REACTIVITY OF MOUSE SPLENIC B LYMPHOCYTES TO ADRENALIN DURING THE IMMUNE RESPONSE

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Conflicting results have been obtained from many investigations to study how changes in the functional state of the nervous system may affect the intensity of immune responses and how injection of an antigen changes the activity of different parts of the nervous system [1]. However, no attempt whatsoever has been made to study the state of reactivity of individual populations and subpopulations of lymphocytes to neurotransmitters in the course of the immune response, although possible changes in reactivity of the effector may largely determine the degree of influence of the regulating system on it.

The object of this investigation was to study reactivity of splenic B lymphocytes of mice to the action of adrenalin at various times after sensitization of the mice with ovalbumin.

EXPERIMENTAL METHOD

Mice of strain C57BL/6 were immunized by a single intraperitoneal injection of 5, 50, and 1000 μg ovalbumin with 5 mg $Al(OH)_3$ in 0.5 ml physiological saline. The animals were decapitated in groups of 8-10 mice 1, 2, 3, 5, and 8 days after immunization. Intact animals served as the control. The spleens of the mice of each group were gently homogenized to isolate lymphocytes. To free the suspension from T lymphocytes the cells were incubated for 1 h at 37°C with anti-Thy-serum in a final dilution of 1:100 and for 30 min with guinea pig complement. The anti-Thy-serum was obtained by immunization of guinea pigs with a brain homogenate from mice of the same strain [6, 8]. The number of viable cells before and after treatment with the antiserum was determined by staining with toluidine blue and by the decrease in incorporation of [^{3}H]thymidine into the antiserum-treated cells. Adrenalin was then added to the washed suspension of B lymphocytes in medium 199 in a final concentration of 10^{-6} M. The reaction was stopped after 10 min by addition of 4 N HCl, protein was sedimented by centrifugation, and the concentration of cyclic AMP in the supernatant was determined by a radiological method (using the appropriate kit from the Radiochemical Centre, Amersham, England). To assess the intensity of proliferation of the splenic lymphocytes, at different times of immunization B lymphocytes were cultured with 2 μCi of [^{3}H]thymidine for 2 h in medium 199 [4]. Involvement of the adrenoreceptors of the cell in the reaction was determined by abolition of changes in the cAMP concentration in response to adrenalin by the β-adrenoblocker obsidan and the α-adrenoblocker droperidol in a final concentration of 10^{-4} M.

Reactivity of the suspension to adrenalin was estimated from the increase in the concentration of cAMP in picomoles/10^6 viable mononuclear cells.

EXPERIMENTAL RESULTS

Treatment of the suspension of splenocytes with anti-Thy-serum and complement led to a decrease of 35-46% in the number of viable cells. According to data in the literature, this number corresponds to the relative number of T lymphocytes in the mouse spleen [3, 6, 9]. In this connection the splenocyte suspension which remained viable can be regarded as a suspension chiefly of B lymphocytes. The cAMP content in the B lymphocyte suspension of intact animals was 1.47 ± 0.145 pmole. Incubation of the suspension with adrenalin increased the cAMP content to 2.17 ± 0.21 pmole, or by 49%. Immunization with all doses of the antigen caused an increase in the background content of cAMP on the 2nd-3rd day of sensitization, with a peak on the 2nd day (Fig. 1). The cAMP content returned to its initial values 5-8 days later.

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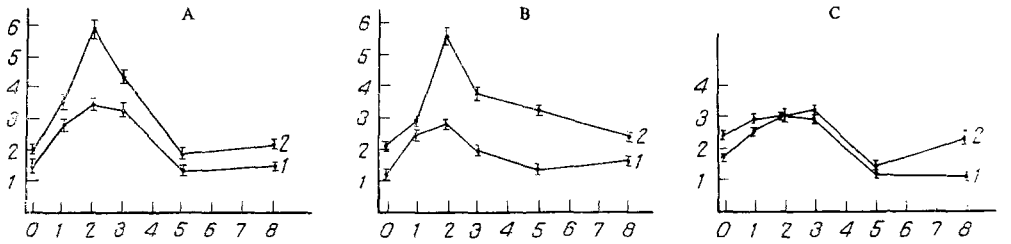

Fig. 1. Changes in cAMP content in mouse spleen B lymphocytes under the influence of adrenalin at different times after immunization. Abscissa, time after immunization (in days); ordinate, cAMP content (in pmoles/10^6 cells). 1) Background cAMP content; 2) cAMP content after incubation with 10^{-6} M adrenalin. A) Immunization with 5 μg ovalbumin; B) immunization with 50 μg ovalbumin; C) immunization with 1000 μg ovalbumin.

