ANTILYMPHOCYTIC ANTIBODIES IN CERTAIN HUMAN DISEASES AS A FACTOR IMPAIRING T-SUPPRESSOR FUNCTION

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UDC 616.153.962.4-097:616.155.32]-078.73

KEY WORDS: T-suppressors; lymphoid cells; complement-dependent lymphotoxic antibodies.

It was reported in [1, 3, 7] that the sera of patients with certain diseases contain antibodies with cytotoxicity against mouse thymocytes. The discovery of such antibodies is regarded as a specific factor in the pathogenesis of schizophrenia [1], Down's syndrome [3], systemic lupus erythematosus, and rheumatoid arthritis [7].

Meanwhile the appearance of antibodies in various diseases may perhaps be a general biological phenomenon reflecting the breakdown of regulatory mechanisms of homeostasis as a whole.

The aim of this investigation was to determine complement-dependent lymphotoxic antibodies in adults and children with various diseases and to study the trend of their effect on lymphoid cells and, in particular, on the T-suppressor population in animals and man.

METHODS

Altogether 157 sera from patients with various diseases, adults and children, were investigated. The control consisted of 49 sera from healthy children and adults.

The cytotoxicity of the patients' sera was studied against lymphoid cells of CBA mice and of a 25-26-day human fetus and peripheral blood lymphocytes from a healthy person, in the presence of fresh guinea pig complement [1, 3]. In each test no fewer than 200 nucleated cells, whose viability was assessed with the aid of a 0.2% aqueous solution of Trypan Blue, were mounted.

Total T-RFC were determined by the method of spontaneous rosette formation with sheep's red blood cells (SRBC) [5]. T-theophylline-sensitive and T-theophylline-resistant lymphocytes (T-TSL and T-TRL respectively) were determined by the method in [8]. T suppressors were induced in CBA mice by intravenous injection of a supraoptimal dose ($5 \cdot 10^9$ of SRBC [2], and the splenocytes of these animals, 10 days after immunization, were transplanted intravenously in a dose of $2 \cdot 10^7$ together with $2 \cdot 10^8$ SRBC into syngeneic recipients. After 4 days the number of antibody-forming cells (AFC) in the spleen of the recipient mice was determined by the method in [4] and their number calculated per 10^6 nucleated cells.

The effect of the patients' sera on the number of total T-RFC, T-TSL, and T-TRL of a healthy donor and on T-suppressor activity in mice was studied by treatment (37°C, 45 min) of the donor's lymphocytes or splenocytes of the mice with the patients' sera in a dilution of 1:20, with which, in the presence of complement (final concentration 1:10) 47.0 ± 3.5-87.0 ± 2.4% of thymocytes of CBA mice died.

RESULTS

Healthy human sera had no cytotoxic action either on thymocytes or on bone marrow cells of mice. Of the 63 patients' sera 38 had a cytotoxic action on thymocytes but not on bone marrow cells. Sera from patients with various diseases possessed cytotoxic properties (Table 1). This was most characteristic of the sera of patients with rheumatoid arthritis, which, as we know, is a disease associated with immune dysfunction.

Unlike thymocytes, lymphoid cells of the mouse spleen and lymph nodes were not subjected to any cytotoxic action of the patients' sera but were agglutinated by them. Absorption of

Department of Immunology, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR A. A. Smorodintsev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 101, No. 6, pp. 730-733, June, 1986. Original article submitted April 9, 1985.

0007-4888/86/0006-0821$12.50

TABLE 1. Cytotoxic Activity of Sera from Patients and Healthy Subjects Toward Mouse Thymus and Bone Marrow Cells

Diagnosis	Number of investigations	Cytotoxic index of sera (in %) tested in dilutions specified				
		with thymus cells				bone with marrow cells
		1:10	1:20	1:40	1:80	1:10
Rheumatic fever	9	12,2±1,3	5,8±0,9	0	0	0
(children)	8	38,0±1,8	30,9±2,3	13,4±1,0	—	0
Myocarditis, arrhythmic form (children)	2	58,1±2,5	45,2±2,5	—	—	0
Neurodermatitis (children)	2	59,5±2,4	45,2±2,5	—	—	0
Bronchial asthma (children)	12	16,5±1,9	4,8±0,6	—	—	0
	7	47,6±1,3	24,1±1,2	7,5±0,6	—	0
	6	75,4±3,9	65,8±3,0	26,6±2,5	0,9±0,8	0
	2	85,0±3,8	81,1±4,3	78,7±4,2	20,4±2,6	0
Rheumatoid arthritis (adults)	5	80,0±4,4	81,1±4,2	56,2±3,8	11,0±1,8	1,2±0,7
Wound infection (adults	2	10,9±1,6	0	—	—	0
	3	41,2±2,0	—	—	—	0
Obesity, II degree (adults)	2	17,5±1,9	—	—	—	0
	3	37,0±2,0	12,0±1,3	—	—	0
Healthy children	5	0	—	—	—	0
Number of invetigations	11	0	—	—	—	0
	5	9,1±1,8	0	—	—	0

Legend. Viability of lymphoid cells on treatment with normal serum (control) was 85-95%. —) Not tested.

sera from patients with bronchial asthma or rheumatoid arthritis twice by CBA mouse thymocytes lowered the cytotoxicity index for thymocytes from 74.8 ± 5.1 to 8.5 ± 0.7% and deprived the sera of their ability to agglutinate lymphoid cells of the spleen and lymph nodes.

