MUSCARINIC CHOLINERGIC RECEPTORS OF B LYMPHOCYTES DURING THE IMMUNE RESPONSE IN MICE

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There is evidence that cholinergic agents affect lymphocyte function [5, 7, 9]. In turn, this has stimulated investigators to study the number of muscarinic and nicotinic cholinergic receptors on the surface of lymphocytes with the aid of corresponding radioactive blocking agents [4, 6, 8]. The data on functional relations between muscarinic cholinergic (MC) and immunoglobulin (antigen-binding) receptors on splenic B lymphocytes of mice immunized with sheep's red blood cells, are particularly interesting [1, 2].

The aim of this investigation was to study the effect of a specific antigen on expression of MC receptors on the surface of splenic B lymphocytes of mice after their immunization.

EXPERIMENTAL METHODS

Experiments were carried out on 550 female CBA mice weighing 18-20 g. The animals were immunized by a single intraperitoneal injection of 250 μg of crystalline ovalbumin with 5 mg of $Al(OH)_3$. The animals were killed 3, 4, and 8 days after immunization. Control animals received the corresponding injections but without ovalbumin. Spleens from 12-15 mice for each group were homogenized and the cell suspension filtered through nylon. To obtain an enriched suspension of B lymphocytes the splenocytes were treated with rabbit anti-Thy-serum and guinea pig complement for 45 min at 37°C. To remove dead T lymphocytes and erythrocytes, the samples were centrifuged on a Ficoll-Verografin density gradient of 1.09 kg/liter for 30 min at 1500 rpm. Cells in interphase were then drawn off and washed twice in Eagle's medium at 4°C, after which viable cells were counted with the aid of a 0.1% solution of trypan blue. Suspensions containing at least 95% of living cells were used in the experiments.

To determine the number of MC receptors on the B lymphocytes, ^{3}H-quinuclidinyl benzilate (^{3}H-QNB; from Amersham Corporation, England) with specific radioactivity of 36 Ci/mmole (106 mCi/mg), a specific blocker of these receptors, was used. In preliminary experiments to study the physicochemical properties of the MC receptors, ^{3}H-QNB was used in concentrations of 0.5 to 12 nM, and the number of cells in the test sample varied from $10 \cdot 10^6$ to $150 \cdot 10^6$. In the main experiments ^{3}H-QNB was used in a concentration of 8 nM and the number of cells in the sample was 70.10^6 to $90 \cdot 10^6$. The degree of total binding was determined by incubation with ligand for 60 min at 22°C, after which the lymphocytes were washed twice with buffer, pH 7.4, at 4°C. To determine the degree of nonspecific binding the samples were preincubated for 20 min at 37°C with atropine sulfate, a nonradioactive blocker of MC receptors, in a concentration of 10^{-4} M, and to determine the effect of the specific antigen on expression of MC receptors, the samples were preincubated with ovalbumin in a concentration of 10 mg/liter under these same conditions. Samples with cells washed free from unbound ligand were lysed in 1 N NaOH solution, 4 ml of scintillation fluid was added to each sample, and bound radioactivity was counted on an LKB 1215 RackBeta 2 counter.

The degree of specific binding was determined from the difference between total and nonspecific binding for samples with preincubation with ovalbumin and without preincubation, and expressed in cpm. The number of receptors on the surface of a B lymphocyte was calculated in all cases by the formula:

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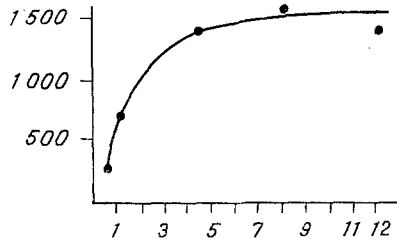

