COMPARISON OF COMPLEMENT-FIXING ACTIVITY OF MODEL IMMUNE COMPLEXES OF DIFFERENT MOLECULAR WEIGHT

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Much attention is now being paid to the study of the pathogenetic role of immune complexes (IC) in various diseases. It has been shown [4, 7] that the fate and action of IC *in vivo* depend primarily on the site of their formation, the nature of the antigen (Ag) and antibody (Ab), and the concentration of these components in the complex. If Ag, infectious agents, or infectious products, are bound with the cell membrane, IC are formed on the membrane. These IC in some virus infections are responsible for subsequent secretion of newly formed IC into the extracellular medium [9].

Binding of Ab with free Ag leads to the formation of circulating IC (CIC), which takes place in bacterial or parasitic responses and autoimmune diseases. The role of IC in the pathogenesis of these diseases depends on the quantity, qualitative composition, charge, and size or molecular weight of the IC and their ability to fix complement [4-6]. According to one view [5, 10], IC clearance is mainly determined by size. For instance, IC of average size with sedimentation constant of 11 S to 19 S, which do not fix complement, have the longest circulating time. Larger complexes (over 19 S) are almost completely removed from the blood stream in a few minutes. The writers showed previously [2] that the complement-fixing activity of small IC also depends on their molecular weight. With an increase in molecular weight of the complex, its ability to fix complement increases.

The circulating time of IC is thus largely determined by the size of the complex and its ability to fix complement.

The aim of this investigation was to compare the complement-fixing activity of IC with different molecular weights.

EXPERIMENTAL METHOD

Aggregated IgG isolated from human blood serum by ion-exchange chromatography on DEAE-cellulose, equilibrated with 0.02 M phosphate buffer, pH 7.5, served as the model of IC. To obtain complexes of different molecular weights, the IgG were aggregated at 63°C for 20 min in different initial IgG concentrations (from 1 to 7 mg/ml).

The mean molecular weights of the model IC thus formed were determined by laser nephelometry [3, 8]. The molecular weight of the aggregates was found to vary from 10 to 30 IgG.

Complement-fixing activity was determined by two methods: thermistography [1] and laser nephelometry [3]. In the latter case the ability of the aggregates to fix complement was assessed from the increase in intensity of scattering of light (ΔR) after addition of complement to the solution of aggregated immunoglobulins, by the equation $\Delta R = R_3 - [R_1 + R_2]$, where R_1 is the intensity of scattering of light by the solution of model IC, R_2 the intensity of scattering of light of complement added in the equivalent quantity of buffer, and R_3 the intensity of scattering of light of the mixture of aggregated IgG and complement. The value of ΔR was 0 if there was no reaction, positive if a fixation reaction took place, and negative if destruction of the complex was observed. The higher the complement-fixing activity of the complexes, the higher the value of ΔR.

In the thermistography method [1], based on measurement of changes in the thermophysical characteristics of the medium as a result of the complex formation reaction, the intensity of the process was assessed in terms of tan α, where α is the change in internal resistance of the temperature transducer during the reaction of complex formation compared with its control value.

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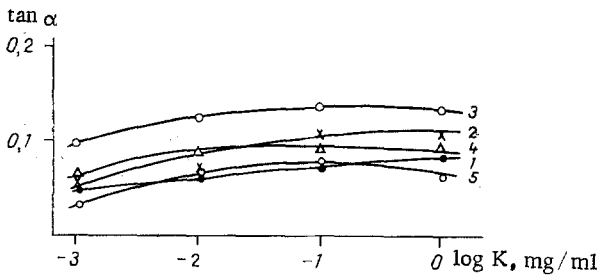

Fig. 1. Dependence of tan α of complement-fixing activity of model IC on logarithm of concentration (log K) of these complexes on addition of 0.02 ml complement in a dilution of 1:50. 1) 10 IgG; 2) 15 IgG; 3) 20 IgG; 4) 25 IgG; 5) 30 IgG.

