CYCLIC NUCLEOTIDES AND CALCIUM IONS IN ACTIVATION OF MOUSE B LYMPHOCYTE MOTILITY ACTIVATED BY ANTI-IMMUNOGLOBULIN SERUM

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

UDC 612.112.94:577.123.3]
.014.46:615.373.6

KEY WORDS: B lymphocytes; cyclic nucleotides; calcium.

The ability of antiserum against immunoglobulins to induce functional activity of B lymphocytes is known. Such serum can increase the proliferative activity of B lymphocytes in the same way as the action of mitogens and specific antigen [11], and can also induce an increase in B lymphocyte motility [13]; motility is increased in a similar way by acetylcholine and exogenous cGMP [1, 13]. The concrete mechanisms of activation of B lymphocyte motility by antiserum are not sufficiently clear.

The aim of this investigation was to study the role of cyclic nucleotides and Ca^{++} ions in activation of B lymphocytes by antiserum against immunoglobulins, with particular reference to induction of motility of these cells by serum, on the basis of the concept of the central role of cyclic nucleotides and calcium in processes of cellular proliferation and motility [8, 12].

EXPERIMENTAL METHOD

Experiments were carried out on 75 male C57BL/6 mice weighing 20-25 g. The mice were decapitated and the spleen homogenized in a Potter's homogenizer and the cell suspension filtered through nylon. Lymphocytes were obtained by the standard procedure of centrifugation in a Ficoll gradient [4]. To obtain a suspension of lymphocytes rich in B lymphocytes, spleen cells were treated with anti-Thy-serum and complement as described previously [7]. Anti-Thy-serum was obtained by immunizing rabbits with mouse brain homogenate [7]. Anti-immunoglobulin serum was obtained by immunizing rabbits with a normal mouse serum fraction salted out at 25-35% saturation with ammonium sulfate, using the scheme of immunization suggested for immunizing rabbits with serum proteins [3]. The serum thus obtained, after absorption by erythrocytes and thymocytes, reacted with 38-45% of spleen cells but did not react with thymocytes. The titer of the serum in the complement-dependent cytolysis test was 1:128. Activation of lymphocytes with antiserum and also with acetylcholine was carried out in accordance with the original method [13]. To study the cyclic nucleotide levels in B-lymphocytes under the influence of antiserum, B lymphocytes of intact animals were cultured in medium 199 for different times in the presence of antiserum in a dilution of 1:128; the reaction was stopped by addition of 5 volumes of 0.1 M HCl in alcohol; the cells were homogenized, protein denatured by heating for 5 min at 100°C, and precipitated by centrifugation, and the levels of cAMP and cGMP in the supernatant were determined after evaporation and dissolving of the residue in buffer by radioimmunoassay (using kits from "Amersham Corporation," England). To obtain the mitochondrial fraction B lymphocytes were homogenized and subjected to differential ultracentrifugation as described in [2]. The isolated mitochondria were tested for their ability to accumulate Ca^{++} ions added to the medium under conditions described for this process, using fluorescence of a tetracycline probe [6].

The viability of the cells was estimated at all stages on the basis of staining with 0.1% trypan blue.

The numerical results were subjected to statistical analysis by Student's t test.

*Academy of Medical Sciences of the USSR.

Research Institute of Immunology, Ministry of Health of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 12, pp. 692-694, December, 1984. Original article submitted February 7, 1984.

0007-4888/84/9812-1673$08.50

TABLE 1. Induction of Motility of Mouse B Lymphocytes by Anti-Immunoglobulin Serum and Acetylcholine under Different Conditions

Experimental conditions	Percentage of motile forms of cells
Medium 199	10±3,0
Physiological saline	8,5±2,5
0,1 M KCl, 0,04 M NaCl	7,0±2,0
Medium 199 + trifluoperazine (10^{-5} M)	6,0±2,0
Medium 199 + antiserum	49,0±3,0
Physiological saline + antiserum	45,5±3,5
0,1 M KCl, 0,04 M NaCl + antiserum	19,5±2,5
Medium 199 + antiserum + trifluoperazine	17,5±5,5
Medium 199 + acetylcholine	42,0±3,0
Physiological saline + acetylcholine	44,0±4,0
0,1 M KCl, 0,04 M NaCl + acetylcholine	39,0±4,0
Medium 199 + acetylcholine + trifluoperazine	15,0±2,0

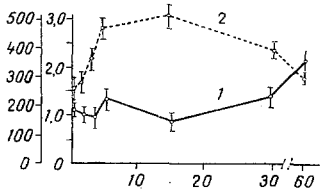

Fig. 1

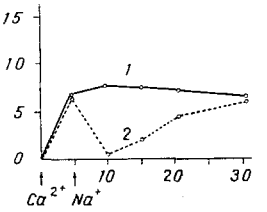

Fig. 2

Fig. 1. Effect of anti-immunoglobulin serum on cAMP (1) and c(GMP (2) levels in mouse B lymphocytes. Abscissa, duration of incubation (in min); ordinate: on left — cGMP level (in fmoles/10^8 cells, on right — cAMP level (in pmoles/10^6 cells).

