BINDING OF MONOCLONAL ANTIBODIES TO CYTOPLASMIC TETRODOTOXIN-SENSITIVE BRAIN PROTEIN WITH HUMAN LYMPHOCYTES

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Water-soluble cell proteins of the mammalian nervous system which, if inserted into an artificial lipid bilayer, can form tetrodotoxin-sensitive sodium channels, were first described in 1979 [5]. It is claimed that these proteins are precursors of voltage-dependent sodium channels, which play a highly important role in the functioning of excitable cells [12]. Recently a cytoplasmic tetrodotoxin-sensitive protein (CTSP), possessing these properties, has been isolated from bovine brain tissue and purified to a homogeneous state [6]. With the aid of monoclonal antibodies (McAb) obtained by immunization with purified CTSP [8], expression of the antigenic determinants of CTSP on membrane cells of varied origin has been studied. The aim of the present investigation was to discover whether determinants of CTSP are expressed on lymphocyte membranes, and if so, whether application of mitogenic (activating) stimuli to the cells affects ability to discover these determinants with the aid of McAb.

EXPERIMENTAL METHOD

Human peripheral blood lymphocytes (HPBL) were isolated by Böyum's method [1]. The HPBL were used as antigen either immediately after isolation or after incubation in medium RPMI-1640 ("Serva") with the addition of HEPES-Na (15 mM) buffer and 10% fetal calf serum ("Sigma"). The HPBL to be incubated were divided into two experimental groups. HPBL of the first group were incubated in the presence of concanavalin II (con A, "Serva," final concentration 6.5 μg/ml). HPBL incubated in the absence of con A served as the control to this group (control 1). HPBL of the second group were incubated in the presence of the supernatant of a two-way culture of mixed lymphocytes from unrelated donors in a final dilution of 1:4. HPBL incubated in the presence of supernatant of a lymphocyte culture from one of the donors served as the control to this group (control 2). The mixed lymphocyte culture (MLC) was set up by the method of Schwenke and Kerneder [9]. Incubation continued for 18 h at 37°C in a humid atmosphere containing 5% CO_2, in wells of 24-well cell culture panels ("Flow Laboratories"). The cell concentration was 2×10^6/ml. At the end of incubation the HPBL were washed 3 times with medium RPMI-1640. For washing the HPBL of the first group and of control 1, N-acetylgalactosamine (NAGA; from "Serva"), which can displace lectins bound with the cell membrane [6], was added to the medium. The HPBL were then absorbed on wells of microchambers (Leningrad Medical Polymers Factory) by Baumgarten's method [10], to be followed by ELISA, using the indirect peroxidase method in the modification of Pateraki et al. [13]. Immunoglobulin fractions of the supernatant of IFB-4 hybridoma (obtained by immunization with CTSP [8]), ascites fluid of hybridoma B2 (obtained by immunization with HPBL [2]) or blood serum from intact BALB/c mice, diluted in medium RPMI-1640, served as the source of the antibodies for ELISA. The immunoglobulin fraction was obtained by the standard procedure of salting out with ammonium sulfate at 50% saturation. Serial dilutions of the immunoglobulin fraction with an initial protein concentration of 1 mg/ml were used. The enzyme-linked immunosorbent was obtained from "Calbiochem" (1:1000). A "Morphoquant" instrument [3] was used for photometry. The results of ELISA were expressed in extinction units at 492 nm with a correction for specificity and were subjected to statistical analysis by Student's test for small samples [4].

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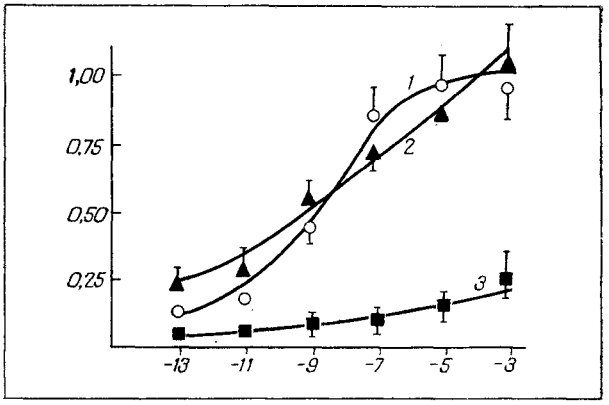

