Deaggregated preparations of human globulin thus have reduced ability to interact with complement *in vitro*. Tolerogenic properties of the deaggregated immunoglobulin preparation correlate with their inability to interact with complement, and this criterion can be used for selection and preliminary testing of tolerogenic preparations.

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VARIABILITY IN SENSITIVITY OF HUMAN LYMPHOCYTES TO THE ANTIPROLIFERATIVE ACTION OF ALKYLATING AGENTS

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The urgency of the study of pharmacogenetic aspects of the action of antitumor and immunodepressive agents has increased considerably in recent years. The fate of a drug in the body depends on many parameters — its absorption, binding with proteins, accumulation in the tissues, biotransformation, transport through membranes, elimination, and interaction with target cells (binding with receptors or other mechanisms). Each of these parameters may be the basis for genetic variability [8]. Interaction between drug and target cell largely determines the observed therapeutic effect of the drug.

Grounds for the present investigation were data obtained previously showing differences in the sensitivity of immunocompetent target cells in mice of different genotypes to the immunodepressive action of cyclophosphamide (CP) [4]. Individual sensitivity of human peripheral blood lymphocytes (PBL) to the antiproliferative action of CP and thiophosphamide (thiotepa), widely used antitumor and immunodepressive agents, was studied. These preparations were chosen because they belong to classes of alkylating agents with different structure and different mechanisms of pharmacological action (CP, unlike thiotepa, requires metabolic activation in the body before it can exert its action [6]).

EXPERIMENTAL METHOD

The Soviet preparation of CP (cyclophosphan, from Saransk Medical Preparations Factory) and thiotepa (from the S. Ordzhonikidze All-Union Pharmaceutical Chemical Research Institute) were used as antiproliferative agents. Serum from male BALB/cISto mice, receiving an intra-

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TABLE 1. Inhibition of Proliferative Response of PBL to Phytohemagglutinin by CP Activated *In Vivo*

Donor	Type of sensitivity	Percentage inhibition of proliferative response with different concentrations of "active" serum			Criterion of uniformity U(ρ^2)
		0,2	0,4	0,6	
A.V	Very low	0,98	2,00	36,18	0
S.T.	Low	18,20	46,93	59,14	5,90064
L.L.		19,68	31,13	45,60	
T.G.		22,10	45,87	60,99	
D.A.		28,61	38,42	80,12	
D.S.		29,13	41,19	63,59	
S.S.		35,02	44,61	32,93	
Yu.V		36,41	43,11	83,74	
A.N.		37,90	32,03	41,11	
Yu.P.	Average	44,63	65,20	75,42	6,03674
V.P.		49,72	56,38	95,10	
S.Yu.		51,13	68,29	70,83	
V.G.		55,50	68,81	79,42	
P.P.		59,43	64,11	69,89	
S.P.	High	69,36	76,90	80,53	5,99496
A.A.		69,60	77,61	80,33	
L.S.		84,70	90,60	93,20	

Legend. Concentration of active serum given in optical density units/ml of incubation mixture. Average values of five measurements given here and in Table 2.

TABLE 2. Inhibition of Proliferative Response to PBL to PHA by Thiophosphamide

Donor	Type of sensitivity	Percentage inhibition of proliferative response with different concentrations of thiotepa			Criterion of uniformity U(ρ^2)
		25 μg/ml	50 μg/ml	100 μg/ml	
S.T.	Low	9,30	45,40	30,70	5,27037
L.L.		14,31	41,18	43,26	
D.S.		21,81	28,86	47,11	
Yu.V.		22,72	47,03	45,57	
Yu.P.	Average	44,63	65,20	75,42	4,21027
T.G.		32,41	35,92	30,35	
A.V.		41,31	55,11	57,18	
S.Yu.		43,33	52,78	51,63	
S.S.		43,90	28,80	27,72	
P.P.		59,43	64,11	69,89	
D.A.	Intermediate	52,40	66,70	70,61	3,0
A.N.		57,21	54,72	67,10	
V.G.	High	61,10	72,21	77,27	6,22932
S.P.		61,78	73,48	82,20	
V.P.		63,52	71,36	86,02	
A.A.		74,91	77,30	78,12	
	Very high	88,96	86,10	89,21	0

peritoneal injection of CP in a dose of 300 mg/kg 30 min before sacrifice ("active" serum) [5], was used as the source of active metabolites of CP. The concentration of active alkylating metabolites of CP in the serum (in optical density units/ml) was determined by the NBP test [7], in the modification described in [3]. The serum was kept at −70°C for not more than 3 days [9].

