SPECTRUM OF REACTIVITY OF MONOCLONAL ANTIBODIES AGAINST SPECIFIC SUPPRESSOR T CELLS

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The use of monoclonal antibodies (MCAB) to characterize the various stages of differentiation of T lymphocytes has provided new opportunities for the description of individual functional subpopulations. In particular, markers of human and murine helper T cells — T4 and L3T4 respectively [6, 14], and human (T8) and murine (Lyt-2,3) killer/suppressor cells have been found [11, 15]. Meanwhile previous ideas on correspondence between differential antigens and functional populations have been revised: for example, antigen Lyt-1 has been found not only on helper T cells, but also on all mature peripheral T cells and on some B cells [10].

In this investigation reactivity of MCAB C1 and C4 relative to cells of different origin and to intracellular structures was studied. It was recalled that the name of "antisuppressor T cells" given to MCAB C1 and C4 is exceedingly functional and evidently reflects the particular features of expression by suppressor T cells of determinants which cannot be revealed on the surface of lymphocytes belonging to other T subclasses: killer cells, proliferating T cells, and producers of macrophage migration inhibition factor [4].

EXPERIMENTAL METHOD

Mice of lines BALB/c and CBA were obtained from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR. MCAB C1 ($\mu,\varkappa$) and C4 ($\mu,\varkappa$), both obtained by ourselves, were used; both were rat antibodies [2] against markers of specific suppressor T cells and MCAB F 9.9 of AKR mice against antigen Lyt-3.2 ($\mu, \varkappa$) [3]. MCAB HO134 and HO22 (against Thy-1.2 and Thy-1.1, respectively) [13] and MCAB against Lyt-1.1 and Lyt-2.1 also were used. Activity of the antibodies was assayed in the complement-dependent cytotoxicity test (CT) [3], using rabbit complement (from "Cedarlane," Canada), and in the immunofluorescence (IF) test in two modifications: either on slides with parafilm wells, covered with poly-L-lysine [5], or in suspension. In the first case the frequency of fluorescent cells was estimated under the microscope (Opton, West Germany), in the second case on a 50-H flow cytometer ("Ortho," USA) [3]. Rabbit antibodies against mouse immunoglobulins (IG) conjugated with fluorescein isothiocyanate (FITC; produced by the N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR), were used as "developing" antibodies in a two-stage test, whereas rabbit antibodies against rat IG absorbed beforehand by mouse IG, and goat antibodies against rabbit IG, conjugated with FITC, were used consecutively in a three-stage test.

T cells were isolated from lymph nodes and spleen by the method described previously [12]. B cells were purified by treatment with anti-Thy-1,2-serum (from "Searle," England). For this purpose $5 \cdot 10^7$ cells were incubated successively with 5 ml of antiserum in a dilution of 1:20 at room temperature for 30 min and with complement for 1 h at 37°C, followed by removal of the killed cells on a Ficoll–Hypaque gradient (1.09 g/cm^3). Thymus cells were fractionated into medullary and cortical by means of peanut agglutinin (PNA; from "Boehringer," West Germany) [3]. The purity of the resulting fractions was estimated by a fluorescence method, by treating the thymocytes with PNA, conjugated with FITC. The degree of purity was not less than 95%. Medullary thymocytes also were obtained by injecting 2.5 mg of hydrocortisone (HC) intraperitoneally into mice 48 h before the experiment. To study cytoplasmic antigens in the IF test

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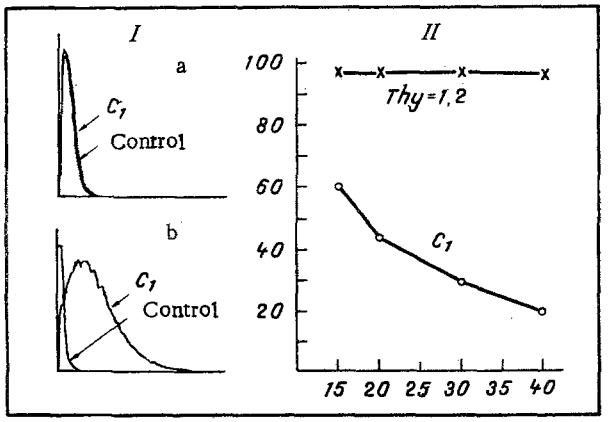

