DIMINISHED ANAPHYLACTOGENIC PROPERTIES OF DEAGGREGATED γ-GLOBULIN

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The comparative immunologic activity of native, aggregated, and deaggregated (tolerogenic) human γ-globulin was studied in experiments on mice and guinea pigs. Diminished activity of the deaggregated preparation was found in reactions of local and general hypersensitivity in sensitized animals, together with diminished ability to stimulate the secondary humoral immune response.

KEY WORDS: *deaggregated γ-globulin; immunogenicity; immunologic tolerance.*

Investigations have shown [1, 4-6, 9] that serum globulins, after ultracentrifugation, lose their immunogenic properties (completely or partially) and, if injected into animals, can induce a state of immunologic tolerance. The same properties can be conferred on proteins by passage through a normal animal or by treatment with macrophages *in vitro* [2, 3, 7, 8].

Diminished immunogenicity of deaggregated protein may be associated either with its inability to stimulate the early (nonspecific) stages of the immune response and, in particular, to be ingested by macrophages, as Frei et al. [7] suggest, or with diminished ability to react with the specific structures of the cells and immunoglobulins.

The study of this problem could help to discover the specific activity of a deaggregated protein in a sensitized recipient.

The object of this investigation was to compare the immunologic activity of native, aggregated, and deaggregated human globulin. Their ability to give rise to reactions of local hypersensitivity and of general anaphylaxis in sensitized animals and their ability to stimulate the secondary immune response were assessed.

EXPERIMENTAL METHODS

Experiments were carried out on CBA or noninbred mice weighing 16-18 g and on noninbred guinea pigs weighing 200-250 g. Commercial human γ-globulin (HGG), containing 10% protein in 1 ml, was used as the antigen. In some experiments γ-globulin additionally purified on Sephadex-DEAE A-50 was used. To obtain deaggregated HGG (tolerogen) the commercial preparation was ultracentrifuged for 3 h on a Beckman L2-65B centrifuge, with the ti-50 rotor, at 150,000g. Aggregated HGG was prepared by heating to 63°C for 30 min. The immune response of the animals was assessed by Boyden's hemagglutination test.

EXPERIMENTAL RESULTS

The tolerogenic properties of ultracentrifuged γ-globulin were tested in preliminary experiments. Intravenous injection of 0.5-2.5 mg of the preparation was found to cause a reactivity of the animal to injection of 2 mg HGG in Freund's complete adjuvant 7 days later. Smaller doses (0.1 and 0.02 mg) gave a partial effect: The level of antibodies was reduced by 75-80% compared with the control.

To discover the ability of the test preparations to stimulate antibody formation, they were injected as a single dose into mice 1 month after primary immunization with native HGG. The original antibody level at this time was 1:768±277 (Table 1). Injection of deaggregated antigen in a dose of 0.5 mg, inducing tolerance in native mice, led to some increase in anti-

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TABLE 1. Effect of Tolerogen on Secondary Immune Response to HGG (M±m)

Immunization	Mean antibody titer 1 month after immunization	Reimmunization	Mean antibody titer 7 days after immunization
2 mg native HGG in Freund's adjuvant	1:768±227	Deaggregated HGG	1:2176±995
		Native HGG	1:17066±3413
		Physiological saline	1:672±249

TABLE 2. Local Allergic Reaction in Sensitized Guinea Pigs (M±m)

Experiment	Native globulin	Aggregated globulin	Deaggregated globulin
1	7,2±2,4	6,8±3.0	0
2	22,2±1,0	22,7±1,6	13,5±2.0
3	29,5±1,6	24,9±1,7	18,8±1.2

Note. Mean diameter of local reaction in sensitized guinea pigs given in millimeters.

body formation (not statistically significant; $P > 0.1$). Meanwhile, the same dose of native globulin has a much more marked effect ($P < 0.01$).

The ability of deaggregated and aggregated HGG to induce a local anaphylactic reaction was studied by intradermal injection of the corresponding preparations into guinea pigs sensitized by native HGG. Table 2 shows the results of three experiments in which the reaction of animals with different degrees of sensitization was tested. Clearly the tolerogenic preparation possessed diminished allergic activity, whereas the native and aggregated proteins induced marked local reactions, not significantly different from each other.

