ANAPHYLACTOGENICITY OF SERUM GLOBULINS CONJUGATED WITH DEXTRANS

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An essential obstacle to the widespread therapeutic and prophylactic use of heterologous immune sera is their allergenicity, which often leads to the development of immunopathological disturbances in patients, the most threatening of which is anaphylactic shock. Traditionally, the reactogenicity of immune antitoxic sera is reduced by treating them by the "Diaferm" method [1]. However, this method cannot be used to reduce the allergenicity of antimicrobial, antiviral, and antilymphocytic sera, because of the important role of the immunoglobulin Fc-fragment in the mechanism of the protective function of antibodies. It is thus an urgent task to develop ways of reducing the allergenicity and reactogenicity of heterologous immunoglobulins without subjecting them to enzymic degradation. According to data in the literature, modification of proteins by polymers can lead to a decrease in their antigenicity [12] and allergenicity [6] and can significantly improve their pharmacological properties [8, 11, 14].

The aim of the present investigation was to study the possibility of reducing anaphylactogenicity of immunoglobulins by means of dextrans.

EXPERIMENTAL METHOD

Experiments were carried out on 238 guinea pigs of both sexes weighing 350-450 g. The anaphylactogenicity of horse blood serum IgG, obtained by the method in [3], of horse blood serum treated by the method in [1] (enzyme-treated serum — ETS), and of conjugates of these proteins with dextrans was investigated. The conjugates were obtained by a method based on the scheme suggested previously [9]. The writers have shown that this method of conjugation with dextran does not impair the antigen-binding activity of IgG [4], nor does it affect its ability to stimulate the humoral immune response [5].

The sedimentation properties of the conjugates were studied by ultracentrifugation [13]. During investigation of the anaphylactogenicity of ETS or the conjugates, on that basis, guinea pigs were sensitized in a dose of 0.1 or 0.5 mg, subcutaneously; they were sensitized intraperitoneally with IgG in a dose of 10 mg protein per animal. The reacting injection was given into the femoral vein in a volume of 1 ml in a dose of 1 or 5 mg of ETS or of the corresponding conjugates and of IgG or its conjugates in a dose of 5 mg 20 days after sensitization. The intensity of the reactions was estimated by the usual method [2]. The number of animals in the experimental groups varied from nine to 34, on average 14. Significance of the differences was estimated by the method described in [10].

EXPERIMENTAL RESULTS

Interaction of ETS and IgG with activated dextrans led to the formation of conjugates with a molecular weight greater than that of the original protein. With the scheme of conjugation chosen, virtually no unbound proteins were left in the mixture. It was shown by ultracentrifugation that the average sedimentation coefficients of the conjugates was 15S, whereas the sedimentation coefficient of native IgG is 7.5S and of the ETS proteins 5.5S. Data on the anaphylactogenic properties of the protein dextran conjugates are given in Figs. 1 and 2.

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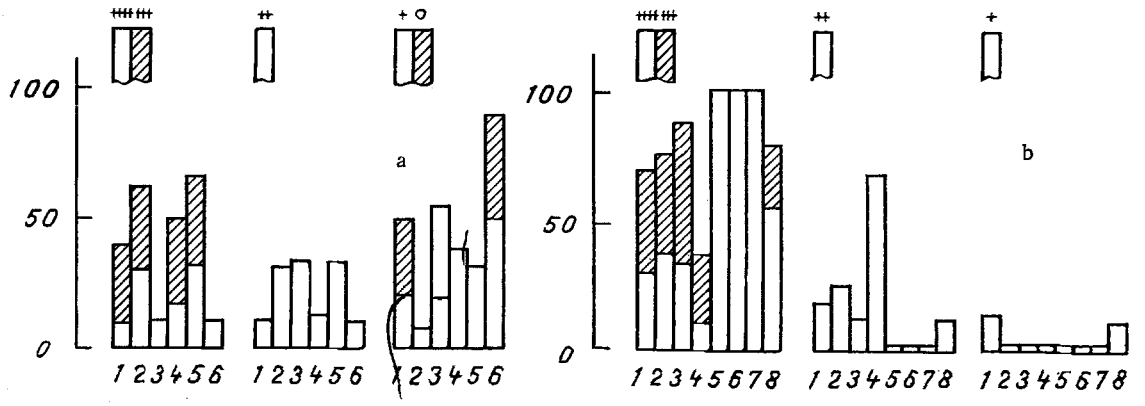

