EFFECT OF ANTIGLOBULIN SERUM ON MUSCARINIC ACETYLCHOLINE RECEPTOR EXPRESSION IN SPLENIC LYMPHOCYTES OF INTACT AND IMMUNIZED MICE

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If specific agonists and antagonists act on muscarinic acetylcholine receptors (MAR) of mouse spleen B lymphocytes, they modify the function of their immunoglobulin receptors [1]. This provided the basis for the conclusion that interaction between immunoglobulin receptors and MAR on B lymphocytes may occur. While developing this idea, the writers obtained evidence that incubation with specific antigen in vitro reduces expression of MAR on mouse splenic B lymphocytes during the immune response to ovalbumin [2].

The aim of this investigation was to study the effect of rabbit antiserum to mouse globulins in MAR expression on lymphocytes of control and immunized mice.

EXPERIMENTAL METHOD

Experiments were carried out on CBA and BALB/c mice. After decapitation of the animals all suspensions were obtained from their spleens and filtered through nylon wadding. To isolate lymphocytes the suspension of splenocytes was lowered above a Ficoll–Verografin gradient with density of 1.09 kg/liter and centrifuged for 30 min at 4°C. Cells in the interphase were withdrawn and washed twice, after which they were counted morphologically. No fewer than 95% of the cells were viable.

Suspensions of B lymphocytes were obtained by exhaustion of the splenocyte suspension from T lymphocytes, by acting on the splenocytes with rabbit antiserum to mouse Thy-antigen and guinea pig complement, after which the suspension of viable B lymphocytes was isolated on a Ficoll–Verografin gradient [2].

The animals were immunized with ovalbumin in a dose of 250 μg with $Al(OH)_3$ or 10^8 washed sheep's red cells, given as a single intraperitoneal injection. The animals were destroyed 3, 4, and 14 days after immunixation.

In each test 75×10^6 cells were taken from each unseparated suspension of lymphocytes or B lymphocytes. All tests were duplicated. To determine the number of MAR on the lymphocytes a radioactively labeled blocker of these receptors – ^{3}H-quenuclidyl benzylate (from Amersham International, England) – with specific activity of 36 Ci/mmole, were used [2].

To study the effect of antiglobulin serum on expression of MAR rabbit antiserum to mouse globulins, obtained from the N. F. Gamaleya Research Institute of Microbiology and Epidemiology, Academy of Medical Sciences of the USSR, were used. The samples of lymphocytes were incubated at 23°C in a volume of 1 ml with antiglobulin serum in dilutions from 1:10 to 1:320 for 15 min, after which the radioactive ligands were added. Normal rabbit serum, heated for 45 min to 56°C and adsorbed on mouse red blood cells, in similar dilutions, was used as the control.

EXPERIMENTAL RESULTS

The numbers of MAR on splenic lymphocytes of CBA mice for an undiluted suspension and B lymphocytes were closely similar, namely 160 ± 21 and 206 ± 34 per lymphocyte respectively for the control. The antiglobulin serum reduced expression of MAR depending on the concentration, maximally by 14% for the undiluted suspension and by 32.5% for B lymphocytes (Fig. 1).

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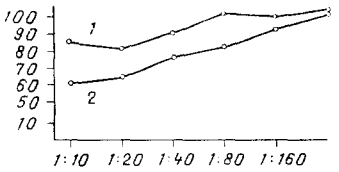

Fig. 1

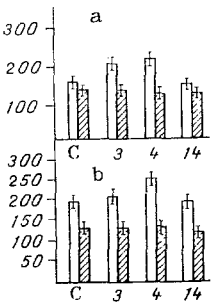

Fig. 2

Fig. 1. Effect of concentration of rabbit antiserum with respect to mouse globulin on MAR expression on lymphocytes of an undiluted splenocyte suspension (1) and on B lymphocytes (2). Abscissa, dilution of antiserum; ordinate, number of MAR (in percent of control).