Investigations of the ability of the deaggregated preparations to induce a general anaphylactic reaction were carried out on adenalectomized mice, which are much more sensitive to the anaphylactic reaction than intact animals. The mice were sensitized 3 times with native HGG: 7, 17, and 3 mg. The interval between the 1st and 2nd injections was 3 days, and between the 2nd and 3rd injections 3 weeks. The adrenals were removed 19 days after the last injection of HGG into the mice, and 2 days later aggregated or deaggregated globulin was injected intravenously. After injection of 4 mg aggregated HGG, 6 of the 8 sensitized animals died from anaphylactic shock, whereas after injection of the same dose of ultracentrifuged HGG into the same number of sensitized mice none of the animals died and no signs of anaphylaxis were observed ($P < 0.05$). In the unsensitized control animals aggregated globulin caused no disturbances, even in twice the dose.

The results confirm the well established fact that deaggregated tolerogenic protein induces tolerance in the native organism and has no tolerogenic action in the immune recipient. Meanwhile the activity of this antigen in the sensitized organism was significantly reduced. However, investigations of the ability of deaggregated protein to bind with antibodies *in vitro* showed that this antigen was indistinguishable from the native form [1]. Consequently, it can be concluded that either the deaggregated preparation binds less readily with antibodies *in vivo* than the native preparation, or that the immune complex formed with the participation of the tolerogen *in vivo* differs in biological activity from the complex formed by the native antigen.

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DEATH OF ADHERENT SPLEEN CELLS (MACROPHAGES) *in vitro* IN HYPERSENSITIVITY OF DELAYED TYPE TO MICROBIAL ANTIGENS

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The cytotoxic action of immune lymphocytes on adherent spleen cells obtained from unsensitized guinea pigs or guinea pigs sensitized with BCG was studied in autologous and allogeneic systems. The low cytotoxic effect found during culture of a suspension of spleen cells of sensitized guinea pigs with tuberculin was greatly increased after the addition of lymph node cells obtained from the same animal. Determination of death of adherent spleen cells, as also of adherent lymph node cells, can be used as a sensitive method for the detection of hypersensitivity of delayed type. The use of spleen cells as target cells is more convenient, for there are many more adherent cells in the spleen than in a suspension of lymph node cells.

KEY WORDS: *hypersensitivity of delayed type; cytotoxic action; immune lymphocytes; adherent cells.*

One of the tests for the detection of hypersensitivity of delayed type (HDP) *in vitro* is the cytotoxic action of immune lymphocytes or their supernatants (lymphotoxins) on target cells of different origins [4-7]. A previous investigation showed that lymph node cells (LNC) obtained from guinea pigs with HDT to streptococcal antigens or to tuberculin, in the presence of the specific antigen, caused death of many peritoneal exudate macrophages in monolayer culture [2]. A high percentage mortality of adherent cells (AC; macrophages) of lymph nodes also has been established in an autologous system during culture of LNC from animals sensitized by a streptococcal culture of BCG, in the presence of the specific antigen [3]. The sensitivity and specificity of this test are such that it can be used to determine HDT in experimental animals. However, the number of AC in a suspension of LNC is relatively small and if the time elapsing after sensitization is long, their number falls to 10-15 per field of vision. Meanwhile in the spleen, besides lymphocytes many AC are found.

The object of this investigation was to study whether AC of the spleen can be used as target cells for the determination of the cytotoxic effect, and also to study the cytotoxic activity of splenic lymphocytes in animals with HDT to microbial antigens.

EXPERIMENTAL METHODS

Noninbred guinea pigs were sensitized in the footpads with Freund's complete adjuvant (600 μg BCG per guinea pig). Tuberculin (from the Leningrad Institute of Vaccines and Sera) was used as the antigen in a dose of 25 μg/ml culture medium (medium No. 199 with the addition of 20% bovine serum, inactivated by heating at 56°C for 30 min, and also with 50 units penicillin and 50 μg streptomycin to 1 ml medium). The source of AC and lymphocytes was a suspension of spleen cells (SC) and LNC (inguinal, popliteal, femoral, and subclavian), taken from animals sensitized with BCG and unsensitized (control) animals. Cells obtained from each animal were washed separately in a large volume of medium No. 199 and, after centrifugation, the cell residue was resuspended in the culture medium. The suspension of LNC, after vital staining was adjusted to a concentration of $20 \cdot 10^6$ living lymphocytes/ml and divided

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