PROLIFERATION OF SPLEEN CELLS IN RESPONSE TO *Bordetella pertussis*

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The adjuvant properties of *Bordetella pertussis* (BP) are widely used for experimental, clinical, and prophylactic purposes. However, the mechanism of its adjuvant action on immunocompetent cells has not been adequately studied. Facts have been obtained to show that whole BP cells can influence proliferative activity of lymphocytes [4, 5]. To study this problem is important in connection with ways of regulating anti-infectious and antitumor immunity [7, 8].

The investigation described below accordingly was undertaken to study the action of BP on proliferative activity of mouse spleen lymphocytes and to compare it with the corresponding effect of nonspecific B and C mitogens.

EXPERIMENTAL METHOD

Standard formalinized vaccine of BP strain 305, obtained from the Laboratory of Physiology and Biotechnology of Microorganisms, I. I. Mechnikov Research Institute of Vaccines and Sera, was used. BP was freed from formalin by long-term dialysis against medium 199 and washed 3 times in culture medium prepared for the lymphocyte blast transformation test (LBTT) [2].

Experiments were carried out on BALB/c mice (from the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR) and nude (athymic) mice of the same line (All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR). Spleen cells were obtained and cultured in the LBTT by the method described previously [3]. Proliferative activity of the spleen cells was estimated by measuring incorporation of [^{3}H]thymidine ([^{3}H]T) into DNA. The duration of culture varied from 24 to 96 h. [^{3}H]-T was added in a dose of 0.5 μCi per microplate well 16 h before the end of culture of the cell suspensions [2]. The suspension of spleen cells was incubated in the presence of various concentrations of BP from 10^6 to 10^{11} microbial cells (MC) to 1 ml of either B or T mitogens. Phytohemagglutinin P (PHA, from Difco, USA) and concanavalin A (con A, from Sigma, USA) were used as T mitogens, and tuberculin (PPD, USSR origin), lipopolysaccharides (LPS) from *Salmonella typhimurium* (from Sigma, USA), and dextran sulfate (SD) (from Serva, West Germany) as B mitogens.

EXPERIMENTAL RESULTS

The vaccine strain of BP had a mitogenic action on spleen cells in doses of 10^8-10^{11} MC/ml (Fig. 1a). The minimal stimulating effect was observed in a dose of 10^8 MC/ml, maximal in a dose of 10^{10} MC/ml. A dose of 10^{11} MC/ml caused nonspecific inhibition of DNA synthesis in the spleen cells on account of their death, by contrast with a dose of 10^{10} MC/ml. Maximal synthesis of DNA in spleen cells in response to BP was observed with a dose of 10^8-10^{10} MC/ml after culture for 48 and 72 h (Fig. 1b). When spleen cells were tested for 96 h, the mitogenic effect of BP was reduced.

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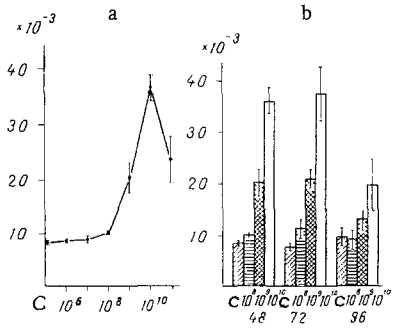

Fig. 1

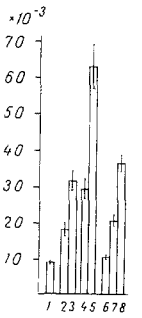

Fig. 2

Fig. 1. Stimulating effect of BP on DNA synthesis in spleen cells depending on dose and duration of culture. a) Dependence on dose. Abscissa, BP concentration, in MC/ml culture medium (C); ordinate (here and in Figs. 2 and 3) time, in min ($\times 10^3$). b) Dependence on duration of culture. Abscissa, duration of culture of cells (in h). 10^8, 10^9, 10^{10}) Number of MC of BP in 1 ml culture medium (C).

Fig. 2. Mitogenic effect of BP compared with nonspecific T mitogens on spleen cells. Abscissa, ingredients: 1) Culture medium (C); 2) PHA 0.2 μl/ml; 3) PHA 0.6 μl/ml; 4) con A 5 μg/ml; 5) con A 20 μg/ml; 6) BP in dose of 10^8 MC/ml; 7) BP in dose of 10^9 MC/ml; 8) BP in dose of 10^{10} MC/ml.

