COMPLEMENT CONSUMPTION AS A TEST OF TOLERANCE OF GLOBULIN PREPARATIONS

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Complement is known to play an essential role in induction of the immune response. In complement deficiency the immune response develops more weakly [4, 10]. Meanwhile aggregated antigens, with increased ability to activate complement, have increased immunogenicity [12]. However, the role of complement in the development of the immune response has on the whole been studied insufficiently [1]. In particular, its role in the induction of tolerance and realization of the tolerogenic function of an antigen is not clear [3, 8].

The aim of the present investigation was to discover the connection between ability of an antigen to activate complement and its immunogenic and tolerogenic properties.

EXPERIMENTAL METHOD

Commercial anti-influenzal human immunoglobulin, obtained from donated blood and standardized with respect to protein, was used as the antigenic and tolerogenic preparations.

To endow the immunoglobulin with tolerogenic properties it was ultracentrifuged for 3 h on a Beckman L2-65B centrifuge (ti = 50 rotor, 150,000g, 47,000 rpm). The upper third of the supernatant was used as the tolerogen. The ability of the preparation to adsorb complement was estimated relative to 50% hemolysis ($C'H_{50}$) [2] or to 100% hemolysis ($C'H_{100}$) by a modified method [5, 9, 11].

The tolerogenicity of the preparation was estimated by tests on 296 (C57BL/CBA)F_1 mice weighing 16-18 g, obtained from the "Rappolovo" Nursery. The animals were given an intravenous injection of 0.5 mg of the test immunoglobulin (tolerogen). Seven days later they were given an intraperitoneal injection of 4 mg human γ-globulin in Freund's adjuvant (test injection). Circulating antibodies were detected by Boyden's passive hemagglutination test (PHT) on the 20th and 40th days after immunization with antigen and adjuvant.

EXPERIMENTAL RESULTS

Direct testing of the ability of the preparations to adsorb complement showed that this ability was reduced in the ultracentrifuged preparation but enhanced in the aggregated preparation compared with that of the native preparation. For instance, equal doses of each preparation (0.5 mg/ml protein) adsorbed the following quantity of complement: ultracentrifuged — 1.2 $C'H_{50}$ units, aggregated — 5 units, native — 3.9 units. A similar relationship was observed when the anticomplementary activity of other doses of immunoglobulin was determined.

The results of experiments on mice into which the different batches of human globulins, either ultracentrifuged or native, were injected are summarized in Table 1. All mice except the controls were given an injection of 0.5 mg of the preparation. The animals were grouped together depending on the level of anticomplementary activity of the administered globulin. As Table 1 shows, injection of globulin with minimal anticomplementary activity led to the creation of tolerance, manifested as inability of the mice to respond to injection of antigen and Freund's adjuvant, or (some animals) the production of a minimal quantity of antibodies. As the level of anticomplementary activity of the injected globulin increased, it produced ever-higher antibody titers.

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TABLE 1. Relations between Anticomplementary and Tolerogenic Activity of Human Immunoglobulin Preparations

Anticomplementary activity, $C'H_{100}$ units	Number of animals in group	Number of animals producing antibodies	Antibody titer in PHA (M ± m)
3,5	32	32	280,0±57,5
3	54	54	83,3±4,5
2,5	20	20	77,5±2,7
2	12	12	66,7±7,3
1,5	12	12	34,2±6,7
1,5	50	5	1,7±0,8
Control	56	56	591±67,0

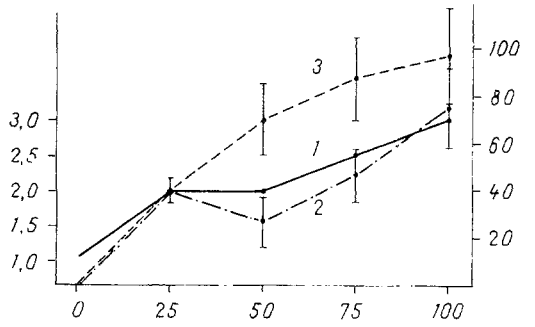

Fig. Anticomplementary and tolerogenic activity of mixtures of ultracentrifuged and native globulin. Abscissa, content of native globulin, %; ordinate: on left — complement consumption (in $C'H_{100}$ units), on right — antibody titers in PHA. 1) Level of anticomplementary activity; 2) antibody titer on 20th day after test injection; 3) antibody titer on 40th day after test injection.

To determine the anticomplementary activity of the preparation more accurately, mixtures of ultracentrifuged and native globulin, with the preparations in different proportions, were tested.

The results showed that the anticomplementary activity of the mixtures increased with an increase in the content of native immunoglobulin (Fig. 1). There was a corresponding increase in the intensity of the animals' immune response. Only the ultracentrifuged preparation, which had no anticomplementary activity, led to the development of a reactivity to subsequent immunization. Addition of 25, 50, and 75% of native globulin to the ultracentrifuged preparation reduced its tolerogenic activity in proportion to the quantity of preparation added and to the anticomplementary activity of the resulting mixture.

It can be concluded from the facts described above that the ability of immunoglobulin preparations to adsorb complement reflects their ability to induce immunologic tolerance and an immune response. The inability of deaggregated globulin to induce an immune response and to interact with the complement system is evidently an interconnected process, for an important role in the induction of the immune response is played by receptors for complement, which are possessed by several cells concerned in the formation of the immune response [6, 7].

The present investigation did not yield any direct evidence that tolerogenic properties of globulin are connected with inability to adsorb complement. These two properties probably coincide. However, it is clear that inability to adsorb complement may serve as a marker of its tolerogenicity. We observed that in cases when, because of technical errors, ultracentrifugation did not lead to elimination of aggregates and, consequently, to ability to adsorb complement, this preparation had no tolerogenic action.

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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