Fig. 1. Curves characterizing binding of McAb with HPBL. Abscissa, logarithm (to base 2) of dilution of sample of immunoglobulin fraction; 1) IFB-4, 2) B2, 3) immunoglobulins of intact mouse; ordinate, extinction at 492 nm (units) after deducting extinction with nonspecific binding [3, 10]. Results of one typical experiment (symbols indicate mean values, short vertical lines give error of mean values of extinction in three parallel tests).

TABLE 1. Effect of Treatment with Con A on Binding of IFB-4 McAb with HPBL

Group	NAGA concentration, mM		
	0,00	20,0	100,0
Control	0,39±0,10 (0,07÷0,71)	0,39±0,08 (0,13÷0,65)	0,41±0,10 (0,09÷0,73)
Treatment with conA	1,96±0,14* (1,51÷2,41)	1,49±0,32* (0,47÷2,51)	1,46±0,20* (0,82÷2,10)

Legend. Values of extinction at 492 nm (units) given here and in Table 2 are shown after deduction of extinction with nonspecific binding. Mean results of four experiments: mean values and their errors and 95% confidence intervals shown in parentheses. Asterisk indicates values differing significantly from control ($p < 0.05$).

TABLE 2. Effect of Treatment with MLC Supernatant on Binding of IFB-4 McAb with HPBL

Group	Day of lymphocyte culture		
	1st	2nd	3rd
Control	0,42±0,06 (0,23÷0,61)	0,40±0,03 (0,30÷0,50)	0,47±0,03 (0,37÷0,57)
Treatment with MLC supernatant	0,35±0,08 (0,09÷0,61)	0,89±0,09* (0,60÷1,18)	0,68±0,08 (0,42÷0,94)

EXPERIMENTAL RESULTS

The results of investigation of binding of immunoglobulins produced by hybridomas IFP-4 and B2 with freshly isolated HPBL are shown in Fig. 1. IFB-4 McAb exhibited dose-dependent binding with HPBL with an intensity not less to that of B2 McAb. Binding of IFB-4 McAb with HPBL treated with con A or MLC supernatant was carried out with a standard dilution of McAb, giving half-maximal binding with intact HPBL (1:200, Fig. 1). The experimental

results are given in Tables 1 and 2. As Table 1 shows, binding of IFB-4 McAb with HPBL incubated with con A was significantly stronger than binding in control 1. IF NAGA was added to the medium used for washing the cells, the difference with control 1 still remained. As will be clear from Table 2, binding of IFB-4 McAb with HPBL, treated with MLC supernatant, was identical with control 2 if the MLC supernatant was taken on the 1st or the 3rd day of culture of the allogeneic cells. If, however, the MLC supernatant was taken on the 2nd day of culture in MLC, binding of IFB-4 McAb was significantly stronger after treatment of the cells than binding in control 2.

The results are evidence that antigenic determinants identical with or very similar to fragments of the CTSP molecule in structure, are expressed on HPBL. Similar determinants were found previously on mammalian brain cell membranes but were not found on liver cell membranes [7]. Expression of such determinants by cell membranes is evidence in support of the kinship of CTSP with membrane sodium channels, for this fact may reflect the structural kinship of CTSP with a particular integral membrane protein. On the other hand, expression of such determinants by lymphocytes is not fully compatible with this hypothesis, for blood cells are not electrically excitable and proteins of voltage-dependent sodium channels have not been isolated from them [11]. The common nature of the antigenic determinants between CTSP and lymphocyte membranes may perhaps reflect the structural kinship of CTSP with other types of ion-selective membrane channels. It must also be noted that strengthening of binding of anti-CTSP antibodies with lymphocyte membranes after exposure of these cells to mitogenic stimuli of lectin and nonlectin nature may be evidence that the membrane protein with which these antibodies bind may play a definite role in mechanisms activating immunocompetent cells.

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