The results of these experiments show that whole killed BP cells have a mitogenic action on mouse spleen cells. In view of these results, in the next experiments BP was used in a concentration of 10^8-10^{10} MC/ml with an incubation time of 48 h: after incubation for this time, besides a high mitogenic action of BP, a lower cell mortality was observed than after 72 and 96 h; culture for 48 h also enabled the action of BP on short-living spleen cells to be assessed.

In a dose of 10^{10} MC/ml BP had a stronger mitogenic action than PHA in concentrations of 0.2 and 0.6 μl/ml (Fig. 2). The mitogenic effect of BP on spleen cells was lower than that of con A in concentrations of 5 and 20 μg/ml (Fig. 3). These results suggested that BP acts on a population of B lymphocytes or T_1 lymphocytes on which PHA does not act, since con A acts on both T_1 and T_2 lymphocytes [1]. BP can evidently stimulate a subpopulation of T_1 lymphocytes which is insensitive to PHA, but which reacts to con A.

To study the possible action of BP on B lymphocytes, nude mice, which have no T lymphocytes, were used. Spleen cells of nude mice after culture for 48 and 72 h did not in fact respond to PHA or con A. The mitogenic action of BP on spleen cells of nude mice was very strong after culture for 48 h (Fig. 3a). In a dose of 10^{10} MC/ml BP had a stronger mitogenic action than LPS (20 and 100 μg/ml) and SD (50 and 250 μg/ml. This confirms that BP has a powerful stimulant action on subpopulations of B lymphocytes and suggests that BP probably acts on less mature subpopulations of B lymphocytes than the B mitogens mentioned above.

To identify the subpopulation of B lymphocytes on which BP acts, mitogens whose action is linked with the degree of differentiation of B cells were chosen. PPD (5 μg/ml), which acts on mature B lymphocytes caused a statistically significant increase in DNA synthesis in the spleen cells, but it was considerably less than that caused by the action of BP in doses of 10^8-10^{10} MC/ml (Fig. 3b). This indicates that BP is able to act on a less mature subpopulation of B lymphocytes, which includes the Ig^+ and Fc^+ lymphocytes [6] that are sensitive to LPS. LPS, in doses of 4 and 20 μg/ml, caused a much smaller increase in DNA synthesis in the spleen cells than BP in doses of 10^9-10^{10} MC/ml (Fig. 3). This supports the view that BP acts on even less mature lymphocytes than LPS. To test this hypothesis SD (50 and 250 μg/ml), which influences immature B lymphocytes, was used. SD was found to give a weaker stimulating effect on DNA synthesis than BP in a dose of 10^{10} MC/ml (Fig. 3). This probably indicates that BP can induce proliferation of precursors of B lymphocytes.

It can be concluded from these results that BP is a B mitogen. Its mitogenic action is probably linked with immature B cells and (or) their precursors. The possibility cannot be ruled out that pertussis vaccine has

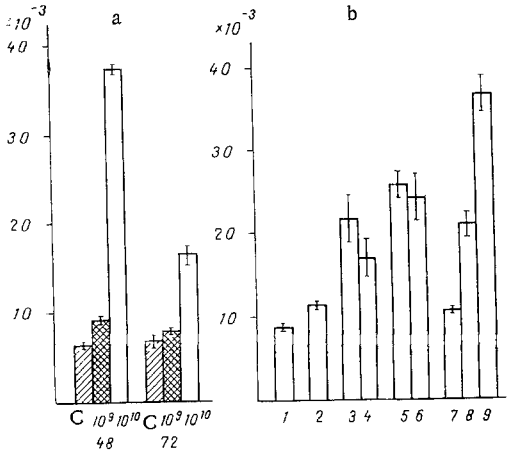

Fig. 3. DNA synthesis in response to BP in spleen cells of nude mice (a). Mitogenic effect of BP compared with nonspecific B mitogens on spleen cells (b). a) Abscissa, duration of culture of cells (in h). 10^9, 10^{10}) Number of MC of BP. b) Abscissa, ingredients: 1) culture medium (C), 2) PPD 5 μg/ml, 3) LPS 100 μg/ml, 4) LPS 20 μg/ml, 5) SD 50 μg/ml, 6) SD 250 μg ml, 7) BP in a dose of 10^8 MC/ml, 8) BP in a dose of 10^9 MC/ml, 9) BP in a dose of 10^{10} MC/ml.

a mitogenic action also on T_1 lymphocyte populations and (or) their precursors. These hypotheses require further experimental verification.

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