Fig. 1. Comparative characteristics of MCAB C1 and C4 in CT and IF tests. I) flow fluorometry of thymocytes (a) and thymoma BW5147 cells (b). In control — treatment with growth medium from myeloma strain. Abscissa, relative intensity of fluorescence ordinate, relative number of cells analyzed; II) CT for MCAB C1 and anti-Thy-1.2 on BALB/c thymocytes with dilution of complement. Abscissa, dilutions of rabbit complement; ordinate, cytotoxic index = $[(a - b)/a] \times 100\%$, where a stands for the fraction of living cells in the control and b for the fraction of living cells in the experiment.

the cells were treated with a solution of 1% Triton X-100 and fixed with formalin [1]. Vinblastine was added to a culture of quail embryonic fibroblasts (QEF) for 12 h in a concentration of 10 μg/ml.

EXPERIMENTAL RESULTS

Although both MCAB studied (C1 and C4) belong to the same class (IgM, κ), they differ in the character of their reaction with target cells (TC). MCAB C1 interact with cells of normal lymphoid organs in CT only, and not in the IF test. By means of flow cytometry, C1 antigen was found on thymona BW5147 cells, but not on thymus cells (Fig. 1, I). The fraction of $C1^+$-cells in the thymus depends on the dilution of complement in the CT test. It will be clear from Fig. 1, II that the fraction of thymocytes killed by MCAB with complement is reduced on dilution of the complement, whereas for MCAB against Thy-1.2 no such dependence was observed. These particular features of the reaction of C1 with TC can perhaps be explained by the low density of the corresponding antigenic determinants on the surface of normal cells and (or) the insufficient mobility of these determinants, which is essential for the recording of IF. MCAB C4, on the other hand, react with the surface of lymphoid cells in the IF test, but have no cytotoxic action, if judged by incorporation of the dye into killed cells. Since the anti-suppressor-T-cell action of MCAB C4 is exhibited only in the presence of complement [4], the possibility cannot be ruled out that this antigenic structure differs in its arrangement on the surface of suppressor T cells compared with other lymphoid cells.

C1 and C4 antigens were found in CT and IF tests respectively on T and B cells from the spleen and lymph nodes, and also on thymocytes. It is shown in Fig. 2a that MCAB C1 interact with 64-68% of intact or cortical (PNA^+) thymocytes and with a smaller fraction (half the size) of the population of medullary thymocytes. Although MCAB C4, like C1, react with different thymocyte populations, among the medullary thymocytes there were three times more $C4^+$-cells than among the cortical thymocytes (21 and 7%, respectively). This means that C1 is expressed mainly on immature thymocytes, and C4 on mature (medullary) thymocytes. The importance of the results given above is confirmed by the fact that interaction of MCAB against Thy-1.2, Lyt-1, Lyt-2, and Lyt-3 with thymocytes did not differ from that described in the literature [8]. It will be clear from Fig. 2b that C1 and C4 antigens are found on purified T and B cells from the spleen and lymph nodes; the fraction of C1- and C4-positive cells in the spleen, moreover, is twice as high among T than among B lymphocytes (about 40 and 20%, respectively), whereas in lymph nodes there was no significant difference (25 and 29%). The purity of separation of the T and B lymphocytes was confirmed by treating them with anti-Thy-1.2 MCAB in the CT test and with antibodies to mouse Ig, conjugated with FITC, in the IF test (Fig. 2b).

