EFFECT OF SOME CHOLINE COMPOUNDS ON LYMPHOCYTE ROSETTE FORMATION IN MICE

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Recent investigations have shown that antigen-binding receptors of B lymphocytes are proteins of sIgM and sIgD type, mounted in the lipid base of the cell membrane [7]. These receptors are mobile, they interact with Fc-receptors, have a molecular weight of 800,000-160,000 daltons, and their peptide chains are linked by sulfide bonds [2]. In their physicochemical properties they have much in common with glycoproteins that carry the properties of choline receptors. The lymphocyte membrane is dynamic and interaction of its proteins, carrying the properties of receptors of different kinds, suggests that agents of both immune and mediator profile have a mutual influence on each other.

The aim of the present investigation was accordingly to study relations between immune and choline receptors of lymphocytes.

EXPERIMENTAL METHOD

Experiments were carried out on 150 BALB/c mice. The animals were immunized with a 10% suspension of sheep's red blood cells (SRBC), in a dose of 0.2 ml intravenously [3]. The strength of the immune response of the mice was determined by measuring the hemagglutinin titer, which varied from 1:40 to 1:640. Five days after immunization the mice were killed and cell suspensions in medium 199 were made from their spleens. Suspensions of lymphocytes were filtered through Kapron gauze, layered on a Ficoll-Verografin density gradient [1], and centrifuged at room temperature for 30 min at 1500 rpm. After centrifugation the resulting whitish ring of cells was removed and washed twice with medium 199. The washed lymphocytes were tested for viability using 0.1% trypan blue solution. The number of viable cells was 96-98%. Phosphorylcholine chloride was obtained from Sigma, USA, acetylcholine chloride from the Moscow Chemapol pharmaceutical chemicals factory, and atropine sulfate also was used. The dilutions of the substances were prepared before use in Hanks' solution. For testing for toxicity, the choline preparations, in the concentrations used (from 10^{-14} to 10^{-5} M) were added in a volume of 0.05 ml to 0.05 ml of lymphocytes (10^7 cells/ml) and the tubes were incubated for 1 h. It was shown that choline-containing preparations in the concentrations used have no toxic action and the percentage of viable lymphocytes was the same after treatment with these preparations as in the control.

Rosette-forming cells (RFC) were detected by the method in [8]. When the percentage of viable cells had been calculated the suspension was made up with medium 199 to a final concentration of 10^7 cells/ml and a 0.5% suspension of SRBC (10^8 cells/ml), washed three times with physiological saline, was prepared.

For the reaction agglutination tubes were used. Into each tube, containing 0.05 ml of the cell suspension, 0.05 ml of the corresponding dilution of the choline-containing preparation was added, the tubes were shaken, placed in a revolving drum, and incubated for 60 min at 37°C. To each tube 0.05 ml of the 0.5% suspension of SRBC was then added, the contents of the tubes shaken, and incubated for 30 min at 37°C, after which the cell mixture was kept at 8-10°C for 18-24 h, and the number of rosettes was then counted. For this purpose the residue was carefully resuspended, the Goryaev's chamber was filled, and the number of rosettes to 10^6 lymphocytes was counted. Usually a high level of RFC in the mouse spleen (4.3×10^3, 5.6×10^3, or 6.1×10^3) per 10^6 cells correlated with a relatively high hemagglutinin titer in the blood serum (1:320-1:640). In some experi-

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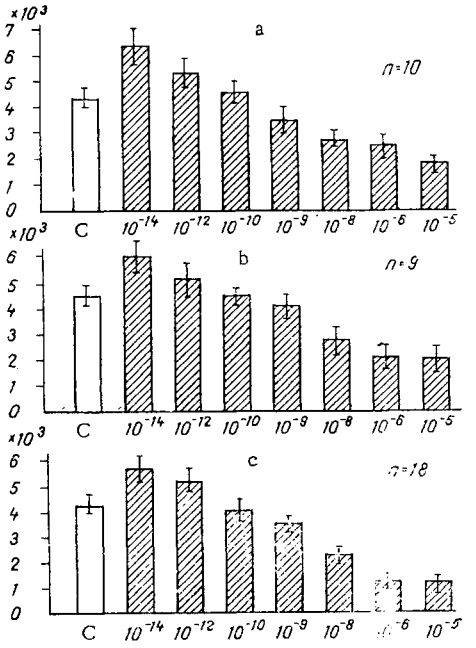

Fig. 1

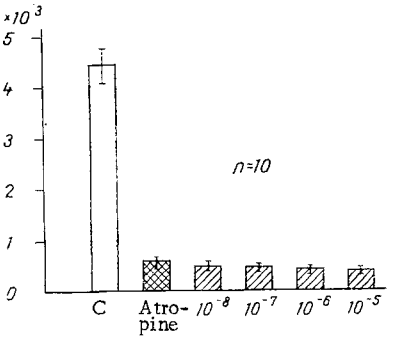

Fig. 2

Fig. 1. RFC in mice under the influence of acetylcholine (a), benzoylcholine (b), and phosphorylcholine (c). Abscissa, dilutions of acetylcholine, benzoylcholine, and phosphorylcholine (in M); ordinate, number of RFC per 10^6 lymphocytes. C) Control.