Fig. 2. Effect of Na^+ ions on accumulation of Ca^{++} ions by mitochondria of mouse B lymphocytes. Abscissa, time (in min), ordinate, relative increase in fluorescence of tetracycline (in %). 1) Background accumulation, 2) accumulation in presence of Na^+ ions. Arrows indicate time of addition of Ca^{++} and Na^+.

EXPERIMENTAL RESULTS

Lymphocytes isolated from mouse spleens were able to increase their motility greatly through the action of both acetylcholine and antiserum against mouse immunoglobulins (Table 1). The effect was preserved in physiological saline in the absence of exogenous Ca^{++} ions, but in the case of antiserum it was abolished in medium containing 0.05 M NaCl and 0.1 M KCl. The effect of acetylcholine was preserved in sodium-free medium. The effect of both substances was abolished in the presence of trifluoperazine, a blocker of calmodulin.

The action of antiserum on B lymphocytes was accompanied by elevation of the cGMP level in the first 3-30 min of its action, whereas a considerable increase in the cAMP concentration was not observed until 1 h after addition of the serum (Fig. 1). Elevation of the cGMP level was preserved in calcium-free medium, but was abolished in medium with a low Na^+ ion concentration.

THe use of fluorescent tetracycline probe sensitive to accumulation of Ca^{++} ions in biological microvesicles showed that, under the conditions used, mitochondria of B lymphocytes could accumulate calcium, and this was accompanied by an increase in tetracycline fluorescence. Addition of Na^+ ions to the system led to release of calcium which had accumulated and was contained in the mitochondria, with recovery of tetracycline fluorescence after incubation for 30 min. No such effect was recorded when mitochondria from mouse thymocytes were used (Fig. 2).

Antiserum against mouse immunoglobulins thus increased the motility of mouse B lymphocytes only in the presence of Na^+ ions in the incubation medium, but calcium ions were not

necessary, although abolition of the effect by the calmodulin blocker trifluoperazine indicates that Ca^{++} ions do take part in this process. The ability of anti-immunoglobulin serum to depolarize B lymphotyces has been described in the literature [10]. Induction of inflow of Na^{+} ions into the B lymphocyte by antiserum outhgt to be accompanied, unlike with T lymphocytes, by outflow of Ca^{++} from the mitochondria, and this was demonstrated by the writers by the calcium-sensitive fluorescent tetracycline probe method. We know that B lymphocytes, but not T lymphocytes, can be activated by ionophores of monovalent metals, causing an inflow of Na^{+} ions into the cell [5]. It has also been shown that B lymphocytes are not required for activation by B mitogens and by antigen in the presence of Ca^{++} ions in the early stages of activation, although participation of Ca^{++} ions in the activation of cells of this type also is not disputed [9]. An increase in the cytoplasmic calcium concentration must be accompanied by an increase in the cGMP content of the B lymphocytes; in this case, moreover, the time course of the increase in cGMP concentration trails behind that of activation of motility of B lymphocytes by antiserum.

The probable mechanism of activation of B lymphocyte motility by antiserum against immunoglobulins is one of induction of the inflow of Na^{+} ions into the cell and stimulation of the outflow of Ca^{++} from the intracellular depot in the mitochondria, and this is accompanied by elevation of the cell cGMP level.

The mechanism of mobilization of intracellular calcium described above makes B lymphocytes independent of extracellular Ca^{++} in the processes of activation of B lymphocytes by immunogenic and other stimuli.

LITERATURE CITED

1. A. D. Ado. M. M. Gol'dshtein, and S. I. Dontsov, Byull. Éksp. Biol. Med., No. 4, 66 (1983).
2. H. Werner, in: Immunologic Methods [Russian translation], Moscow (1979), p. 234.
3. L. A. Zil'ber, in: Immunochemical Analysis [in Russian], Moscow (1968), p. 12.
4. A. Boyum, Scand. J. Clin. Lab. Invest., 21, 97 (1968).
5. L. Hammerstrom and E. Smith, Scand. J. Immunol., 5, 37 (1976).
6. J. M. Gimbe, M. Gustin, D. B. P. Goodman, et al., Biochim. Biophys. Acta, 685, 253 (1982).
7. E. G. Golub, Cell. Immunol., 2, 353 (1971).
8. L. Kurmichi, S. Z. Baranok, and H. Wakonisezy, Eur. J. Cell. Biol., 24, 327 (1980).
9. R. M. Lyall, J. H. Dubois, and M. J. Crumpton, Biochem. Soc. Trans., 8, 720 (1980).
10. J. G. Monroe and J. C. Cambier, Immunobiology, 163, 181 (1982).
11. D. Primi, G. K. Lewis, and J. W. Goodman, J. Immunol., 125, 1286 (1980).
12. H. Rasmussen and D. Goodman, Physiol. Rev., 57, 421 (1977).
13. G. F. Schreiner and E. R. Unanue, J. Immunol., 114, 802 (1975).