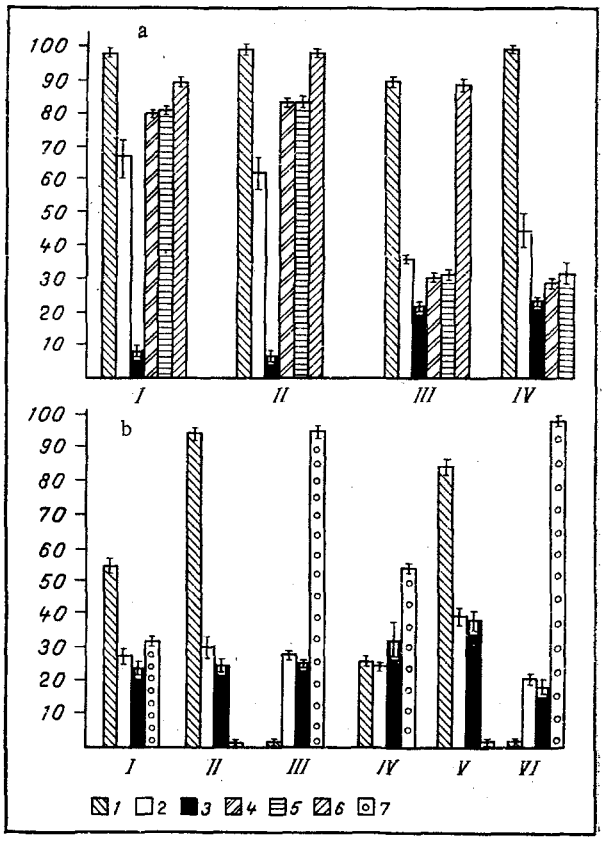

Fig. 2. Interaction of MCAB with thymocytes (a), and lymph node and spleen cells (b) of normal CBA mice. a) Thymocytes: intact (I), cortical PNA^+ (II), medullary PNA^- (III), HC-resistant (IV); b) peripheral lymphoid cells: intact (I), T (II), and B (III) from lymph nodes; intact (IV), T (V), and B (VI) from spleen. Ordinate, fraction of cells reacting with antibodies against Thy-1.2 (1), Lyt-1 (6), Lyt-2 (4), Lyt-3 (5), and C1 (2) in CT test, and against C4 (3) in IF test; t) mouse anti-Ig in IF test.

TABLE 1. Reactivity of MCAB Against Tumor Cell Lines Cultures In Vitro (Cytotoxic Index)

MCAB (whole culture fluids)	TC				
	P815	BW5147	BL4	RDM4	LBRM33
C1	—2.9	95.3	87.8	4.5	99
Thy-1.2	3.7	0	63.2	6	99.8
Thy-1.1	—1	98.8	—5.4	27.3	—5.4
Line of mice which were tumor donors	DBA/2	AKR	C57Bl/6	AKR	B10.BR

During the study of expression of C1 and C4 antigens on tumor cell lines cultured in vitro it was found that MCAB C1 react in the CT test with BW5147, EL4, and LBRM33 T lymphocytes, but not with RDM4 and P815 mastocytoma T lymphocytes (Table 1). MCAB against Thy-1.2 and Thy-1.1 were used parallel with C1 in the same experiments. The results (a selective reaction of each tumor with the corresponding anti-Thy-1-antibodies) indicate the specific character of the reaction with MCAB C1. Unlike C1, MCAB C4 did not react with the membrane of any of the lines tested. Since the same MCAB stain internal structures of evidently nonviable cells brightly, fixed thymoma cells, mouse fibroblasts, and QEF were stained. It was found that MCAB C4 react with elements of the cytoskeleton (Fig. 3a). After destruction of

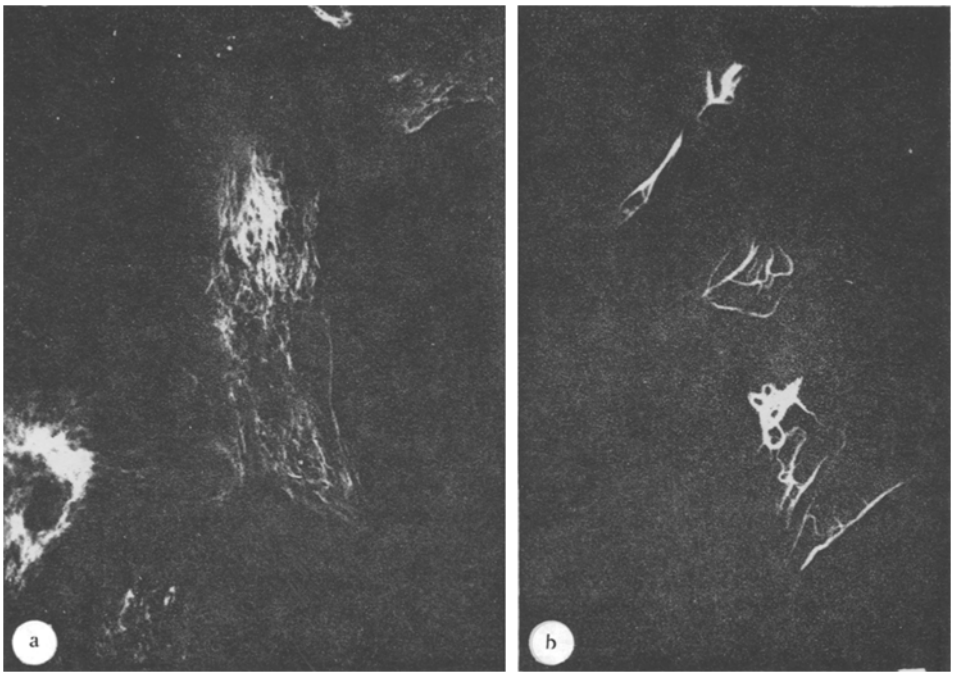

Fig. 3. Immunofluorescence of MCAB C4 on quail embryonic fibroblasts: a) fixation with formalin. Membrane destroyed by Triton X-100. Magnification: ocular 15, objective 40, immersion; b) The same after preliminary treatment of the cells with vinblastine.