Fig. 1. Intensity of anaphylactic reaction in guinea pigs sensitized with original ETS proteins (a) or conjugates of ETS with dextrans (b). a: 1) Sensitization with 0.1 mg ETS, reaction to 1 mg ETS protein; 2) sensitization with 0.5 mg ETS, reaction to 5 mg ETS protein; 3) sensitization with 0.1 mg ETS, reaction to 1 mg ETS protein conjugated with dextran with mol. wt of 150,000 daltons (D), with ratio of protein to dextran of 1:4; 4) sensitization with 0.5 mg ETS, reaction to 5 mg of ETS protein conjugated with dextran with mol. wt. of 35,000-50,000 D, with ratio of protein to dextran of 1:9; 5) sensitization with 0.5 mg ETS, reaction to 5 mg ETS protein conjugated with dextran with mol. wt. of 35,000-50,000 D, with ratio of protein to dextran of 1:2; 6) sensitization with 0.5 mg ETS, reaction to 5 mg ETS protein conjugated with dextran with mol. wt. of 150,000 D, with ratio of protein to dextran of 1:4; b: 1) sensitization with 0.5 mg ETS protein conjugated with dextran with mol. wt. of 35,000-50,000 D with ratio of protein to dextran of 1:2, reaction to 5 mg ETS protein; 2) sensitization with 0.5 mg ETS protein conjugated with dextran with mol. wt. of 35,000-50,000 D, with ratio of protein to dextran of 1:2, reaction to 5 mg ETS protein conjugated by the same method; 3) sensitization with 0.5 mg ETS protein conjugated with dextran with mol. wt. of 35,000-50,000 D, with ratio of protein to dextran of 1:9, reaction to 5 mg of ETS protein; 4) sensitization as in 3, reaction to 5 mg ETS protein conjugated with dextran with mol. wt. of 35,000-50,000 D, with ratio of protein to dextran of 1:9; 5) sensitization with 0.5 mg ETS conjugated with dextran with mol. wt. of 150,000 D, with ratio of protein to dextran of 1:4, reaction to 5 mg ETS; 6) sensitization as in 5, reaction to 5 mg of ETS protein conjugated by the same method; 7) sensitization with 0.1 mg ETS protein conjugated with dextran with mol. wt. of 150,000 D, with ratio of protein to dextran of 1:4, reaction to 1 mg ETS protein; 8) sensitization as in 7, reaction to 1 mg ETS protein conjugated with dextran with mol. wt. of 150,000 D, with ratio of protein to dextran of 1:4. Abscissa, data grouped by intensity of reaction from ++++ to 0 in different experimental groups.

It will be clear from Fig. 1a that increasing the sensitizing dose of ETS from 0.1 to 0.5 mg, accompanied by an increase in the reacting dose from 1 to 5 mg, led to an increase in the intensity of the anaphylactic reaction. The data in Fig. 1a demonstrate the ability of dextrans, especially with mol. wt. of 150,000 D, to reduce the anaphylactogenicity of ETS with the use of protein–dextran conjugates to trigger anaphylactic shock ($P \leq 0.01$). This property was weaker in the case of conjugates obtained with dextran with mol. wt. of 35,000-50,000 D. In that case also, however, the effect was positive in all three groups of data.

The principles demonstrated in Fig. 1a are confirmed to a certain extent by Fig. 1b. Conjugates of ETS with dextran with mol. wt. of 35,000-50,000 D, prepared with protein to dextran in the ratio of 1:9, have reduced reactogenic activity. Changing the ratio of protein to dextran to 1:2 abolished this effect. The data in Fig. 1b show that modification of protein by dextran not only reduces its ability to induce an anaphylactic reaction, but also leads to a marked increase in its sensitizing activity during immunization of the animal with conjugate (compared with native protein). This effect was most marked when dextran with mol. wt. of 150,000 D was used. This effect was weaker in the case of ETS proteins conjugated with dextran with mol. wt. of 45,000-50,000 D. Meanwhile, the results in Fig. 1b show that hypersensitization developed only to antigenic determinants of protein and not to antigenic determinants of the protein–dextran conjugate. The results are evidence that modification of ETS by