Fig. 1

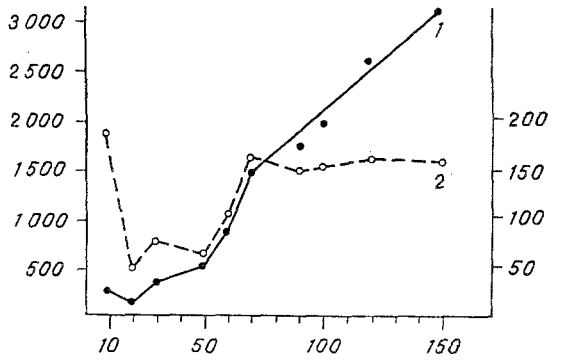

Fig. 2

Fig. 1. Degree of specific binding of ^{3}H-QNB by intact B lymphocytes ($70 \cdot 10^6$ cells per sample) depending on radioligand concentration. Abscissa, concentration of ^{3}H-QNB (in nM); ordinate, degree of specific binding (in cpm).

Fig. 2. Degree of specific binding (1) and number of binding sites (2) for ^{3}H-QNB (8 nM) on a B lymphocyte depending on number of cells in sample. Abscissa, number of cells in sample $\times 10^6$; ordinate: on left, specific binding (in cpm); on right, number of binding sites per cell.

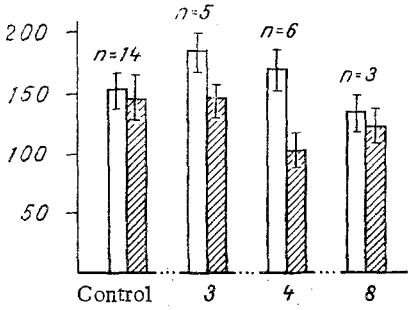

Fig. 3. Number of MC receptors on B lymphocytes during immune response. Abscissa, days after immunization; ordinate, number of MC receptors. Unshaded columns, without antigen; shaded columns, under influence of antigen. n) Number of experiments.

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

where P is the number of receptors on the cell, a the level of specific binding, in cpm, N Avogadro's number, A specific activity of the radioligand, and b the number of cells in the sample.

RESULTS

Preliminary experiments showed that the most reproducible results of counting of ^{3}H-QNB-binding sites were obtained by incubation of lymphocytes with the ligand at 22°C for 60 min. The degree of specific binding increased with an increase in the ^{3}H-QNB concentration during incubation with lymphocytes from 0.5 to 4 nM, after which it remained constant up to 12 nM (Fig. 1). The main experiments were accordingly carried out with ^{3}H-QNB in a concentration of 8 nM.

Since the density of MC receptors on B lymphocytes was very low (130-187 per cell), it was important to determine the optimal number of cells in the sample. It was shown that, only starting from $70 \cdot 10^6$ cells in the sample did the degree of specific binding increase proportionally to the increase in the number of lymphocytes (Fig. 2). When fewer than $70 \cdot 10^6$ lymphocytes were used in the sample, because of the low level of specific binding of the ligand, its radioactivity became comparable with the background, and artefacts could arise.

The number of MC receptors on a B lymphocyte from the control and intact animals was 158 ± 17. Their number increased 3 and 4 days after immunization by 14-31% (Fig. 3). This increase in the number of MC receptors coincided with the peaks of proliferative activity of B lymphocytes, induced by immunization [3]. The number of MC receptors on B lymphocytes 8 days after immunization fell rather below the initial level.

Preincubation of lymphocytes of the control animals with a solution of ovalbumin in the concentration specified had hardly any effect on expression of MC receptors, whereas preincubation of lymphocytes of immunized animals with ovalbumin 3 days and, in particular, 4 days after immunization considerably reduced the expression of MC receptors on them (Fig. 3).

The presence of MC receptors on the surface of B lymphocytes in the mouse spleen has been discussed in the literature [8]. The results of the present investigations, like those of others [4], are evidence that these receptors are definitely present on B lymphocytes.

The conclusion, which has been drawn, that the number of MC receptors on lymphocytes is extremely labile for various reasons [6] is evidently the result of the fact that the authors cited used too few lymphocytes in the sample for determination.

The present results are evidence of the possibility of stearic interaction between MC receptors and immunoglobulin receptors binding specific antigen on B lymphocytes during the immune response, and this is important as a contribution to the understanding of the mechanism of nervous regulation of immunogenesis.

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