Fig. 2. Action of antiglobulin serum on MAR expression during immunization with ovalbumin. Abscissa, time after immunization (in days); ordinate, number of MAR per lymphocyte. a) Undiluted suspension of splenocytes, b) B lymphocytes. Unshaded columns — incubation with normal rabbit serum in a dilution of 1:20; shaded columns — incubation with antiglobulin serum in a dilution of 1:20, c) control.

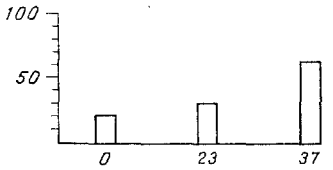

Fig. 3. Effect of antiglobulin serum in dilution of 1:20 on MAR expression on B lymphocytes depending on temperature. Abscissa, temperature (in °C); ordinate, reduction of MAR expression (in percent).

The effect of the antiglobulin in the serum on the state of MAR of the undiluted suspension of lymphocytes was considerably greater 3 and 4 days after immunization with ovalbumin. The number of MAR fell to 30% after incubation of these lymphocytes with antiglobulin serum (Fig. 2a). The action of antiglobulin serum on MAR of the undiluted lymphocyte suspension 14 days after immunization was the same as in the unimmunized control.

Immunization increased the absolute number of MAR on B lymphocytes to 240-270 per cell. However, it hardly changed the effect of the antiglobulin serum (Fig. 2b).

To study the mechanism of action of the antiglobulin serum on MAR expression of B lymphocytes, incubation was carried out with the serum and radioligand at 23°C, and also in parallel tests at 0 and 37°C.

The action of the serum was somewhat weaker at 0°C than at 23°C; and at 37°C it was considerably stronger (Fig. 3).

Very similar data were obtained on immunization of CBA mice with sheep's red blood cells and also during the action of antiglobulin serum on B lymphocytes of immunized and unimmunized BALB/c mice. Thus the ability of antiglobulin serum to modify MAR expression on lymphocytes of intact mice and by an even greater degree, on immunized mice, is a sufficiently universal process.

We know that B lymphocytes synthesize immunoglobulins of different classes, which are incorporated into their surface, where they perform the function of antigen-recognizing and antigen-binding receptors. Antiserum to globulins can bind specifically with these receptors, modifying the function of the lymphocytes. These data are evidence that antiglobulin

serum can also affect MAR expression on lymphocytes: by a lesser degree on an undiluted splenocyte suspension, by a greater degree on one enriched with B cells. In the course of the immune response this difference almost disappears. The reason evidently is that during the immune response the number of B lymphocytes in the spleen increases relative to the number of T lymphocytes [3].

MAR expression may be reduced by the action of antiglobulin serum on immunoglobulin receptors either on account of screening of adjacent MAR or on account of destruction of this molecular zone, for the Ig–anti-Ig complex is removed from the surface of the B lymphocytes. The process of preparation of the complex depends on the temperature, and at 37°C it takes 15 min [4-6].

The investigation showed that antiglobulin serum changes MAR expression most strongly on B lymphocytes at 37°C and least strongly at 0°C. This means that MAR expression may be influenced by both processes: binding of antiglobulin antibodies to specific receptors (at 0°C and 23°C) and subsequent separation of the Ig–anti-Ig complex (at 37°C). However, since incubation of B lymphocytes with antiglobulin serum at 37°C induces greater changes in the number of MAR, it must be concluded that configurational changes on the lymphocyte membranes connected with separation of the Ig–anti-Ig complex have the strongest influence on the number of free MAR.

Thus whereas previously it was shown that cholinergic agonists and antagonists can influence the function of immunoglobulin receptors [1], in the present investigation the opposite possibility was found: by acting on immunoglobulin receptors of B lymphocytes it is possible to influence MAR expression. This is evidence that there exists a unique kind of interaction between immunoglobulin and neurotransmitter receptors, which is evidently determined by the closeness of their location, and which can therefore be characterized as steric. This type of interaction may be one of the mechanisms of regulation of immune responses by the nervous system.

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