EFFECT OF ANTICHOLINOCEPTOR SERUM ON IMMUNE ROSETTE FORMATION IN MICE

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Some cholinergic antagonists are known to have a definite action on the state of the antigen-binding receptors of lymphocytes. In particular, a dose-dependent effect of acetylcholine, benzoylcholine, and phosphorylcholine on immune rosette formation has been demonstrated in mice [1]. On this basis interaction between immune and mediator receptors of lymphocytes has been postulated.

The aim of this investigation was to study the action of specific anticholinoceptor serum (ARC-serum) on immune rosette formation in mice.

EXPERIMENTAL METHOD

Experiments were carried out on 190 BALB/c mice. The mice were immunized with a 10% suspension of sheep's red blood cells in a volume of 0.2 ml, injected into the caudal vein [6]. The mice were killed 5 days after immunization and a cell suspension prepared from their spleens by mild homogenization; the suspension was filtered through a capron filter and layered on a Ficoll-Verografin density gradient with density coefficient of 1.077 g/ml and centrifuged for 30 min at room temperature [4]. After centrifugation, a white ring of lymphocytes formed in the interphase and was carefully removed and washed twice with medium 199. The viability of the cells was determined with 0.1% trypan blue solution. The washed lymphocytes were used in the immune rosette formation test.

To obtain ACR-serum chinchilla rabbits were immunized with a preparation of membrane fragments enriched with acetylcholine receptors, obtained by the method in [8]. It is stated in the literature that the number of choline receptors is greater in denervated muscles than in normal muscles, and for that reason muscles of BALB/c mice, both normal and, mainly, denervated by division of the sciatic nerve, were used as the source for obtaining membrane fragments enriched with acetylcholine receptors. The titer of the rabbit ACR-serum was 1:1280. In the immune rosette formation tests the AR serum was adsorbed on sheep's red blood cells (SRBC) and lymphocytes obtained from the spleen of mice immunized with SRBC. The serum was adsorbed on mouse lymphocytes by adding $50\text{-}70 \cdot 10^6$ lymphocytes to 1 ml of ACR-serum. The mixture of lymphocytes with ACR-serum was incubated for 30 min with periodic shaking. After centrifugation of the mixture the serum was removed and readsorbed on mouse lymphocytes. The titer of the ACR-serum adsorbed on SRBC and mouse lymphocytes was usually three orders of magnitude below the titer of the unadsorbed serum.

The suspension of washed lymphocytes obtained from the immunized mice was treated with the adsorbed ACR-serum to obtain a final dilution of the serum of 1:20. The mixture of cells with ACR-serum was incubated for 30 min, after which the immune rosette formation test was carried out as described previously [2]. The number of rosette-forming cells (RFC) was counted in a Goryaev's counting chamber. For this purpose the cell residue was carefully resuspended and the chamber filled with the cell suspension and the number of rosettes counted in the whole chamber; knowing the volume of the chamber and the number of lymphocytes in 1 ml, the number of rosettes per 10^6 lymphocytes could be calculated.

EXPERIMENTAL RESULTS

Rabbit serum against acetylcholine receptors from denervated mouse muscles inhibited immune rosette formation by 56.8%, rabbit serum against acetylcholine receptors from normal mouse muscles inhibited the reaction by 40.6%, whereas normal rabbit serum had virtually no effect on rosette formation (inhibition of the reaction by 5.6%).

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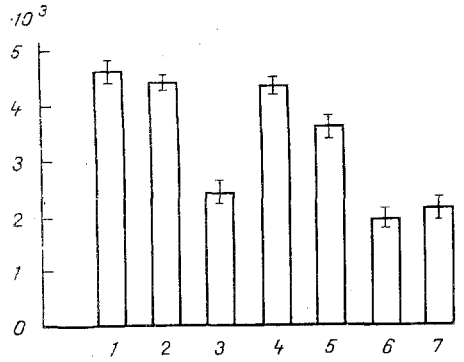

Fig. 1. Action of ACR-serum and of carbachol on immune rosette formation in mice (n = 16). Abscissa: 1) control; 2) normal rabbit serum; 3) ACR-serum adsorbed on SRBC; 4) ACR-serum adsorbed on SRBC and mouse lymphocytes; 5) carbachol (10^{-5} M); 6) ACR-serum adsorbed on SRBC followed by carbachol (10^{-5} M); 7) carbachol (10^{-5} M) followed by ACR-serum, adsorbed on SRBC; ordinate, number of RFC per 10^6 lymphocytes.

The next step was to test the rabbit serum against acetylcholine receptors of denervated muscles, adsorbed on mouse lymphocytes, in the rosette formation test. Since antibodies to choline receptors have been removed from the adsorbed serum, its action caused inhibition of immune rosette formation by only 12.9%.

In a series of experiments the action of the cholinergic agonist carbochol, in a concentration of 10^{-5} M, was tested after treatment of the mouse lymphocytes with ACR-serum. The aim of these experiments was to discover how the cholinergic agent carbachol would behave after blockage of choline receptors by specific serum. The experiments showed that whereas ACR-serum inhibited immune rosette formation by 56.8% and that carbochol, in a concentration of 10^{-5} M, inhibited rosette formation by only 36.6%, as a result of their consecutive action (ACR-serum followed by carbachol) immune rosette formation was inhibited by 50%. Evidently in this case the ACR-serum abolished the action of carbachol, so that it was not exhibited, because the choline receptors were blocked by the serum. If the order was reversed and the lymphocytes were treated with 10^{-5} M carbochol followed by the ACR-serum, the effect was the same as before. The inhibitory action of ACR-serum on immune rosette formation was much stronger than that of carbachol (Fig. 1). The influence of the ACR-serum and carbachol is directed toward interaction with choline receptors of the mouse lymphocytes. When the effect of carbochol on E-rosette formation was studied it was found that preincubation of human peripheral blood lymphocytes with carbachol reduced the number of E-RFC by 30-40% [7]. The authors cited consider that carbachol acts through nicotine-acetylcholine receptors, for its effect is abolished by α-tubocurarine. There is evidence that carbochol increases the number of "early" active E-RFC and acts through muscarinic receptors, because this effect is blocked by atropine [5].

The results of these investigations thus showed that ACR-serum, which blocks the choline receptors of lymphocytes, causes inhibition of immune rosette formation and prevents the manifestation of the action of carbachol on mouse lymphocytes. Since blockage of choline receptors by ACR-serum and by the cholinergic agonist carbachol leads to a decrease in the number of RFC, this may be evidence of interaction between choline and antigen-binding receptors, which makes an important contribution to our understanding of the mechanism of regulation of immune reactions at the level of immunocompetent cells.

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