ANTILYMPHOCYTIC ANTIBODIES IN VARIOUS HUMAN DISEASES AS A FACTOR DEPRESSING SUPPRESSOR T CELL FUNCTION

G. A. Belokrylov and A. P. Suvorovtseva

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Several workers [1, 3, 7] have reported the presence of antibodies possessing cytotoxicity relative to mouse thymocytes in sera from patients with various diseases. The discovery of such antibodies is considered to be 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 different diseases may perhaps be a general biological phenomenon reflecting disturbance of the regulatory mechanisms of homeostasis as a whole.

The aim of this investigation was to determine complement-fixing lymphotoxic antibodies in adults and children with various diseases and to study their effect on the suppressor T-cell population in animals and man.

EXPERIMENTAL METHOD

Tests were carried out on 157 sera from adults and children with various diseases. The control consisted of 49 sera from healthy children and adults.

The cytotoxicity of the patients' sera was investigated on lymphoid cells of CBA mice, a 25-26-week-old fetus, and peripheral blood lymphocytes of an adult person in the presence of fresh guinea pig complement (1:3). In each test no fewer than 200 nucleated cells were counted: Their viability was estimated by means of a 0.2% aqueous solution of trypan blue. The total number of rosette-forming T cells (T-RFC) was determined by the spontaneous rosette formation method with sheep's red blood cells (SRBC) [5]. Theophylline-sensitive and theophylline-resistant T lymphocytes (T-TSL and T-TRL respectively) were determined by the method in [8]. Suppressor T cells were induced in CBA mice by intravenous injection of a supraoptimal dose ($5 \cdot 10^9$) of SRBC [2]; the splenocytes of these mice, 10 days after immunization in a dose

TABLE 1. Cytotoxic Activity of Sera from Patients and Healthy Subjects against Mouse Thymus and Bone Narrow Cells (M ± m)

Group tested	Diagnosis	Number of persons tested	CTI (in %) of sera tested in undermentioned dilutions with				
			thymus cells				bone marrow cells
			1:10	1:20	1:40	1:80	1:10
Children	Rheumatic fever	9	12,2±1,3	5,8±0,9	0	0	0
		8	38,0±1,8	30,9±2,3	13,4±1,0	—	0
	Myocarditis	2	58,1±2,5	45,2±2,5	—	—	0
	Neurodermatitis	2	59,5±2,4	45,2±2,5	—	—	0
	Bronchial asthma	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
Adults	Rheumatoid arthritis	5	80,0±4,4	81,1±4,2	56,2±3,8	11,0±1,8	1,2±0,7
	Wound infection	2	10,9±1,6	0	—	—	0
		3	41,2±2,0	—	—	—	0
	Obesity of degree II	2	17,5±1,9	—	—	—	0
		3	37,0±2,0	12,0±1,3	—	—	0
Children	Healthy subjects	5	0	—	—	—	0
Adults	Healthy subjects	11	0	—	—	—	0
		5	9,1±1,8	0	—	—	0

Note. Viability of lymphoid cells with normal serum (control) was 85-95%; minus sign) no test was undertaken.

Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad.
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TABLE 2. Effect of Patients' Sera on Suppressor T-Cell Activity in Adoptive Transfer Experiment

Donor of splenocytes	Preparation with which splenocytes were treated	Number of recipient mice	Number of IgM-AFC per 10^6 karyocytes in recipient's spleen
Intact mice	Medium 199	24	194,4 ± 19,0
Immune mice		22	37,2 ± 3,6
Intact mice	Healthy human serum	6	345,0 ± 29,2
Immune mice		15	76,3 ± 10,4
Immune mice	Sera from patients with bronchial asthma	34	112,9 ± 5,3*
	wound infection	6	120,0 ± 24,7
	rheumatoid arthritis	6	130,8 ± 20,3*
	obesity of the II degree	12	180,8 ± 15,7*

Note. $P < 0.05$ compared with control (transfer of immune splenocytes treated with healthy human serum).

of $2 \cdot 10^7$ together with $2 \cdot 10^8$ SRBC, were injected intravenously into syngeneic recipients. The number of antibody-forming cells (AFC) in the spleen of the recipient mice was determined 4 days later by the method in [4]. Each mouse was tested individually. The effect of the patients' sera on the total number of RFC and on the number of T-TSL and T-TRL of a healthy adult blood door and on suppressor T-cell activity in mice was estimated by treating the donor's lymphocytes or the mouse splenocytes with the patients' sera at 37°C for 45 min in a dilution of 1:20, at which, in the presence of complement (final concentration 1:10), between 47 ± 3.5 and 87 ± 2.4% of thymocytes of CBA mice died. Antigen–antibody complexes were removed from the sera by precipitation with CO_2.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that healthy human sera had no cytotoxic action either on the thymocytes or on the bone marrow cells of the mice. Of 63 patients' sera 38 were cytotoxic against thymocytes, but not against bone marrow. This property was most characteristic of the sera from patients with rheumatoid arthritis, i.e., a disease connected with immune dysfunction. Unlike thymocytes, the lymphoid cells of the mouse spleen and lymph nodes were not subjected to the cytotoxic action of the patients' sera, but were agglutinated by them. Ability of sera from patients with bronchial asthma and rheumatoid arthritis to agglutinate was abolished by absorption twice with mouse thymocytes. Under these circumstances the cytotoxicity index (CTI) of the sera for thymocytes fell from 74.8 ± 5.5 to 8.5 ± 0.7%. The cytotoxic effect of the sera was observed only in the presence of complement and was not abolished after removal of the antigen–antibody complexes: 67.7 ± 3.2% before elimination of the complexes in sera from patients with rheumatoid arthritis and 66.1 ± 3.3% after their removal.