Fig. 2. Action of phosphorylcholine after atropine (10^6) on immune rosette formation in mice. Abscissa, dilutions of phosphorylcholine (in M). Remainder of legend as to Fig. 1.

ments the suspension of splenic lymphocytes was treated with anti-Thy-serum and complement. The population of B lymphocytes thus obtained had a viability of 92-96%.

There is evidence that about 60% of lymphocytes in the mouse spleen are B cells [6].

EXPERIMENTAL RESULTS

Treatment of splenic lymphocytes with acetylcholine in high concentrations (from 10^{-8} to 10^{-5} M) caused a decrease in the number of cells forming rosettes with SRBC. The decrease was considerable when acetylcholine was used in a concentration of 10^{-5} M, when it was 60.2%, whereas in a concentration of 10^{-8} M acetylcholine inhibited rosette formation by 44%. When acetylcholine was used in low concentrations (from 10^{-14} to 10^{-12} M) a stimulating effect was observed on RFC: In a dose of 10^{-14} M the compound increased the number of RFC by 62.4%, whereas in a dose of 10^{-12} M the increase was 33.2% (Fig. 1a).

The effect of benzoylcholine on rosette formation also depended on the concentration of the compound used. A particularly sharp fall in the number of RFC was observed when it was used in a dose of 10^{-5} M (by 71.3%) but a concentration of 10^{-8} M reduced the number of RFC by only 30.8%. In low doses benzoylcholine stimulated rosette formation. In a concentration of 10^{-14} M it increased the number of RFC by 50.4%, but in a concentration of 10^{-12} M the increase was only 28.9% (Fig. 1b).

Like acetylcholine and benzoylcholine, phosphorylcholine also had an inhibitory effect on rosette formation in concentrations of 10^{-5} to 10^{-8} M. A particularly marked decrease (by 74.4%) in the number of RFC was observed when it was used in a dose of 10^{-5} M, and in a concentration of 10^{-8} M the compound inhibited rosette formation by 51.1%. In low concentrations, on the other hand, phosphorylcholine stimulated rosette formation: by 40% in a dose of 10^{-14} M, by 34.3% in a dose of 10^{-12} M (Fig. 1c).

All the compounds of the choline series tested thus had an inhibitory action in high concentrations on rosette formation; this inhibition was particularly marked when the compounds were used in a dose of 10^{-5} M. Low con-

centrations of choline compounds, however, caused an increase in the number of RFC, especially if used in a dose of 10^{-14} M.

It must be pointed out that a decrease in the number of rosette-forming lymphocytes with a large number of SRBC (more than 10) was found after addition of choline-containing preparations in high concentrations. Since a high density of receptors for SRBC is a characteristic feature of B cells, they can bind a large number of these cells (10 or more) [3]. It can accordingly be postulated that the choline-containing compounds, in concentrations of 10^{-5} to 10^{-8} M, had an inhibitory action chiefly on the B lymphocyte population.

Inhibition of rosette formation by B lymphocytes through the action of cholinergic preparations was observed in an investigation by Ferreira et al. [4].

In a special series of experiments using atropine, an antagonist of acetylcholine, in a concentration of 10^{-6} a sharp decrease in the number of RFC was observed. For instance, after treatment with atropine rosette formation was inhibited by 86.8%. Blocking choline receptors by atropine abolished the action of phosphorylcholine on rosette formation (Fig. 2). Since atropine is a powerful muscarinic antagonist, and after its action on lymphocytes the process of rosette formation is sharply inhibited, it can be postulated that the process of formation of immune rosettes is connected with surface membrane structures of lymphocytes related to the muscarinic receptors of the lymphocytes. The presence of specific cholinergic receptors on mouse lymphocytes was demonstrated by Gordon et al. [5] using labeled 3-quinuclidinyl benzilate, a specific cholinergic muscarinic agent.

Representation of choline-receptor structures on lymphoid cells is an important argument in support of the participation of the corresponding mediators in regulation of the immune response at the level of immunocompetent cells.

The similarity of the physicochemical properties and the probable closeness of location of the two types of receptors (immune and mediator) in the lymphocyte membrane suggest that close interaction exists between the two receptors we have studied on the surface of lymphocytes.

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