LOWERING THE ANTICOMPLEMENTARY ACTIVITY AND INDUCTION OF TOLEROGENICITY OF HUMAN GLOBULIN BY UREA TREATMENT

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The presence of aggregates in globulin preparations is the result of their partial denaturation during isolation and keeping; on the one hand, they make these preparations unfit for intravenous injection, and, on the other hand, they enhance their immunogenic properties.

By ultracentrifugation of the globulins the aggregates can be removed, the preparation deprived of its immunogenicity, and tolerogenicity induced. However, the deaggregated preparation obtained in this way is unstable, and this limits the possibility of its use excessively.

Preparations with a reduced content of aggregates can be obtained by gentle methods of preparative immunochemistry and also by enzymic and combined acid and enzymic hydrolysis of the globulins [3, 6]. Such preparations have many advantages, but even they are not completely and stably deaggregated.

Urea is known to increase the solubility of proteins and to affect the conformation of their molecule, and it is used to dissociate immune complexes [1, 8, 11]. It has been suggested that, under certain conditions, the level of aggregation can be reduced by addition of urea.

The aim of this investigation was to discover whether human globulin can be deaggregated by treatment with urea solutions and to determine some properties of the preparation thus obtained.

The degree of deaggregation of globulin preparations can be judged from loss of their ability to fix complement, and in this way the immunogenicity and tolerogenicity of the globulin can be estimated [4].

EXPERIMENTAL METHOD

The properties of the official preparation of human anti-influenzal globulin (from the Pasteur Scientific-Research Institute of Epidemiology and Microbiology, Leningrad) were studied. Solutions of urea (from Reakhim, Czechoslavakia) of varied molarity, made up in 0.85% NaCl, were used [5].

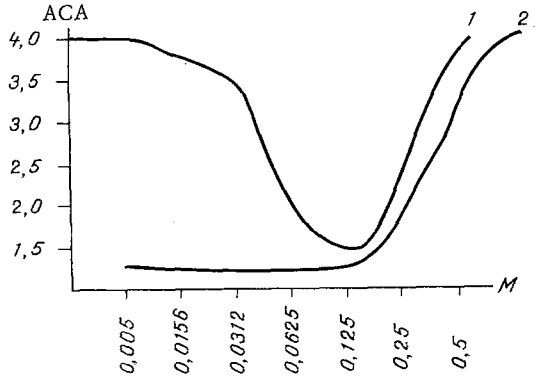

Fig. 1. Dependence of anticomplementary activity (ACA) of globulin on molarity of urea. 1) Immunoglobulin treated with urea; 2) urea solution.

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TABLE 1. Reduction of Complement Fixation after Treatment of Different Concentrations of Globulin Preparation with 0.12 M Urea

		Protein concentration in treated preparation, mg/ml						
Complement consumption of 0.5 mg of test preparation	Control (treatment with physiological saline)	145	75	30	20	10	2,0	1,0
	3,95±0,08	3,5±0,02	2,5±0,02	2,5±0,01	2,2±0,02	1,5±0,16	1,5±0,01	1,5±0,02

To estimate the degree of aggregation of the globulin, its ability to fix complement equivalent to 50% [2] or 100% hemolysis was determined by a modified method [7, 9, 10].

To determine the tolerogenicity of the preparations, experiments were carried out on (B57BR/CBA)F_1 mice weighing 16-18 g (from the Rappolovo nursery, Academy of Medical Sciences of the USSR). The test immunoglobulin was injected intravenously into the animals in a dose of 0.5 mg. After 7 days a test injection of 4 mg of native immunoglobulin in Freund's adjuvant was given intraperitoneally. Antibodies were detected by Boyden's passive hemagglutination test on the 20th and 40th days after the test injection.

To obtain a control tolerogenic preparation the immunoglobulin was subjected to ultracentrifugation on a Beckman L2-65B centrifuge, with ti-50 rotor, at 150,000g and 47,000 rpm for 3 h. The top third of the supernatant was used as tolerogen.