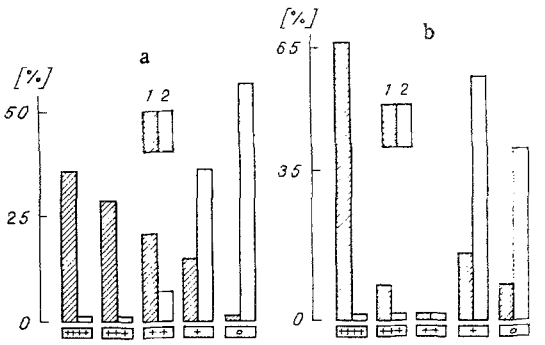

Fig. 2. Intensity of anaphylactic reaction in guinea pigs sensitized with IgG (a) or with IgG conjugated with dextran (b). a: 1) Reaction to IgG; 2) reaction to IgG conjugated with dextran with mol. wt. of 35,000-50,000 D, with protein to dextran in the ratio of 1:6; b: 1) reaction to IgG; 2) reaction to IgG conjugated with dextran with mol. wt. of 35,000-50,000 D, with protein to dextran in the ratio of 1:6. Remainder of legend as to Fig 1.

dextran leads to a marked decrease in activity of the proteins in the composition of the conjugate as an agent triggering the anaphylactic reaction, but also significantly increased their sensitizing activity. These effects were exhibited most clearly by the use of dextran with mol. wt. of 150,000 D to conjugate ETS proteins. The use of dextran with mol. wt. of 35,000-50,000. D is of considerable practical interest, for it enables the ability of the proteins in the conjugates to induce an anaphylactic reaction to be reduced, whereas at the same time the sensitizing activity of the protein in the conjugate is not significantly increased by only a very small degree; the optimal ratio of protein to dextran by weight was found to be 1:9.

Investigation of the effect of conjugation with dextran on the anaphylactogenic activity of ETS proteins met with the obstacle that this protein mixture was heterogeneous for molecular weight. A series of experiments was accordingly undertaken on IgG.

The results of experiments using conjugates of IgG with dextran with mol. wt. of 35,000-50,000 D (Fig. 2) confirmed the conclusions established in previous experiments. A statistically significant ($P \leq 0.01$) decrease in the anaphylactogenicity of IgG conjugated with dextran was observed, when used as the agent precipitating an anaphylactic reaction (Fig. 2a), a small increase in the sensitizing properties of IgG in the composition of a conjugate, and compensation of the adjuvant activity of dextran as a component of the conjugate when the conjugate also was used for the reacting dose (Fig. 2b). It must also be noted that the anaphylactic reaction to injection of the conjugates developed after a longer latent period than that to native protein: 20-30 min after injection of the conjugate whereas the reaction to injection of native protein developed after only 1-3 min. Sensitization to dextran after immunization with the conjugate developed to a minimal degree. A reaction of ++ was observed in only 20% of animals.

In the discussion of these results a number of suggestions may be put forward regarding the mechanism of the effects observed. Reduction of the anaphylactogenic properties of the protein as a component of a conjugate is evidently due to mechanical screening of the most exposed antigenic determinants of the protein, leading to steric hindrances during the reaction of antigen with antibody at the moment of resolution. Hydrolysis of the glycoside residues of dextran, taking place in vivo with the aid of endogenous dextrans, leads to destruction of the dextran matrix and makes the antigenic determinants of the protein more accessible for the antigen or (in the case of sensitization) for receptors of the cells responsible for immunologic recognition of the antigen and development of the immune response. In the case of primary contact sensitization develops, whereas in the case of the reactive dose, a delayed reaction develops, leading to desensitization under certain conditions.

Stimulation of the immune response to protein in the composition of conjugates with dextran may depend, on the one hand, on enlargement of the molecules due to their aggregation on

the matrix, and on the other hand, it may be the result of polyclonal activation of the antibody forming system, in the same way as has been described for polyions [7].

Thus during conjugation of proteins of immunoglobin nature with the aid of dextrans weakening of their challenging activity is responsible for the reduction of their anaphylactogenicity; under these circumstances the decisive factors are the molecular weight of the dextran and the ratio of protein to dextran in the conjugate.

Since the antigen-binding activity of the protein is preserved during conjugation of IgG with dextran [4], this method of reducing anaphylactogenic activity is perfectly suitable for the creation of an "immobilized" immune preparation of the "immobilized" enzyme type, for example, for emergency serum therapy.

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