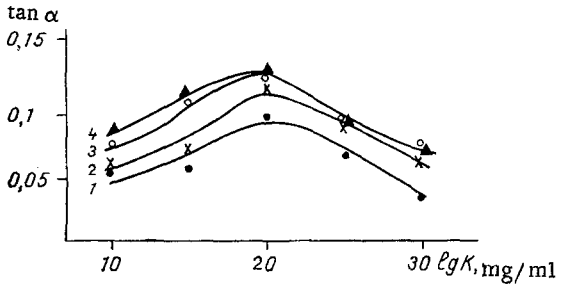

Fig. 2. Dependence of complement-fixing activity of model IC on molecular weight of complex on addition of 0.02 ml complement in dilution of 1.5. 1) $1.6 \cdot 10^{11}$; 2) $1.6 \cdot 10^{10}$; 3) $1.6 \cdot 10^{13}$; 4) $1.6 \cdot 10^{14}$ in 1 ml of solution.

Reactions with inactivated complement and reactions between native immunoglobulins in the initial concentrations, and complement, served as the reaction control. Freshly prepared standard guinea pig serum, produced by the I. I. Mechnikov Moscow Research Institute of Vaccines and Sera, was used as complement.

EXPERIMENTAL RESULTS

Curves showing dependence of the complement-fixing activity of model IC of different molecular weights (from 10 to 30 IgG) on addition of 0.02 ml complement in a dilution of 1:5 as a function of logarithm of protein concentration, are given in Fig. 1. The dependence of tan α on log K, as Fig. 1 shows, is exponential in character with a change of molecular weight of the aggregates from 10 to 30 IgG. In the case of transition to large complexes (over 20 IgG) both the rate of fixation and the quantity of fixed complement were reduced. Similar relationships were obtained on addition of the same quantity of complement in a dilution of 1:50. The only difference is a decrease in the absolute values of tan α at the assigned protein concentration.

It was shown previously [2] that in the case of aggregation of IgG solutions with different initial protein concentration, on increasing the IgG concentration from 0.5 to 2 mg/ml both the molecular weight of the aggregates and their number increase. The same protein concentration (Fig. 1) thus corresponds to different quantities of aggregates, and interpretation of the data in Fig. 1 is made more difficult. Accordingly the data shown in Fig. 1 were recalculated, to allow for an equal number of interacting aggregates. The fraction of aggregated protein relative to total protein in the solution was estimated [2]. The mean molecular weights of the aggregates [8] were determined by nephelometry. Since the weight of one IgG molecule ($M_0 = \frac{m}{N}$, where m is the molecular weight of 1 g-mole of the substance and N the Avogadro number) is 2.5×10^{-19} g, the mean number of complexes formed in the case of heat aggregation in an assigned initial protein concentration can be calculated. The

TABLE 1. Characteristics of Model IC of Different Molecular Weights of IgG

Initial IgG concentration in solution, mg/ml	Mean molecular weight of complex, IgG	Weight of single aggregate ($m \times 10^{-16}$), mg
1	10	25,0
2	15	37,5
3,5	20	50,0
5,0	25	62,5
7	30	75,0

results of the calculations are given in Table 1. They show that with an increase in protein concentration from 1 to 7 mg/ml the molecular weight of the aggregates increased threefold and their absolute number by 2.4 times.

Plotting the data in Fig. 1 between new coordinates (tan α and log $\varkappa$ where $\varkappa$ denotes the concentration of aggregates of assigned molecular weight) led to no significant change in character of the curves, which indicates that dependence of the complement-fixing activity of the aggregates on their concentration is comparatively weak.

