LITERATURE CITED

1. V. I. Gavrilov and R. S. Dreizin, Vopr. Virusol., No. 1, 43 (1966).
2. B. S. Naroditskii, E. V. Karamov, T. I. Felitsina, et al., Dokl. Akad. Nauk SSSR, 237, 228 (1977).
3. B. S. Naroditskii, B. A. Zavizion, E. V. Karamov, et al., Vopr. Virusol., No. 1, 36 (1978).
4. L. J. Bello and H. S. Ginzberg, J. Virol., 3, 106 (1969).
5. F. L. Graham and A. J. Van der Eb, Virology, 54, 536 (1973).
6. M. Green and M. Pina, Virology, 20, 199 (1963).
7. R. N. Hull, I. S. Johnson, C. G. Culbertson, et al., Science, 150, 1044 (1965).
8. R. M. McAllister, J. L. Riggs, C. Reed, et al., Proc. Soc. Exp. Biol. (New York), 131, 1442 (1969).

ROLE OF T- AND B-LYMPHOCYTES IN HETEROGENEITY OF CELL-MEDIATED REACTIONS TO BACTERIAL ANTIGENS IN MAN

D. K. Novikov and V. I. Novikova

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Blood leukocytes from 30 patients with allergy to tuberculin and bacterial antigens were treated with antithymus (ATS) or anti-immunoglobulin serum (AIGS), after which the leukocyte migration inhibition (LMI) test was carried out with these antigens. ATS abolished LMI by tuberculin and sometimes by bacterial antigens (staphylococcal, streptococcal, etc.). AIGS frequently abolished LMI caused by bacterial antigens but not by tuberculin. In other cases treatment with any serum abolished LMI by antigens or, conversely, it was abolished only by treatment with both antisera in turn. The type of lymphocytes (T or B) determining the reaction to the same antigen in the secondary immune response differed in different patients and also differed in the same patient for different antigens. Five types of interaction between lymphocytes and antigen in the LMI test were distinguished.

KEY WORDS: T- and B-lymphocytes; cell-mediated reaction; antithymus and anti-immunoglobulin serum; immune response to bacterial antigens; hypersensitivity of delayed type (HDT).

To induce an immune response to thymus-dependent antigen, interaction between two types of lymphocytes and macrophages is necessary [7]. The role of these cells in hypersensitivity of delayed type (HDT) reactions, developing in patients, is less clear. Stimulation of human and animal lymphocytes by an antigen to which they exhibit HDT causes the formation of biologically active substances (mediators) in vitro, especially a factor inhibiting migration (MIF) of macrophages and polymorphs [1, 2, 9]. By abolishing the function of T- or B-lymphocytes by treatment with specific antisera and by analyzing MIF formation attempts were made to discover which lymphocytes react, and by means of which receptors, with bacterial antigens and tuberculin during the secondary immune response in vitro.

EXPERIMENTAL MATERIAL AND METHOD

To block receptors of T-lymphocytes or to eliminate these cells from the suspension of leukocytes it was treated with monospecific rabbit antiserum against human thymus (ATS). The antiserum was obtained by the method in [4] after immunization of rabbits (two or three cycles) with thymus cells from healthy fetuses, which had died as a result of complications during labor. The rabbits were immunized with thymocytes (50-100 million per injection, 50-80% of the cells were viable), treated with rabbit antiserum against human serum proteins, and 1-2 days later they received an intravenous injection of 1-2 ml of the same antiserum in order to suppress synthesis of antispecific antibodies and so to increase the specificity of the ATS. The antisera

Department of Morphology and Immunology, Central Research Laboratory, Vitebsk Medical Institute.

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TABLE 1. Assessment of Specificity of ATS and AIGS

Method	Treatment of lymphocyte suspension		
	untreated	ATS (1:4)	AIGS (1:10)
Stimulation of MIF synthesis[1]			
By phytohemagglutinin (10 μg/ml)	4±0,2	0	4±0,3
By AIGS (1:200)	2±0,1	2±0,2	1±0,4
Rosette formation[2]			
RFC	66±4,5	8±6,2	70±8,4
EAC-RFC	14±5,1	13±3,1	6±4,2
Immunofluorescence[3]			
Indirect method E-RFC	2±1,9	84±5,7	3±2,4
RFC with mouse erythrocytes	0,6±1,2	1±1,4	64±9,5
Direct method Treatment with AIGS labeled with FITC	21±6,3	22±4,4	2±4,8
Cytotoxic test[4]			
Blood T-lymphocytes[5]	2±0,6	88±6,5	—
Thymus cells	2±0,6	87±1,8	—
Bone marrow cells	3±0,9	5±2,4	—
Blood B-lymphocytes[5]	3±0,5	4±1,7	—

