CHARACTERISTICS OF β-ADRENORECEPTORS ON SPLENIC B LYMPHOCYTES OF MICE IMMUNIZED WITH A PROTEIN ANTIGEN

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In the last decade much research has been published on the structure and functional role of receptors for adrenergic agonists on lymphocytes [3, 4, 6, 9]. It has been shown that receptor structures for neurotransmitters on lymphocytes are synthesized and eliminated at different rates depending on the functional state of the lymphocytes; their number is therefore labile and varies with exposure to different physiological and immunological conditions [5, 7, 8].

The writer showed previously that reactivity of mouse spleen B lymphocytes to adrenalin varies in the course of the immune response [1] and suggested that this enhanced reactivity is connected both with an increase in the number of receptors for adrenalin on lymphatic cells and also with increased adenylate cyclase activity during interaction of the ligand with β-adrenoreceptors.

The aim of this investigation was to study the physicochemical characteristics and number of β-adrenoreceptors on mouse spleen B lymphocytes before and after immunization with a protein antigen.

EXPERIMENTAL METHOD

Experiments were carried out on female C57BL/6 mice weighing 18-20 g. To obtain a suspension of B lymphocytes the animals were decapitated and the spleens homogenized in medium 199 and filtered through nylon wadding. The resulting suspension of lymphocytes was incubated for 45 min at 37°C with rabbit anti-Thy-serum and guinea pig complement, after which dead cells and erythrocyte residues were removed on a Ficoll–Verografin density gradient (1.09 g/cm^3). The enriched B lymphocyte suspension, in interphase, was washed 3 times in medium 199 at 4°C. The number of living cells, which was usually not less than 95%, was counted with the aid of toluidine blue. To determine the number and characteristics of the binding sites (receptors) of adrenergic agonists, the labeled β-adrenoblocker ^{3}H-dihydroalprenolol (^{3}H-DHA), with a specific activity of 82 Ci/mmole (Amersham International, England), was used. The cells (5×10^6) were incubated with ^{3}H-DHA in 0.5 ml of culture medium for 20 min at 23°C to determine total binding. The suspension was then washed twice with cold buffer, pH 7.2. To determine nonspecific binding, the cell suspension was incubated with unlabeled propranolol, in a concentration of 10^{-5} M, for 15 min before incubation with the radioligand. Cells washed to remove unbound radioligand were solubilized by the addition of 3% Triton X-100 to the samples. After the residue had been dissolved, 4 ml of ZhS-8 scintillation fluid was added to the samples and radioactivity (cpm) measured on a scintillation counter. All tests were carried out in duplicate or triplicate.

To determine the number of receptors during formation of the immune response, the animals were given a single intraperitoneal injection of 250 μg ovalbumin with 5 mg $Al(OH)_3$ and killed 2-8 days after immunization.

In preliminary experiments to study the physicochemical properties of the β-adrenoreceptors and to choose optimal conditions for obtaining the most stable and reliable results, the B lymphocytes were incubated with ^{3}H-DHA at different temperatures, for different exposures, after pre-incubation with different concentrations of nonradioactive propranolol.

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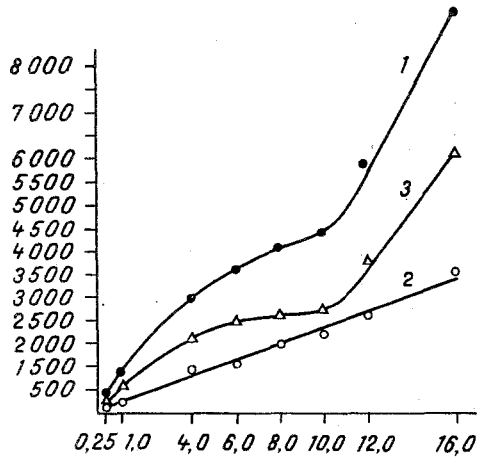

Fig. 1

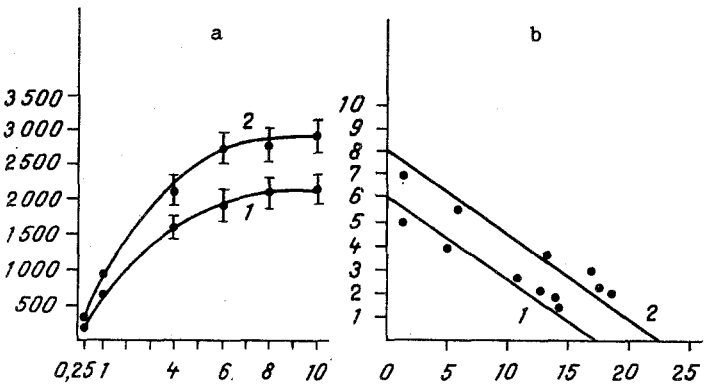

Fig. 2

Fig. 1. Binding of ^{3}H-DHA on mouse spleen B lymphocytes depending on concentration of radioligand during incubation. Abscissa, concentration of ligands (in mM); ordinate, binding (in cpm/5 × 10^6 cells). 1) Total binding, 2) nonspecific binding, 3) specific binding.

