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Effect of Immune "Sessile" Receptors on the Choline Receptors of the Lymphocyte Membrane in Mice Immunized with Certain Antigens

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Earlier [1-3] we showed that multiple bioactive substances, neurotransmitters, hormones, and drugs have a substantial effect on the immunocompetent cell receptors and on the antigen-driven reactions. in this context we studied acetylcholine, benzoylcholine, cholynolytics (atropine, quinuclidinyl benzilate [^{3}HQNB]), epinephrine, histamine, pilocarpine, and other ligands. In experiments with mice of the BALB/c strain immunized with sheep erythrocytes or ovalbumin it was shown that blocking of the M-choline receptors on spleen B cells with the mentioned ligands drastically inhibits rosette formation with the specific antigen. The quantity of fluorescein isocyanate-stained fluorescent cells was also decreased. A similar effect could be obtained by blocking the M-choline receptors of BALB/c mouse spleen B lymphocytes with rabbit antibodies to choline receptors [3]. The number of free choline receptors on the surface of spleen B lymphocytes from mice immunized with albumin falls after the addition of the specific antigen. The blocking of mouse spleen β-adrenoreceptors induces a similar, though less pronounced effect. These data suggest that the ligands studied have an effect on the immune receptors of lymphocytes via the corresponding choline receptors. In this way a "receptor-receptor" system is organized. Such systems nave been described for various low-molecular-weight ligands [12]. We studied this system under conditions where one of the components was a macromolecular stimulus (antigen), while the other (low-molecular-weight) components were represented by transmitters or drugs (atropine, pilocarpine). The interaction between immune and transmitter (choline) receptors on the lymphocyte surface can be demonstrated in inverse experiments as well.

Therefore, the goal of this study was to attempt to determine the effect of the immune receptor functional state before and after the interaction with specific antigen on the capacity of the choline receptor to bind acetylcholine and its analogs (carbacholine, etc.). In other words, there arises the question: could choline receptor blocking be fulfilled via immune receptor interactions? In the course of this study it was necessary to determine whether a change occurs in the acetylcholine binding to the choline receptor following the binding of an antigen to the immune receptor.

MATERIALS AND METHODS

Lymphocytes were obtained from 100 mice of the BALB/c strain immunized with ovalbumin or bovine

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serum albumin. The animals were sacrificed by bleeding, and a spleen cell suspension was obtained after mild homogenization. The cells were separated after Boyum [11] and passed through a capron filter; the resulting suspension was layered on a Ficoll-Verographin gradient (density coefficient 1.079 g/ml) and centrifuged at 1500 g for 30 min at room temperature. The lymphocytes accumulating in the whitish interphase layer were separated and washed twice with medium 199. In order to obtain a B lymphocyte-enriched population, T-cells were eliminated by treating the spleen cell suspension with anti-Thy serum in the final dilution 1:64 and complement (final dilution 1:10). Anti-Thy serum was produced in rabbits immunized with brain homogenate from mice of the strain used. The principle of the method of choline receptor blocking via immune receptors lies in that antigen (ovalbumin) binding to the "sessile" immune receptors interferes with ("blocks") the capacity of the choline receptors to bind acetylcholine and its analogs (carbocholine, phosphorylcholine). The lymphocytes were treated with specific antigen using techniques elaborated earlier. Immunochemical study employed the method devised by Turpaev *et al.* in 1961-1965 [5]. It is based on the capacity of acetylcholine and its analogs to quench the fluorescence of lymphocyte membrane proteins excited with ultraviolet light of wavelength 260-300 nm, the maximal quenching (hypochromic effect) being observed at wavelength 296 nm. The hypochromic effect was induced by the blocking of the choline receptors with acetylcholine. It is thought that acetylcholine binding to fluorescent proteins of choline receptors inhibits the fluorescence, the degree of inhibition depending on the quantity of bound acetylcholine. The quenching was recorded using a Simadzu Qv-50 photoelectromultiplier and expressed as the number of pulses per minute in the output signal. To the suspension of lymphocytes (10^5 cells per milliliter), untreated or pretreated with specific antigen (ovalbumin, 2 μg per 10^5 cells per ml) and placed in the cells, an acetylcholine solution of various concentration (10^{-14}-10^{-3} M) was added, and the cells were incubated at 37°C for 30 min. The intensity of fluorescence was then recorded. The quenching phenomenon was calculated according to the formula:

$$\frac{[(F_i-1)-F_i]-[(0.01i/(2.5+0.01i))-F_i]}{F_i}$$

where $F=I_o\phi\varepsilon dc$ is the sum of the effects of all energies per second;

ϕ is the ratio of released to bound quanta per second (quantum output);

ε is the molar coefficient of light absorption;

d is the length of the light path in the solution;

c is the concentration of a substance in the solution;

L, the quantum output, is equal to T/T_o (T is the lifetime of excited molecules;

I_o is the additive effect of photons of all energies [10].