[1] $\log_2$ of titer of supernatant from culture of stimulated lymphocytes causing inhibition of leukocyte migration.
[2] Percentages of RFC: E-RFC) with sheep's erythrocytes, EAC-RFC) with pigeon's erythrocytes coated with C_3-complement.
[3] Percentage of fluorescent rosette-forming lymphocytes.
[4] Percentage of dying cells.
[5] Isolation of lymphocytes by sedimentation of RFC.

were absorbed with fetal erythrocytes and bone marrow cells and with human blood leukocytes after removal of T-lymphocytes [5]. The specificity of the antisera was verified by several methods (Table 1).

Leukocytes were isolated from 8-15 ml of blood from 30 children and adults with pneumonia or recovering from suppurative infections and allergic to bacterial antigens, as shown by intradermal tests (carried out after blood sampling), and they were suspended (5-10 million cells/ml) in medium No. 199 [4]. This suspension was divided into four parts. One part was treated with ATS (1:8) and guinea pig complement, a second with a mixture of antisera against human immunoglobulins of classes G, M, and A (from the N. F. Gamaleya Institute of Epidemiology and Microbiology, batch 2, dilution 1:6-1:10), the third part was untreated or was incubated with complement alone, the fourth with ATS alone. Incubation continued for 30 min at 37°C with the antisera and 20 min with complement. The cells were washed twice with medium No. 199, suspended in the same medium with 20% inactivated bovine serum, and divided into portions corresponding to the number of antigens to be tested. Bacterial allergens, obtained from Kazan Research Institute of Microbiology, were added to the leukocyte suspension in a final dilution of 0.04 skin dose/ml, old tuberculin in a dilution of 1:800, and BCG vaccine in a dose of 0.05 mg/ml. These doses of antigens did not affect migration of unsensitized leukocytes. Glass capillary tubes with one end closed were filled with the leukocyte suspensions, with and without antigens, and the leukocyte migration inhibition (LMI) test was performed [3]. After incubation for 24 h at 37°C the capillary tubes were removed from the wells in the plates, the number of migrating cells was counted, and LMI was calculated as a percentage [3].

EXPERIMENTAL RESULTS AND DISCUSSION

Migration of blood leukocytes obtained from patients and not treated with antisera was inhibited by one or, more frequently, by several antigens. LMI due to synthesis of MIF could be abolished after treatment of the leukocytes with ATS or with anti-immunoglobulin serum (AIGS). The ATS usually abolished LMI due to tuberculin and BCG vaccine, and only in two subjects was their effect abolished by AIGS. Abolition of LMI by

TABLE 2. Types of Interaction of Patients' Lymphocytes with Antigens in LMI Test

Antigens tested	No. of patients with LMI	Abolition of LMI after treatment with				Change in reactivity of leukocytes, type V	
		ATS type I	AIGS type II	ATS + AIGS type III	ATS + AIGS type IV	ATS	AIGS
Tuberculin	23	16	2	4	2	3	—
Hemolytic staphylococcus	7	1	1	1	4	2	4
Hemolytic streptococcus	13	4	4	0	5	3	0
Pneumococcus (group)	14	4	4	3	3	2	0
Proteus mirabilis	7	2	1	3	1	3	4

Legend. Altogether 30 patients were tested; I-V) type of reaction of patients' lymphocytes to bacterial antigens and tuberculin.

ATS indicated that the reaction depended on the presence of intact T-lymphocytes, whereas the same effect of AIGS proved that lymphocytes with immunoglobulin receptors (type B) also were essential for it. ATS could abolish LMI even without the addition of guinea pig complement. Possibly its thermolabile components were synthesized by leukocytes.

LMI by bacterial antigens was abolished in 11 cases only by treatment with ATS, in 10 cases by AIGS, and in 13 cases by either of these antisera (Table 2). This fact indicated that interaction between T- and B-lymphocytes is necessary for MIF formation. In seven cases only successive treatment with both antisera abolished LMI (type III), which proves that MIF may be formed in these patients by both T- and B-lymphocytes. In another seven cases treatment of the leukocytes with ATS made them capable of reacting to tuberculin and bacterial antigens by inhibition, and in six cases, by stimulation of migration (type V; Table 2). Probably in these cases ATS abolished the suppressive action of T-cells on MIF synthesis by B-lymphocytes. AIGS likewise could modify the reactivity of the leukocytes.