Fig. 2. Specific binding of ^{3}H-DHA (a) and Scatchard plot (b) for control B lymphocytes (1) and 3 days after immunization (2). Abscissa: a) concentration of ligand (in nM), b) quantity of specifically bound ligand (in fmoles); ordinate: a) specific binding (in cpm/5 × 10^6 cells), b) ratio of specifically bound to free ligand (in fmoles/nmoles).

Specific binding of ^{3}H-DHA by B lymphocytes was calculated as the difference between total and nonspecific binding (in cpm). The results were used to construct a Scatchard plot. The number of binding sites (receptors) was determined from the specific binding, using ^{3}H-DHA in a concentration of 8 nM, by the standard method, by the equation:

$$P = \frac{a \cdot N}{A \cdot b},$$

where P is the number of receptors, N is Avogadro's number, A the specific activity of the ligand, a) specific binding in the sample, and b) the number of cells in the sample.

EXPERIMENTAL RESULTS

Mouse spleen B lymphocytes can specifically bind the labeled β-adrenoblocker ^{3}H-DHA. The degree of specific binding depended on the duration of incubation with the ligand: it increased during the first 15 min, and thereafter remained constant for 60 min. Heating the cells for 15 min at 70°C, which caused their death, completely prevented them from specifically binding ^{3}H-DHA, evidence of the thermolability of the β-adrenoreceptors.

Specific binding increased during incubation of the B lymphocytes with ^{3}H-DHA in concentrations rising from 0.25 to 6 nM. Later, up to a concentration of 10 nM, it remained almost constant, after which it rose sharply (Fig. 1) due to binding of the ligands by low-affinity receptors [2]. To determine the number of β-adrenoreceptors, we therefore used the specific binding during incubation of B lymphocytes with ^{3}H-DHA in a concentration of 8 nM.

The Scatchard plot (Fig. 2b) shows that high-affinity β-adrenoreceptors, which can be saturated by low (under 10 nM) concentrations of ligands, are homogeneous structures with a dissociation constant (K_d) of 0.34 nM ^{3}H-DHA, and their number per B lymphocyte in the control animals was 1640 ± 171.

The number of β-adrenoreceptors changed after immunization (Fig. 3). For instance, it increased by 20% 2 days after immunization and by 32% 3 days after. Later the number of β-adrenoreceptors decreased and fell below the control level, but after 8 days it returned to its original value.

The change in the number of receptors on B lymphocytes induced by immunization was not accompanied by any changes in their affinity: values of K_d in the control and in lymphocytes

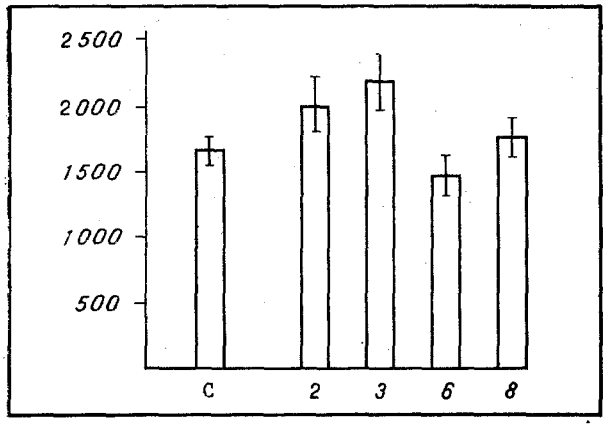

Fig. 3. Number of β-adrenoreceptors of a B lymphocyte before and after immunization. Abscissa, time after immunization (in days); ordinate, number of receptors. C) Control.

isolated 3 days after immunization, at a time of the maximal increase of proliferative activity induced by the antigen [1], were closely similar (Fig. 2a, b).

These results confirm the writer's hypothesis [1] that increased reactivity of splenic B lymphocytes to adrenalin during the formation of immune responses is associated, at least in part, with an increase in the number of β-adrenoreceptors on them.

Increased expression of β-adrenergic receptors on B lymphocytes evidently reflects the process of their proliferation and differentiation, aimed at the formation of B-producers from B-precursors.

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