The ovalbumin solution in saline was added in a dose of 2 μg per 10^5 lymphocytes, after which the cells were incubated for 20 min at 37°C and washed twice with and resuspended in medium 199 (final volume 2.5 ml). Proserine was used as acetylcholine esterase inhibitor. The output signal of the device was measured in the regime of λ_{excit}=296 nm, $\lambda_{fluoresc}$=369 nm; titration was performed with 10^{-5} M acetylcholine solution and with analogous solutions of other agents. The control lymphocyte samples were treated similarly except for the addition of antigen. The numerical result was expressed as the relative decrease in the intensity of the fluorescence maximum in percentage to the initial level (before the addition of acetylcholine), i.e., the output signal of the photoelectromultiplier was measured in the presence and in the absence of various preparations. We measured the relative fluorescence intensity twice under different conditions (for example, in the presence and in the absence of quenching) and used the data of two spectra; the ratio of these relative maximum values is equal to the ratio of the absolute fluorescence intensities. The results were statistically evaluated using Student's t test. The following reagents were used in this study: acetylcholine (Karpov Chemical Pharmaceutical Factory, Moscow), benzoylcholine iodide and butyrylcholine bromide (Chemapol), phosphorylcholine chloride and L-α-glycerophospho-

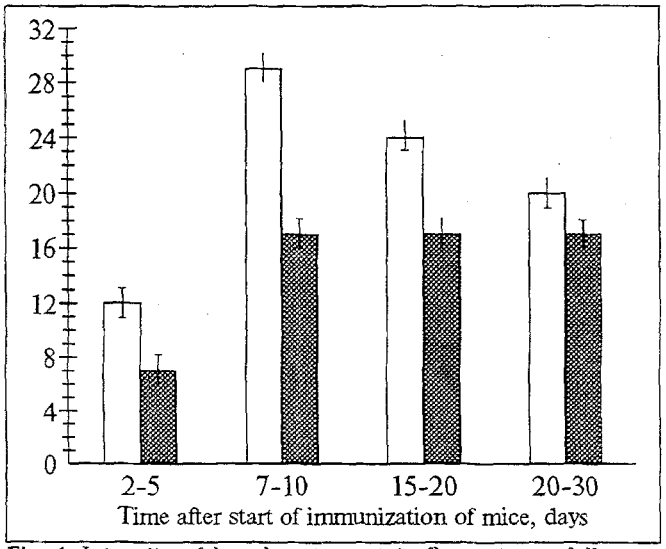

Fig. 1. Intensity of lymphocyte protein fluorescence following acetylcholine treatment. Ordinate: fluorescence of lymphocyte proteins before (open bars) and after (hatched bars) addition of acetylcholine, %, $(\Delta F/F)\times 100$.

rylcholine (Sigma), histamine (Calbiochem), atropine sulfate (Chemical Pharmaceutical Factory, Moscow), proserine 0.05% (Chemical Pharmaceutical Factory, St. Petersburg), and phycoline and epinephrine (Serva).

RESULTS

The main result of the experiments (see Fig. 1) is that the hypochromic effect of acetylcholine is reduced after treatment of immune lymphocytes with the specific antigen (in our case ovalbumin). The degree of reduction was expressed in percentage to the hypochromic effect observed on the same lymphocytes untreated with antigen. The hypochromic effect in these lymphocytes was taken as 100. The time interval between the immunization and the performance of the experiments was also taken into account (Fig. 1).

F is the hypochromic effect of acetylcholine (fluorescence intensity) in percentage to the intensity of fluorescence of immune proteins before and after treatment with the specific antigen. The fluorescence intensity is expressed in absolute units as the number of quanta per second or, using the photoelectromultiplier, as the number of pulses per minute. It can be considered that the smaller the difference in the fluorescence level before and after acetylcholine treatment, the higher the choline receptor activity and, accordingly, the smaller the effect of the antigen-binding receptors upon the choline receptors and on the excitation or inhibition of the latter by acetylcholine. On the other hand, the greater the difference in the acetylcholine hypochromic effect, the more powerful the influence of the antigen-binding receptor on the choline receptors and the more efficient the blocking of the latter with specific antigen acting via the corresponding receptor. The molecular mechanism of antigen-binding and choline receptors interaction is still poorly understood. It may be speculated that conformational, electrostatic, and chemical (e.g., carbamide) forms of linkage play a role.

The maximum acetylcholine-induced quenching of the fluorescence of lymphocyte proteins registered before contact with the antigen is observed 10 days after immunization. This attests to the maximum expression of choline receptors at this time. The choline receptor expression also accompanies the expression of immmune antigen-binding receptors on the lymphocytes in the course of immunization. However, the first few days following antigen priming are characterized by a much weaker hypochromic effect of acetylcholine. On the 10th-20th day post-immunization this initial phase of the immune response goes over the maximum and then slopes down, in parallel with a decrease of the acetylcholine-induced hypochromic effect.

The results presented here prove, in our opinion, the existence of a close functional relationship between the antigen-binding and acetylcholine receptors in the immune response. Further investigation is required.

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