Although the type of lymphocytes reacting to antigen depended to some degree on the type of antigen, this factor was not always decisive. Usually T-lymphocytes reacted only to tuberculin. LMI by streptococcal allergen depended in four of 13 cases on the presence of intact immunoglobulin-carrying lymphocytes; the reaction to staphylococcal allergen in four of seven cases required interaction between T- and B-lymphocytes; the reaction to pneumococcal allergen was determined equally often by T- and B-lymphocytes. Retesting of the patients 2 weeks to 3 months later showed that the type of cells which responded to the antigen remained constant. If ATS abolished LMI by tuberculin, after 1-3 months the same result was observed in the 11 subjects tested. Similar results were obtained when the reaction of leukocytes from five patients to streptococcal allergen was abolished by AIGS, and also in three cases when it was abolished by treatment with either antiserum.

Lymphocytes of patients with agammaglobulinemia, but with no defects of cellular immunity can form MIF, which indicates that T-lymphocytes are essential for its synthesis [11, 13]. Experiments with purified T- and B-lymphocytes gave inconsistent results. Both T- and B-lymphocytes obtained by fractionation on columns [7, 12] or by isolation of rosette-forming cells (RFC) [10] always formed MIF in response to stimulation by antigens. However, there is evidence [9, 14] that only T-RFC produce this factor in response to stimulation by tuberculin PPD also, but with different antigens. The possibility cannot be ruled out that other leukocytes may have the same property. The present results indicate that although both T- and B-lymphocytes are able to form MIF, the type of cells which determines the HDT reaction in vitro to the same antigen often differs in different patients and also in the same patient to different antigens.

Inhibition of migration of human lymphocytes by tuberculin and bacterial antigens was the result of interaction of different lymphocyte populations in different patients with them. The lymphocyte population in a given patient which reacts to a concrete antigen is evidently determined genetically [6, 5]. We distinguished several types of reaction of lymphocytes to antigens in the LMI test (Table 2): 1) T-lymphocytes react to the antigen. This is observed in most patients with allergy to tuberculin and in some with allergy to bacterial antigens. The reaction is abolished by ATS but not by AIGS. For that reason the Ig-receptors, if they exist on

T-lymphocytes also [6], do not determine their reaction with antigen; 2) lymphocytes with Ig-receptors (type B) react to the antigen. LMI is abolished by AIGS but not by ATS; 3) both lymphocyte populations react to the antigen. LMI is abolished by treatment with both antisera only; 4) interaction between T- and B-lymphocytes is essential for the reaction to antigen. Either antiserum abolishes LMI; 5) one lymphocyte population modifies or inhibits the response of the other population to the antigen. Treatment with one antiserum "induces" or modifies (suppression–stimulation) the response to antigen. Similar variants of interaction between lymphocytes and antigens in the secondary immune response also are observed, evidently, when other cellular tests in vitro and intradermal tests are used. The results indicate the heterogeneity of cell-mediated reactions in man to bacterial antigens, as a result of differences in the role of T- and B-lymphocytes, for which immunogenetic mechanisms are evidently responsible.

LITERATURE CITED

1. M. M. Averbakh (editor), Mediators of Cellular Immunity [in Russian], Moscow (1976).
2. A. A. Mikhailova and R. V. Petrov, in: General Problems in Pathology [in Russian], Vol. 5, Moscow (1977), p. 7.
3. D. K. Novikov, G. P. Adamenko, and V. I. Novikova, Byull. Éksp. Biol. Med., No. 6, 707 (1976).
4. D. K. Novikov and V. I. Novikova, in: Inventions and Efficiency Suggestions in Medicine [in Russian], Leningrad (1975), p. 59.
5. R. V. Petrov, Vestn. Akad. Med. Nauk SSSR, No. 1, 41 (1974).
6. R. V. Petrov, Immunology and Immunogenetics [in Russian], Moscow (1976).
7. L. Chess et al., J. Immunol., 115, 315 (1975).
8. D. S. Dumonde et al., Nature, 224, 38 (1969).
9. P. J. Fimmel, J. Immunol., 115, 135 (1975).
10. B. H. Littman, J. R. David, and R. E. Rocklin, Cell. Immunol., 24, 241 (1976).
11. R. E. Rocklin et al., New Engl. J. Med., 282, 1340 (1970).
12. R. E. Rocklin et al., J. Exp. Med., 140, 1303 (1974).
13. H. Valdimarsson et al., Lancet, 1, 1259 (1970).
14. J. Wybran et al., New Engl. J. Med., 288, 710 (1973).