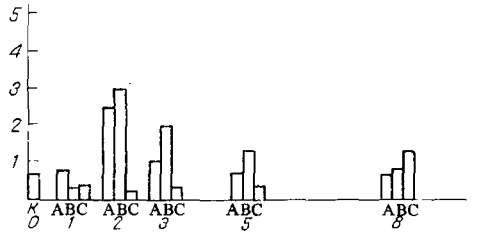

Fig. 2. Increase in cAMP content in mouse spleen B lymphocytes at different times after immunization in response to adrenalin. K) Intact control. Remainder of legend as to Fig. 1.

Definite differences were observed in the kinetics of changes in the reactivity of B lymphocytes to adrenalin depending on the time of sensitization and the dose of the antigen (Fig. 2). After immunization with 5 μg ovalbumin the increase in cAMP in the B lymphocytes on the 1st day after immunization in response to adrenalin was reduced somewhat compared with the control, it increased on the 2nd-3rd day of immunization, and returned to normal later. Immunization with 50 μg ovalbumin was accompanied by a more marked rise in the cAMP content in the B lymphocytes on the 2nd day after incubation with adrenalin. After 3 days this difference decreased but remained higher than initially, and later it fell rapidly. Immunization with 1000 μg ovalbumin reduced the increase in cAMP in response to adrenalin on the first 3 days of sensitization, but their reactivity returned to its initial value after 5-8 days.

Preincubation of the B lymphocyte suspension with obsidan considerably inhibited the action of adrenalin on a suspension obtained from both immunized and intact animals. Incubation with droperidol had no significant effect on the subsequent action of adrenalin. It can be concluded from these observations that adrenalin exerts its action on B lymphocytes through β-adrenergic receptors, in agreement with data in the literature for different types of cells [2, 7]. We know that β-adrenoreceptors on lymphocytes are linked with the enzyme adenylate cyclase, activation of which leads to an increase in cAMP synthesis, and for that reason the increase in the cAMP content in the lymphocytes after treatment with adrenalin characterizes the state of their reactivity to β-adrenomimetics [5]. The increase of reactivity reached a peak 2 days after immunization with 5 and 50 μg ovalbumin and it preceded the beginning of intensive proliferation of B lymphocytes, the maximum of which was observed on the 3rd day of immunization (Fig. 3).

The intensity and direction of changes in reactivity of B lymphocytes to adrenalin after immunization depended on the dose of antigen. Reactivity was increased on the 2nd-3rd day of immunization with 5 μg, and even more by 50 μg ovalbumin. At the same time, it was reduced after immunization with 1000 μg ovalbumin – a dose which can be regarded as tolerance-inducing, for proliferation of B lymphocytes was suppressed after immunization with this dose.

It can be concluded on the basis of these results that reactivity of mouse spleen B lymphocytes during

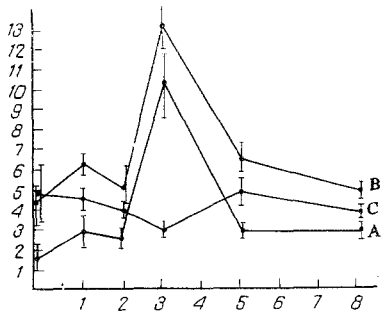

Fig. 3. Incorporation of [^{3}H]thymidine into mouse B lymphocytes at different times after immunization. Abscissa, time after immunization (in days); ordinate, incorporation of [^{3}H]thymidine (in cpm/10^6 cells). Remainder of legend as to Fig. 2.

initiation of immune responses is changed within wide limits compared with the initial value. These changes are evidently connected with a change in the number of β-adrenoreceptors of B lymphocytes during antigen-induced proliferation of B-precursors and their differentiation into antibody-forming cells. Changes described in B lymphocytes in response to adrenalin depend on the dose of antigen and the time elapsing after its administration.

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