A cytotoxic effect of the sera was observed only in the presence of complement, and it was not weakened after removal of antigen–antibody complexes: The cytotoxicity index of the sera of patients with rheumatoid arthritis was 67.7 ± 3.2% before elimination of the complexes from them, and 66.1 ± 3.3% after their removal.

To study the nature of the target cells of the cytotoxic antibodies, the suppressor activity of the splenocytes of immunized mice treated with the patients' sera was estimated. These cells, together with $2 \cdot 10^8$ SRBC, were transferred to syngeneic recipient mice, in which the number of IgM-AFC was determined.

The results showed that splenocytes from immunized mice reduced the number of AFC in the recipient mice by 4.5 times compared with transplanted intact splenocytes. Treatment of the splenocytes of immunized mice before transfer with sera possessing cytotoxicity toward mouse thymocytes led to a significant decrease in the suppressor effect of the immune splenocytes. Transfer of intact splenocytes, treated with normal human serum, increased by 1.7 times ($P < 0.001$) the number of AFC in the recipient mice compared with the number of AFC after transfer of intact splenocytes in medium 199 (Table 2).

The data described above show that antilymphocytic antibodies contained in sera from patients with various diseases act on the T-suppressor population of the mouse spleen. The effect of the antibodies was manifested as a significant weakening of suppressor activity of the T-lymphocytes on the thymus-dependent immune response.

To study the ability of antilymphocytic antibodies of human patients to act on human lymphocytes, the cytotoxic effect of the sera from children with bronchial asthma was studied on thymus, spleen, and lymph node cells from a human fetus at 25-26 weeks. In the human fetus, unlike in the mouse, it was found that not only lymph node and spleen cells, but also thymocytes were insensitive to the toxic action of the patients' sera. CI of the sera of patients with bronchial asthma for the above-mentioned fetal lymphoid cells was between 2.8 ± 1.2 and 13.1 ± 2.4% compared with between 45.7 ± 3.5 and 79.8 ± 2.8% for mouse thymocytes. However, human lymphoid cells were agglutinated by the sera. This activity depended directly on the value of CI for mouse thymocytes. Ability to agglutinate was abolished by absorption of the sera twice with mouse thymocytes.

TABLE 2. Effects of Patients' Sera on Activity of T-Suppressors in Adoptive Transfer Experiment (M ± m)

Donors of splenocytes	Preparation with which splenocytes were treated	Number of mice in experiment	Number of IgM-AFC in recipient mice per 10^6 splenic karyocytes
Intact mice	Medium 199	24	194,4±19,0
Immunized mice		22	37,2±3,6
Intact mice	Serum from healthy donors	6	345,0±29,2**
Immunized mice		15	76,3±10,4
Immunized mice	Serum of patients with:		
	bronchial asthma	34	112,9±5,3*
	wound infection	6	120,0±24,7
	rheumatoid arthritis	6	130,8±20,3*
	obestiy, II degree	12	180,8±15,7*

Legend. Number of AFC was determined individually in each mouse. Number of AFC per 10^6 splenic karyocytes in recipient mice immunized with $2 \cdot 10^8$ SRBC was 417.8 ± 17.0. *P < 0.05 compared with corresponding values after transfer of immune splenocytes treated with serum from healthy blood donors; †P < 0.01 compared with corresponding value for transfer of intact splenocytes in medium 199.

The fact that human lymphocytes are agglutinated by patients' sera is evidence that antibodies interact with lymphoid cells, as a result of which the number and function of the T lymphocytes may be reduced. Investigation of these parameters in 94 children with bronchial asthma showed a decrease in the number of total T-RFC from 51.3 ± 1.8% in the control (28 healthy children donors) to 46.8 ± 1.0% (P < 0.05). This decrease was on account of T-TSL (19.5 ± 1.1% compared with 33.7 ± 1.7% in healthy children, P < 0.01). Since the T-TSL population consists mainly of T-suppressors [8], it can be tentatively suggested that the decrease in the number of T-theophylline-sensitive cells takes place under the influence of antilymphocytic antibodies found in the sera of patients with bronchial asthma in large quantities (Table 1), and inhibiting T-suppressor function in mice (Table 2). To test this hypothesis, we studied the effect of sera from patients with bronchial asthma on the number of T-lymphocytes and of their separate subpopulations. Incubation of the donor's peripheral blood lymphocytes with sera active against mouse thymocytes (CI = 49.8 ± 2.8%), reduced the number of total T-RFC from 62.5 ± 3.4 to 44.0 ± 3.5% (P < 0.01). Under these circumstances the number of T-TSL was significantly (P < 0.01) reduced: 30.0 ± 3.2% after treatment of the cells with the patient's serum compared with 44.0 ± 3.5% after incubation of the lymphocytes with normal serum. Treatment of lymphocytes with the latter increased the number of T-RFC from 52.5 ± 2.5% (cells in medium 199) to 62.5 ± 2.4% (P < 0.01), which can probably be explained, just as in the case of stimulation of the immune response by normal serum in adoptive transfer (Table 2), by the presence of a thymic factor in the serum. Patients' sera not possessing cytotoxicity toward mouse thymocytes acted on human T-lymphocytes in the same way as normal serum.