the microtubules by vinblastine, the stained filaments were gathered into perinuclear rings (Fig. 3b), evidence that they belong to the class of intermediate filaments.

The distribution of antigens detected by MCAB C1 and C4 on cells of various lymphoid organs, on T and B cells, and on tumor cell lines in culture, was thus investigated. Since C1 reacts mainly with cortical and C4 with medullary thymocytes and since it is absent on the membrane of $C1^+$ tumor cells, it can be postulated that C1 is the marker of precursors of suppressor T cells, which is preserved on some functioning suppressor T cells, whereas C4 is the marker only of mature suppressor T cells, which may perhaps not be identical with $C1^+$ suppressor T cells. A similar situation was found during an investigation of polyclonal rat antisuppressor antibodies [2]. Some such antibodies, exhausted by BW5147 and EL4 cells, containing the C1 marker on their surface, stimulate suppressor T cell formation in vivo. Conversely, other monoclonal antibodies which, like C4, do not react with the membranes of the above-mentioned thymomas, inactivate the suppressor function in vivo.

The cross-reactivity of MCAB C4 with intermediate filaments, revealed by these experiments, is not the only case of this kind: MCAB against Thy-1.1 and membrane protein of human T-lymphoma interact with the same proteins [7, 9]. In all cases the reaction can be explained by the existence of common determinants on membrane proteins of lymphocytes and on intermediate filaments, or by expression of the actual proteins of the intermediate filaments on the membrane. It is a particularly interesting fact that this cross-reactivity of MCAB C4 extends to the surface of the suppressor T cells. The significance and functional importance of this fact will be topics for further study.

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STIMULATION OF THE IMMUNE RESPONSE IN CYPROHEPTADINE-INDUCED BLOCKADE OF SEROTONIN RECEPTORS

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The existence of several different types of serotonin receptors, involved in the realization of behavioral reactions, temperature regulation, and other functions of the body, is now known [8, 9, 11, 14].

When drugs affecting serotonin turnover and deposition are used, the serotoninergic system has an inhibitory effect on immunogenesis [5, 10]. There is no information in the literature about which receptors are responsible for this effect.

The aim of this investigation was to study the possible role of C_2-receptors in the modulating action of serotonin on immunogenesis and to study the mechanism of this action.

EXPERIMENTAL METHOD

Experiments were carried out on 178 male CBA mice weighing 20-25 g. The immune response was tested on the 5th day after immunization with sheep red blood cells (SRBC) in doses of $5 \cdot 10^6$ or $5 \cdot 10^8$ by counting the number of rosette-forming cells (RFC) [6]. Cyproheptadine (Cyp; from Serva, West Germany) was injected intraperitoneally in doses of 10, 20, and 30 mg/kg in distilled water once 30 min before immunization. 5-Hydroxytryptophan (5-HTP; Serva) in a dose of 300 mg/kg and haloperidol (from Gedeon Richter, Hungary) in a dose of 1 mg/kg were injected intraperitoneally in physiological saline twice a day for 2 days, the first injection being given 30 min before immunization. In the case of combined administration of 5-HTP and Cyp, and also Cyp and haloperidol, the interval between injections of the drugs was 5-10 min.

To study the role of the pituitary and thymus in the modulating action of Cyp on the immune response, the drug was injected into mice with a divided pituitary stalk or into thymectomized mice. Intact and thymectomized mice, and also mice with a divided pituitary stalk, receiving the same volume of physiological saline, served as the controls.

The pituitary stalk was divided in the mice under pentobarbital anesthesia through a transauricular route, and thymectomy was performed through a midline incision in the region of the sternum, by aspiration with a vacuum pump. The accuracy of destruction of the pituitary stalk and of removal of the thymus was estimated visually after decapitation of the animals.

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

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