EXPERIMENTAL RESULTS

In the experiments of series I the ability of the immunoglobulin to fix complement was determined after treatment with different doses of urea, using different incubation times and concentrations of globulin, and the stability of the effect was evaluated.

Different portions of globulin (1 mg/ml) were treated with urea solutions of different concentration, and the degree of lowering of their ability to fix complement was determined.

Urea was shown to have a deaggregating action in concentrations starting with 0.0156 M and the maximal deaggregating effect was observed with concentrations of 0.0625-0.125 M. With higher urea concentrations (>1 M) no deaggregation could be detected, evidently due to the effect of urea on the test system (Fig. 1).

The optimal urea concentration was thus shown to be 0.12 M, and this was used in the subsequent experiments.

Treatment of different quantities (from 1 to 10 mg/ml) of globulin with 0.12 M urea caused the preparation to lose its ability to adsorb complement (within the limit of sensitivity of the method) when used in concentrations of up to 10 mg/ml (Table 1).

It will be clear from Table 1 that treatment with 0.12 M urea had a complete effect with protein concentrations up to 10 mg/ml, but with doses of between 20 and 75 mg/ml anticomplementary activity was only partially reduced.

In a separate experiment the incubation time with urea required to produce deaggregation of globulin was determined. Deaggregation was found to take place after 3 h, and not to increase until the 7th day of incubation. The deaggregation effect was quite stable.

TABLE 2. Tolerogenic Action of Urea-Treated Immunoglobulin

Immunoglobulin used to prime animals	Expt. No.	Response to test injection (average antibody titer, M ± m)	P
Urea-treated	1	2,8±0,03	<0,01
	2	0,05±0,01	<0,01
Ultracentrifuged	1	2,3±0,03	<0,01
	2	3,5±0,17	<0,01
Intact (control)	1	5,5±0,3	—
	2	5,5±0,2	—

The writers showed previously that ability to fix complement correlates with the tolerogenic activity of the preparation [4].

In the experiments of series II the tolerogenicity of urea-treated globulin was evaluated. Table 2 gives the results of an investigation of the tolerogenic properties of the immunoglobulins. The test preparation was injected into mice, and 2 weeks later they were immunized with globulin in Freund's adjuvant. Priming the animals with urea-treated immunoglobulin, just as with ultracentrifuged immunoglobulin, was found not to give a stimulating effect, but to cause significant lowering of the level of response to the immunogenic preparation.

This result indicates induction of tolerance in the primed animal.

Treatment of immunoglobulin with urea solution thus causes its deaggregation, the occurrence of which can be judged from inability to fix complement, and a stable preparation with tolerogenic properties can be created.

LITERATURE CITED

1. E. Ruoslahti, Immunoadsorbents in Protein Purification, ed. by E. Ruoslahti [Russian translation], Moscow (1979), pp. 11-17.
2. I. A. Tarkhanova, in: Immunochemical Analysis, ed. by L. A. Zil'ber [in Russian], Moscow (1968), pp. 79-98.
3. T. G. Shemerovskaya, V. V. Nemov, I. A. Kiseleva, and B. N. Sofronov, Immunologiya, No. 4, 60 (1982).
4. T. G. Shemerovskaya and B. N. Sofronov, Byull. Éksp. Biol. Med., No. 5, 71 (1983).
5. T. G. Shemerovskaya, B. N. Sofronov, A. B. Zhebrun, et al., Immunologiya, No. 6, 60 (1985).
6. L. Frommhagen and H. Fudenberg, J. Immunol., 89, 336 (1962).
7. K. Hoffken, F. Bosse, U. Stein, and C. Schmidt, J. Immunol. Methods, 53, 51 (1982).
8. J. Malgras et al., Rev. Franc. Transf., 13, 173 (1970).
9. J. Romer, A. Gardi, and P. Kistler, Test Method for the Quality Control of Plasma Proteins, Basel (1979), pp. 147-151.
10. H. Pinko, J. Lindgren, and E. Ruoslahti, Immunochemistry, 10, 381 (1973).