INHIBITION OF IMMUNE HEMOLYSIS BY GLYCOPROTEINS OF THE α-FRACTION OF BLOOD SERUM

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UDC 612.124.017.1-064

Glycoproteins of the α-fraction of bovine blood serum possess anticomplementary activity expressed as inhibition of the immune hemolysis reaction. It is postulated that the effect observed is determined by the presence of complement inactivator in the immunoregulatory globulins.

The blood serum of man and certain species of animals (ox, rabbit, rat, mouse) contains proteins with immunosuppressive activity. These proteins with the mobility of α-globulins are described as immunoregulatory globulins (IRG). The IRG prolong the survival of skin grafts, inhibit hemolysin production in experiments in vivo, and depress the blast-transformation reaction of lymphocytes stimulated by phytohemagglutinin and specific antigens [1, 2, 3, 5]. The mechanism of immunodepression has not been studied.

The object of the present investigation was to examine the role of IRG in the antigen–antibody–complement reaction.

EXPERIMENTAL METHOD

IRG of the α-fraction of blood serum was isolated by the method described in [6]. Fraction C, eluted from DEAE-cellulose by 0.5 M acetate buffer, pH 5.0, was used in the experiments. After lyophilization the IRG preparations were kept for 4 weeks at −20°C. Immune hemolysis was carried out in a standard system of sensitized sheep's red cells and complement by the method described by Kabat and Meyer [4]. A 5% suspension of sheep's red cells, complement (1:30), and hemolytic serum in a dilution of 1:400 in veronal buffer, pH 7.3, containing optimal concentrations of magnesium and calcium, were used in the reaction. IRG was added to the samples in a final concentration of 1, 2, 4, 6, 8, and 10 mg/ml at the stage of sensitization of the red cells or during addition of the complement. The intensity of hemolysis was estimated photometrically.

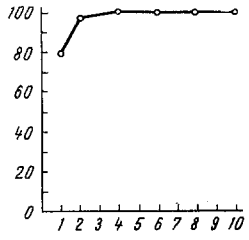

Fig. 1. Inhibition of immune hemolysis by IRG from α-fraction of blood serum. Ordinate, inhibition of hemolysis (percent); abscissa, concentration (in mg/ml).

EXPERIMENTAL RESULTS

On the addition of 0.5 ml complement and IRG to a system containing of 0.5 ml 5% suspension of sensitized red cells, virtually total inhibition of hemolysis was observed at the stage of addition of complement, when the protein concentration was 2 μg/ml (Fig. 1).

To rule out the possibility of nonspecific inhibition of hemolysis with large protein concentrations an experiment was carried out in which crystalline bovine albumen was added to a system of sensitized

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red cells – complement in concentrations of 2, 4, 6, 8, and 10 mg/ml. No difference was found between the intensities of hemolysis in the experimental and control samples.

To assess the stages of immune hemolysis at which IRG exhibits its action, the effect of IRG on the intensity of hemolysis was studied when the protein was added at the stage of sensitization of the red cells. Both in the experiment in which the final protein concentration was 2, 4, 6, 8, or 10 mg/ml and in the control the red cells were not washed after sensitization. The intensity of hemolysis in the experimental and control series was practically identical. Consequently the IRG do not affect the formation of the antigen – antibody complex, and inhibition of hemolysis by IRG is evidently due to inactivation of the complement. Inhibition of hemolysis may evidently be due to the presence of a complement inactivator in the IRG whose electrophoretic mobility is similar to that of one of the IRG fractions during electrophoresis in acrylamide gel.

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