any inappropriateness of choice of the control group of healthy subjects, so that ultimately it would be possible to assess more strictly differences between activity of lymphocytes from patients and normal subjects. A higher level of spontaneous proliferation of MNC from patients with pollinosis than in the control also was found in this series ($n = 5$, 3181 ± 479 cpm and $n = 5$, 1677 ± 401 cpm, respectively; $P < 0.05$). As Fig. 1 shows, no significant differences could be found between the proliferative response of lymphocytes from patients and healthy people to PHA. Just as in tests of concanavalin A [4], we thus showed that the PHA-induced proliferative response of lymphocytes from patients with pollinosis did not differ significantly from that in normal subjects. The absence of differences was confirmed by results obtained by the paired control method. On the basis of these results and of data in the literature [1, 4, 14] it can be postulated that the proliferative response of lymphocytes from patients with pollinosis and from normal healthy subjects to concanavalin A and to PHA does not differ essentially.

Comparison of the proliferative response of B cells to LPS in an MNC culture from patients with pollinosis and normal subjects by the paired control method likewise revealed no significant differences with respect to the parameters tested (Fig. 1D).

As the most constant distinguishing feature in the two series of investigations increased spontaneous proliferation of peripheral blood MNC in patients with pollinosis was thus observed. The absence of differences in the level of proliferative response of MNC to LPS in pollinosis patients and normal subjects can probably be explained on the grounds that immature forms of A cells were involved in the process of enhanced spontaneous proliferation of MNC [15, 16]. Under such conditions the proliferative response of MNC from patients with pollinosis to LPS did not reflect the true proliferative activity of immature forms of B lymphocytes as target cells for lipopolysaccharide [7, 8].

Comparison of the indices of proliferative activity of MNC (the level of spontaneous proliferation, the LPS-induced response) with the total IgE level revealed no statistically significant correlation between these data, possibly due to the independence of the processes concerned. Whereas the proliferative response of lymphocytes to LPS characterizes comparatively immature subpupulations of B cells [7, 8], the hyperproduction of IgE observed in atopic patients is more likely to be connected with changes in the later stages of differentiation of IgE-synthesizing B lymphocytes (at the memory cell level) into IgE-producing plasma cells [10].

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