INDUCTION OF MOBILITY OF MOUSE B LYMPHOCYTES BY ACETYLCHOLINE AND SUBSTANCES RAISING INTRACELLULAR CYCLIC GMP AND CALCIUM LEVELS

Academician A. D. Ado* and V. I. Dontsov

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KEY WORDS: B lymphocytes, cyclic GMP, calcium.

Acetylcholine (ACh) is known to induce mobility of mouse B lymphocytes [10], but the concrete mechanisms of this process have received little study. Since ACh can raise the cyclic GMP (cGMP) level in cells [4, 8] and can also affect ion transport through cell membranes [2, 7, 9], it was decided to investigate induction of mobility of B lymphocytes by ACh, exogenous cGMP, and by concanavalin A (con A), which increases the calcium inflow into widely different cells [5]. The dynamics of accumulation of intracellular cGMP and the participation of calcium in this process were investigated in response to ACh.

EXPERIMENTAL METHOD

The spleen was obtained from 55 C57BL/6 mice and used to prepare a cell suspension by gentle homogenization of the spleen in medium 199 followed by filtration through four layers of Kapron. To remove T lymphocytes the suspension of spleen cells was treated with anti-Thy-serum and complement. The anti-Thy-serum was obtained by immunizing a rabbit with mouse brain homogenate [3], and in the complement-dependent cytolysis test it had a titer of 1:64. The antiserum was toxic for 100% of thymocytes and 35% of spleen cells, but not for mouse bone marrow cells. Dead cells along with debris and nonlymphoid cells were sedimented during subsequent centrifugation of the cells in a Ficoll–Hypaque density gradient [1].

On subsequent investigation of the effect of the various substances on B lymphocyte mobility an original method was used [10]. Lymphocytes in a concentration of $1 \cdot 10^6$ to $2 \cdot 10^6$ cells/ml were incubated for 5 min in plastic dishes in medium 199 in a humid chamber at 37°C with 0.1 μM ACh, 1 mM cGMP, or 50 μM con A (the concentrations of the substances were chosen beforehand in preliminary experiments). The cells were then fixed with 2% formaldehyde and the number of mobile forms estimated, using the phase-contrast microscope. No fewer than 200 cells were counted and those with pseudopodia or possessing an ameba-like morphology were recorded. The mobility stimulation test was carried out under ordinary conditions or in the presence of 1 mM EDTA and 0.1 mM trifluoperazine, after treatment of the cells with chymotrypsin (0.2 mg/ml, 30 min) or neuraminidase (50 U/ml, 30 min).

The cGMP level in the B lymphocytes was studied by the appropriate kit (Amersham Corporation, England) according to the standard procedure. The cells were preincubated in medium 199 for 15–20 min, after which ACh was added and incubation continued at 37°C for different times. The reaction was stopped by the addition of cold 80% ethanol with HCl, the cells were homogenized in the cold, protein was centrifuged, and the supernatant with extracted cGMP was evaporated in vacuo. cGMP was dissolved in buffer (from the same firm) and its level determined by radioimmunoassay. Special experiments showed that such extraction does not lead to loss of doses of cGMP contained in the sample or added from outside.

Statistical analysis of the data was carried out by Student's t test.

EXPERIMENTAL RESULTS

By using anti-Thy-serum it was possible to obtain a suspension of spleen cells free from T lymphocytes and rich in B lymphocytes [3]. The action of all three substances (ACh, cGMP,

*Academy of Medical Sciences of the USSR.

Laboratory of Molecular Mechanisms of Allergy, Research Institute of Immunology, Ministry of Health of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Bioloii i Meditsiny, Vol. 97, No. 2, pp. 177-178, February, 1984. Original article submitted May 18, 1983.

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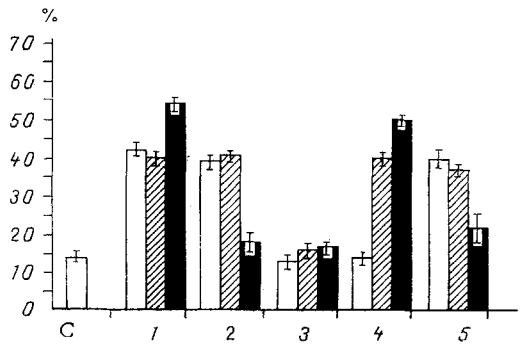

Fig. 1. Mobility of mouse B lymphocytes under different conditions. Abscissa, conditions of testing: C) control. 1) Ordinary conditions, 2) in presence of EDTA, 3) in presence of trifluoperazine, 4) treatment of cells with chymotrypsin, 5) treatment with neuraminidase; ordinate, percentage of mobile forms of B lymphocytes. Unshaded columns — ACh, obliquely shaded — cGMP, shaded black — con A.