Suppressor activity of immune splenocytes treated with patients' sera, cytotoxic for thymocytes, revealed a significant weakening of the suppressor effect of the splenocytes in a syngeneic transfer experiment (Table 2).

In the human fetus, unlike in mice, not only cells of the lymph nodes and spleen, but also thymocytes were insensitive to the toxic action of the patients' sera. Values of CTI for sera from bronchial asthma patients for fetal cells mentioned above fluctuated from 2.8 ± 1.2 to 13.1 ± 2.4%, and for mouse thymocytes from 45.7 ± 3.5 to 79.8 ± 2.8%. However, human lymphoid cells, like the mouse cells, were agglutinated by the sera. This activity was found to be directly dependent on the value of CTI for mouse thymocytes, and it was completely abolished by absorption with these cells twice.

Investigation of the number and function of the T lymphocytes in 94 children with bronchial asthma revealed a decrease in the total number of RFC from 51.3 ± 1.8% in the control (28 healthy children) to 46.8 ± 1.0% ($P < 0.05$). This decrease took place on account of T-TSL (19.5 ± 1.1% compared with 33.7 ± 1.7% in healthy children, $P < 0.01$). Incubation of peripheral blood lymphocytes from a healthy donor with sera active for mouse thymocytes (CTI = 79.8 ± 2.8%) reduced the total number of RFC from 62.5 ± 3.4 to 44.0 ± 3.5% ($P < 0.01$).

Under these circumstances the number of T-TSL, which were mainly suppressor T cells [8] was reduced: 30 ± 3.2% after treatment of the cells with patients' serum and 44 ± 3.5% on incubation of lymphocytes with normal serum.

The results are evidence that the effect of antilymphocytic antibodies obtained from patients with various diseases is aimed at the suppressor T-cell population, whose functional activity is significantly depressed under the influence of antibodies, in agreement with the data in the literature on the antisuppressor orientation of autolymphocytic antibodies in systematic lupus erythematosus [6] and rheumatoid arthritis [10].

The appearance of antibodies against T lymphocytes in various diseases, described by the present writers and others [9], is not pathognomonic for a particular disease but is a general phenomenon, reflecting the state of the regulatory mechanisms of homeostasis as a whole.

LITERATURE CITED

1. M. E. Vartanyan, Vestn. 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 bases of immunologic disturbances in cells with trisomy for chromosome 21," Author's Abstract of Dissertation for the Degree of Doctor of Medical Sciences, 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. Shora, 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).

ROLE OF G_0- AND G_1-SPLENOCYTES AND OF ANTIGEN-BINDING LYMPHOCYTES PRODUCING ANTIGEN-DEPENDENT NONSPECIFIC IMMUNOLOBULINS

M. G. Agadzhanyan, I. N. Smirnova, and E. V. Sidorova

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Induction of the specific immune response and the appearance of antibody-forming cells (AFC) are accompanied both *in vivo* and *in vitro* by an increase in the number of nonspecific immunoglobulin-forming cells (NIGFC) [4, 11]. In particular, we do not know whether antigen-dependent NIGFC (adNIGFC) are formed from resting precursor cells (G_0 cells) or from immunoglobulin-producing cells already present in the body (IGFC). These "background" IGFC in the mouse spleen account for usually 0.1-0.2% of the total number of cells. Most of them are B cells, in the G_1 phase of the cell cycle, i.e., they are blast cells. In addition, the writers showed previously that antigen-binding cells (ABC) participate in the formation of adNIGFC [1]. To elucidate the role of "background" IGFC in the formation of adNIGFC, in the investigation described below NIGFC formation was studied in a suspension of normal splenocytes and of splenocytes from which G_1 cells had been removed. It was also interesting to determine how removal of ABC affects the ability of splenocytes, after exhaustion of all G_1 cells, to form adNIGFC.

Laboratory of Development of Methods of Isolation, Purification, and Concentration of Biologically Active Substances, Moscow Scientific-Research Institute of Virus Preparations, Ministry of Health of the USSR.

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