Curves of dependence of complement-fixing activity of model IC of different concentrations on their molecular weight, on the addition of the same quantity of complement in a dilution of 1:50, are given in Fig. 2. They show that dependence of tan α on m/M_0 is extremal in character. In the region of small and average molecular weights up to 20 IgG (region I) an increase in complement-fixing activity of IC is observed with an increase in molecular weight of the complex, in agreement with data in the literature [2]. However, if m/M_0 is greater than 20 IgG (region II) dependence of tan α on m/M_0 is falling. In region II of values of m/M_0, with an increase in the IC concentration total complement-fixing activity falls, but reaches a certain limit at high concentrations of IC (the confluent branch at $\varkappa = 1.6 \times 10^{13}$ and 1.6×10^{14}). In region I of values of m/M_0 up to 20 IgG this was not observed. Corresponding curves of dependence of tan α on m/M_0 were obtained on addition of the same quantity of complement in a dilution of 1:5. It is also clear from Fig. 2 that the change in complement-fixing activity is determined primarily by the size of the complex, and depends only a little on its concentration (this is particularly characteristic of model IC with mol. wt. of over 20 IgG).

The rising branch of the curve of tan α as a function of m/M_0 may be associated with the fact that with an increase in molecular weight of the complex the number of determinant groups capable of fixing complement also increases. It was not clear why a further increase in molecular weight of IC leads to weakening of complement-fixing activity of the aggregates. It might be supposed that the large complexes formed in the course of aggregation (over 20 IgG) are unstable, and disintegrate on the addition of complement.

To confirm this hypothesis a parallel determination was made of changes in the size of the model IC after addition of complement to it. For complexes with mol. wt. under 20 IgG, on the addition of complement an increase in the intensity of scattering of light ΔR was indeed observed. At m/M_0 over 20 IgG the value of ΔR was negative. This indicates that the process taking place was not complex formation but destruction of IC after the addition of complement. With an increase in the concentration of added complement the number of disintegrating aggregates increases (the absolute value of ΔR increases). These results agree with data in the literature [10, 11], from which it follows that under certain conditions complement can dissolve the precipitate.

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CHANGES IN PROPERTIES OF HUMAN ERYTHROCYTE MEMBRANES IN THE PRESENCE OF ISOIMMUNE ANTIBODIES

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In the modern view cell membranes are complex quasicrystalline structures with a high degree of lability [5, 6, 8]. Several papers have been published on changes in cell membranes under the influence of certain physicochemical factors: temperature, γ-irradiation, and different substances [2]. The action of antibodies on the erythrocyte membrane has not been adequately studied [3, 4]. Little is still known on the pathogenetic importance of low-activity auto- or isoantibodies which may be present in human blood, but which are not detected by immunological methods usually adopted. Research in this direction may be promising from the standpoint of explaining the mechanism of development of antigen–antibody reactions, for injury to the erythrocyte membrane is an organic stage in this process.

The insufficient study paid to changes in the erythrocyte membrane under the influence of antibodies may perhaps be attributable to difficulties of a technical character. Phase structural transitions in artificial phospholipid membranes are usually studied by differential temperature analysis (scanning calorimeter [7, 9]), which is not sufficiently sensitive to study erythrocyte membranes of man and animals because of the high cholesterol content in the erythrocytes. In the present investigation it was therefore decided to use a light scattering technique, so that both the initial density of the erythrocyte membranes and changes in density during phase transitions could be recorded [5].

The aim of this investigation was to study some properties of the erythrocyte membrane of human blood in the presence of isoimmune anti-rhesus antibodies.

EXPERIMENTAL METHOD

Experiments were carried out on 34 samples of rhesus-positive erythrocytes from group O(I) blood donors. Two tubes of blood were taken from each donor: In one tube the blood contained heparin as stabilizer in a dose of 125 U/ml, the other tube was without stabilizer. The same batch of anti-rhesus group O(I) serum with antibody titer of 1:128 was used. The erythrocyte agglutination test was conducted on the blood for investigation, with anti-rhesus serum, by the conglutination method with gelatin and by the indirect Coombs' test. The titer of anti-rhesus serum with the test erythrocytes from different donors varied from 1:16 to 1:256, in most cases from 1:64 to 1:128. To study phase transitions in the erythrocytes 9 parts of heparinized blood were added to 1 part of anti-rhesus serum diluted with 0.9% NaCl solution. In the control, blood from the same donor was treated with similar dilutions of

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