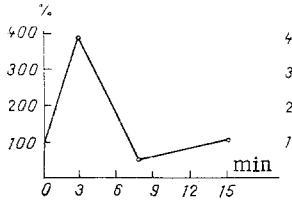

Fig. 2

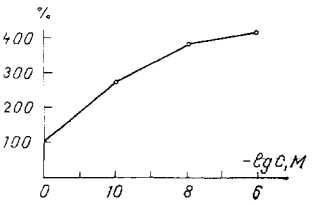

Fig. 3

Fig. 2. cGMP level in mouse B lymphocytes after treatment with ACh. Abscissa, time after treatment with ACh (in min); ordinate, change in cGMP level in B lymphocytes (in %).

Fig. 3. Dose-dependent rise of cGMP level in mouse B lymphocytes under the influence of ACh. Abscissa, ACh concentration (—log C, M); ordinate, change in cGMP level in B lymphocytes (in %).

and con A) was accompanied by a considerable increase in the number of mobile forms of lymphocytes — from 15% in the control to 40-50% during stimulation (Fig. 1). The different conditions were variously reflected in the ability of each of the three agents to stimulate lymphocyte mobility. Binding of intracellular calcium by EDTA abolished the action only of con A, but not of ACh or cGMP. We know that con A binds nonspecifically with the membrane of widely different cells and can modify its permeability for calcium mechanically, and this is true also for B lymphocytes [5]. Since lectins are bound by membrane glycosides, it will be clear that the effect of con A could be abolished by neuraminidase whereas the effect of ACh could be abolished by chymotrypsin. Meanwhile the effect of cGMP was not abolished by these enzymes, thus confirming the view that cGMP, if added to the medium in high concentrations, can penetrate inside cells. The effect of all three substances could be abolished by preincubation of the cells with trifluoperazine, a substance which blocks the effects of calcium through inactivation of calmodulin, the protein through which calcium exerts its effects [6]. This is evidence that all three substances exert their action ultimately through calcium.

By radioimmunoassay a marked increase (by 3-4 times) in the intracellular cGMP concentration was found in the B lymphocytes after treatment with ACh. The effect developed

quickly: the cGMP level reached its maximum 3 min after the beginning of addition of ACh, and after 8 min the cGMP level had fallen below its initial value (Fig. 2). The effect of ACh was abolished by atropine, but depended only a little on extracellular calcium: The rise of the cGMP level during the action of ACh on B lymphocytes was still present in medium with EDTA. The effect of ACh depended on dose: ACh in a concentration of 10^{-8} M was enough to cause the cGMP level to rise close to its peak value (Fig. 3).

Elevation of both intracellular cGMP and calcium levels is thus accompanied by stimulation of B lymphocyte mobility; ultimately elevation of the cGMP level stimulates B lymphocyte mobility through calcium mechanisms. Since the effect of cGMP and of substances raising its level in the cell depends only a little on extracellular calcium, it seems very probable that cGMP can influence the redistribution of calcium in the cell.

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SYNTHESIS OF A HIGH-CAPACITY IMMUNOSOLVENT WITH ORIENTED IMMOBILIZATION OF Fab'-FRAGMENTS FOR ANTIGEN ISOLATION

V. T. Skvortsov and A. E. Gurvich

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Immunosorbents containing immobilized antibodies are being used on an ever-increasing scale for the isolation and determination of antigens [1, 2, 5, 7, 9, 10]. To obtain such immunosorbents various carriers are used, mainly sepharose, to which a fraction of immunoglobulins or pure antibodies is covalently bound.

However, when these methods are used the antigen molecules, on binding with the matrix, are oriented randomly, as a result of which only some (1 of 4) of the active centers react with antigen.

The writers have attempted to overcome this shortcoming by binding Fab'-fragments of antibodies in an oriented manner to the carrier through thio groups, which are located at the opposite end from the active center. This possibility arose in connection with the development of methods of specific binding of thiol-containing molecules to the matrix by thiol-disulfide exchange [3, 8].

Donkey antibodies against rabbit IgG were isolated by means of a cellulose immunosorbent [1]. $F(ab')_2$-fragments were isolated by the method described previously [6] and reduced with 0.02 M dithiothreitol (from Koch-Light, England) in 0.1 M Tris-HCl buffer, pH 8.0, with 0.5 M NaCl and 0.001 M EDTA. The Fab'-fragments thus obtained were separated from dithiothreitol on a column with P6 Bio-Gel (from Bio-Rad, USA), equilibrated with the same buffer, and were

Laboratory of Chemistry and Biosynthesis of Antibodies, N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR O. V. Baroyan.)

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