The results are evidence that the appearance of antibodies against T-lymphocytes in various diseases is not pathognomonic for any particular disease, but is a general phenomenon reflecting the state of the regulatory mechanisms of homeostasis as a whole. This conclusion is confirmed by the discovery of antilymphocytic antibodies not only in a heterologous, but also in a homologous system in a wide spectrum of human diseases: in systemic lupus erythematosus, rheumatoid arthritis, rheumatic fever, scleroderma, infectious mononucleosis, and even in myositis and vaccination [9].

In the experiment with syngeneic transfer into mice the effect of antilymphocytic antibodies from patients with various diseases was found to be directed toward the T-suppressor population, functional activity of which was significantly depressed by the antibodies. Under the influence of sera from patients with bronchial asthma, functional activity of T-suppressors also was depressed in healthy human blood donors, as was shown by a decrease in T-TSL. This is in agreement with data in the literature on the antisuppressor trend of lymphocytic autoantibodies in systemic lupus erythematosus [6] and rheumatoid arthritis [10].

Lymphocytic autoantibodies, by inhibiting T-suppressors, may facilitate the development of a pathological process, and subsequently, by disturbing normalization of the number and function of T-suppressor cells, may lead to its chronic transformation.

LITERATURE CITED

1. M. E. Vartanyan, Vest. Akad. Med. Nauk SSSR, No. 1 51 (1974).
2. A. G. Kalinkovich, B. V. Pinegin, and E. L. Luganskaya, Immunologiya, No. 3, 38 (1983).
3. E. I. Shvarts, "Metabolic basis of immunologic disturbances in cells with trisomy for chromosome 21," Author's Abstract of Dissertation for the Degree of Doctor of Medical Sciences [in Russian], Leningrad (1981).
4. N. K. Jerne and A. A. Nordin, Science, 140, 405 (1963).
5. M. Jondall, G. Holm, and H. Wigzell, J. Exp. Med., 136, 207 (1972).
6. O. Kunio and T. Kiyoaki, Int. Arch. Allergy, 71, 346 (1983).
7. M. T. Lavastida and J. C. Daniels, Fed. Proc., 37, 1669 (1978).
8. S. Limatibul, A. Shore, H. M. Dosch, and E. W. Gelfand, Clin. Exp. Immunol., 33, 503 (1978).
9. P. I. Terasaki, V. D. Mottironi, and E. V. Barnett, New Engl. J. Med., 283, 724 (1970).
10. T. Sakane, S. Takada, Y. Murakawa, et al., J. Immunol., 129, 1972 (1982).

PREPARATION AND PROPERTIES OF MONOCLONAL ANTIBODIES TO INDIVIDUAL PREKERATINS OF SIMPLE RAT EPITHELIUM

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UDC 616-018.7-008.939.6-092.9-07

KEY WORDS: hybridomas; intermediate filaments; prekeratins.

Intermediate filaments (IF) of epithelial cells consist of special proteins called prekeratins (PK). By now 19 PK have been identified by biochemical analysis of human tissues [11]. All are more or less homologous with one another and they evidently constitute an evolutionarily related group of proteins. This same group also includes proteins from IF of other types of cells: vimentin, desmin, and proteins of neurofilaments and glial filaments [5, 8].

Mainly biochemical methods have been used to study the distribution of individual PK in various kinds of cells, but these do not enable tissues with complex cellular composition to be investigated or fine heterogeneities of population to be detected. Nevertheless, even now it can be concluded that the IF of different types of epithelium are composed of different sets of PK. However, this unique multiplicity of the PK and the biological significance of specific sets relative to different types of epithelium await explanation. The complete pattern of distribution of individual PK in epithelium of adult animals has not been obtained. The principles governing the formation of this distribution in ontogeny have not been studied. The solution of these problems requires antibodies specifically recognizing individual PK. It is difficult to obtain such antibodies because of the close degree of affinity between members of the PK family. Polyclonal sera obtained by immunization with individual PK often give cross reactions with other proteins of this family [4, 9]. The preparation of monoclonal antibodies reacting with only one polypeptide also has proved to be a difficult task. Only a few such clones have been described in the liaterature [6, 10, 12].

The aim of this investigation was to obtain and study the properties of a series of hybridoma clones producing antibodies to individual PK from simple types of epithelium.

METHODS

Monoclonal antibodies were obtained by the method in [7] with minor modifications [1, 2]. BALB/c mice were immunized with a preparation of IF isolated from the mucosa of the rat large

Laboratory of Mechanisms of Carcinogenesis and Carcinogenic Substances, Research Institute of Carcinogenesis, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Kraevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 101, No. 6, pp. 733-736, June, 1986. Original article submitted February 11, 1985.

 0007-4888/86/0006-0824$12.50