Lymphocytes were isolated from heparinized peripheral blood of healthy donors of both sexes in a Ficoll—Verografin density gradient. The lymphocytes were then washed twice and resuspended in medium 199. The washed lymphocytes were then incubated in a dose of 2 million cells with various concentrations of "active" serum or thiotepa (solution in medium 199) at 37°C for 1 h. The volume of the incubation mixture was 1 ml. In the control, the cells were incubated with normal mouse serum (in the case of CP) or in medium 199 (in the case of thiotepa). Preliminary experiments showed that the preparations, in the concentrations used, had no lymphocytotoxic action (as shown by staining with trypan blue). At the end of incubation the cells were washed three times with cold medium 199 and then cultured in round-bottomed 96-well plastic plates (Leningrad Medical Polymers Factory) at 37°C in an atmosphere of 5% CO_2 for 72 h. The cells were cultured in medium 199 with the addition of human group AB (IV) blood serum. Each sample, 200 µl in volume, contained $3 \cdot 10^5$ lymphocytes, together with phytohemagglutinin P (from Difco, USA) in a concentration of 2.5 µg/ml culture mixture as the mitogen. Each experimental and control group consisted of five analogous samples. The intensity of proliferation was estimated from incorporation of radioactive label ([^{3}H]thymidine, 1 µCi/ml), which was added 20 h before the end of culture. The results were expressed in percentages of inhibition of proliferation compared with the control.

Statistical analysis of the experimental results included analysis of the initial population in order to divide it into homogeneous groups according to the method in [1]. The data were grouped according to the concentration of the agent at which greatest variability of the results was observed. The criterion of uniformity U (ρ^2) was compared with the critical value of χ^2_3, 0.95 = 7.815 at a level of significance $\alpha = 0.05$ and with three degrees of freedom.

EXPERIMENTAL RESULTS

Table 1 gives data on the sensitivity of PBL of different individuals (17 persons) to the action of "active" serum (CP). Depending on the degree of uniformity of the parameters obtained, four types of sensitivity of the donors' lymphocytes could be distinguished: very low, low, average, and high.

Similar results were obtained when the antiproliferative action of thiotepa was studied (Table 2). In this case it was possible to distinguish five types: low, average, intermediate between average and high, high, and very high. Comparison with the data in Table 1 shows that on the whole the type of sensitivity remained the same, although there were certain deviations.

Confirmation of the equality of sensitivity to the action of CP and thiotepa was given by grouping the values obtained for both parameters together (Table 3).

It must be pointed out that the belonging to a particular type of sensitivity is a stable characteristic. In a repeat control experiment, in which the sensitivity of the lymphocytes of donor A.V. ("very low" type of sensitivity to CP and "average" to thiotepa) and of donor L.S. ("high" type to CP and "very high" to thiotepa) was investigated simultaneously, the types of sensitivity to which the cells belonged remained the same.

The variability of the sensitivity of human lymphocytes to the action of alkylating agents thus revealed did not correlate with the index of stimulation (the ratio between the level of proliferation of lymphocytes after mitogenic stimulation and the level of spontaneous proliferation in control cultures).

The results do correlate with previous experimental data [2] showing the immunodepressive action of CP and thiotepa *in vivo* in mice of different lines. Mice of lines DBA/2 ISto and C3H/SnSto were more sensitive to the action of both CP and thiotepa than mice of line BALB/cISto.

Studies of the immunodepressive action of CP and thiotepa on mice *in vivo* and of their antiproliferative activity on human lymphocytes *in vitro* thus showed that the degree of sensitivity remains the same irrespective of the type of alkylating agent used.

A detailed analysis of the possible causes of individual differences in the sensitivity of lymphocytes to the action of CP and thiotepa will be a task for future research.

TABLE 3. Distribution of Individuals by Types of Sensitivity to the Antiproliferative Action of CP and Thiotepa (grouping of data from Tables 1 and 2)

Donor	Type of sensitivity	Criterion of uniformity $U(\rho^2)$
A.V.	Very low	0
S.T. L.L. T.G. D.A. D.S. S.S. Yu.V. A.N.	Low	10,68760
Yu.P. V.P. S.Yu. V.G. P.P.	Average	7,41634
S.P. A.A. L.S.	High	11,08960

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