Besides myasethenia and rheumatic fever, myopathy is thus another disease in which immune complexes are deposited in the thymus. The location of the complexes in different zones of the thymic lobule is evidence that different subpopulations of thymic lymphocytes undergo changes in these diseases. In conclusion it must be pointed out that, although the causes of the changes in thymic tissues in myopathy are not yet known, the very fact that such changes are present suggests that immunopathological disturbances due to injury to the central organ of the lymphoid system play an important role in the development and course of this disease.

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EFFECT OF MOLECULAR WEIGHT OF AGGREGATED IMMUNOGLOBULINS ON THEIR COMPLEMENT-FIXING ABILITY

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Reliable evidence of the pathogenetic role of immune complexes (IC) in several infectious, immune, and autoimmune diseases has recently been obtained [4, 7, 9]. Despite much research into the role of IC in the pathogenesis of these diseases, the problem of which physicochemical properties determine the pathogenetic properties of the IC, and of what determines their clearance, still remains unexplained.

It was accordingly decided to study the effect of size of IC on their ability to fix complement, and the investigation described below was carried out for this purpose.

EXPERIMENTAL METHOD

As models of IC of different molecular weight (M) aggregated human IgG, obtained from normal human blood serum by ion-exchange chromatography on DEAE-cellulose was used.

Aggregation of the IgG was carried out at 63°C for 20 min. Since M of the aggregates arises during aggregation with an increase in the protein concentration [3, 8], to obtain aggregates of different M original solutions of IgG with different initial concentrations, from 0.5 to 2 mg/ml, were used. M of the aggregates was determined nephelometrically [2]. As a result, solutions with mean weights of aggregates amounting to 7, 10, and 15 times the M of IgG were obtained.

A freshly prepared solution of standard lyophilized guinea pig serum, from the I. I. Mechnikov Moscow Research Institute of Vaccines and Sera, was used as complement.

The complement-fixing activity of IC of different M was determined by thermistography [1]. The intensity of the reaction was assessed by the value of tan α, where α is the angle of slope of the experimental curve relative to the control curve. The complement-fixation reaction of IC with activated complement and the reaction between unaggregated IgG in initial concentrations in the solution and native complement served as the control.

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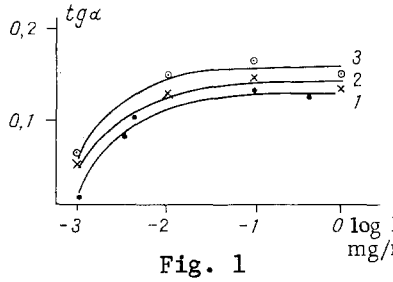

Fig. 1

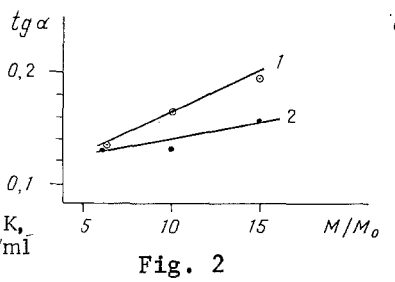

Fig. 2

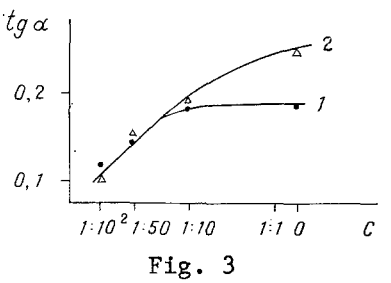

Fig. 3

Fig. 1. Dependence of tan α on log K. K) Concentration of IgG in solution containing aggregated immunoglobulins of different M: 1) seven IgG; 2) 10 IgG; 3) 15 IgG, on addition of standard complement in dilutions of 1:50.

Fig. 2. Dependence of complement-fixing activity of aggregated immunoglobulins on reduced molecular weight of complex (M/M_o). M and M_o) Molecular weights of complex and of IgG molecule respectively on addition of 0.02 ml of standard complement in dilutions of 1:5 (1) and 1:50 (2).

Fig. 3. Dependence of complement-fixing activity of aggregated immunoglublins with mol. wt. of 10 IgG (1) and 15 IgG (2) on concentration of added complement. In all cases the number of complexes in solution was constant at 8×10^{12}.

EXPERIMENTAL RESULTS

Figure 1 gives curves of complement fixation of model IC of different M values as a function of log K, where K is the concentration of IgG in the solution on addition of 0.02 ml complement in a dilution of 1:50. The results (irrespective of M) showed that the complement-fixing ability of the complexes is exponential in character, and that the complement-fixing ability of the model IC increases with increase in M. Similar relationships were obtained when the same quantity (0.02 ml) of complement in a dilution of 1:5 was added to solutions with aggregated IgG of different M. Addition of complement in a concentration 10 times higher led only to an increase in the current and limiting values of tan α for all IC of equal M, but the character of the relationships remained unchanged.

To understand these relationships it is necessary to estimate the fraction of aggregated IgG and the number of complexes thus formed. Accordingly, after aggregation the aggregated protein was separated from free IgG by the polyethylene glycol (PEG; M = 6000) precipitation test at 10% concentration [5, 6]. Having estimated the concentration of immunoglobulin bound into aggregates from its optical density on a spectrophotometer, and knowing the mean M of the complex, the number of complexes of a particular M obtained during aggregation with different initial IgG concentrations in the solution was calculated (Table 1).

As a result of aggregation of IgG solutions with different initial concentrations, with an increase in the IgG concentration in the solution both M of the aggregates and their number increased (Table 1). The difference between the values of tan α (Fig. 1) with the same immunoglobulin concentration in solution may thus be attributable not to a change in M of the aggregate, but to a change in the total number of these aggregates. If the data presented in Fig. 1 are rearranged so that equal numbers of complexes of different M interact with complement, the curves reflecting the relationship (Fig. 1) remain the same in character. The only difference between the rearranged curves and the data given in Fig. 1 is that in high concentrations of model IC the dependence of complement-fixing activity of the complexes on M of the aggregates is expressed most strongly. Figure 2 shows dependence of the complement-fixing activity of model IC on their M on the addition of complement in different concentrations. As will be clear from Fig. 2, dependence of tan α on M is linear; with an increase in the concentration of complement the slope of the lines increases. The reason is that with a change toward complexes of greater M, consisting of a large number of IgG molecules with conformation changed as a result of aggregation, the total number of complement-fixing groups of a single complex capable of fixing complement is increased. The complement-fixing ability of aggregated immunoglobulins increases with a rise in concentration of added complement (with the assigned value of M/M_o values of curve 1 in Fig. 2 lie higher than values of curve 2). Consequently, in this case, on the addition of 0.02 ml complement, not all complement-fixing groups of the aggregated immunoglobulins are saturated. It can therefore be expected that with an increase in the concentration of added complement to aggregates of an assigned molecular weight, their saturation with complement ought to be observed and the curve of tan α as

TABLE 1. Characteristics of Model IC Obtained by Aggregation of Solutions of IgG of Different Initial Concentration (aggregation temperature 63°C, time 20 min)

Initial concen. of IgG in solution, mg/ml	Protein concentration in complex, mg/ml	Mean molecular weight (M) of complex, IgG units	Weight of single aggregates, $M \cdot 10^{-16}$, mg	Concentration of complexes in solution, $\chi \cdot 10^{13}$
2	0,4	15	37,5	10
1	0,2	10	25	8
0,5	0,1	7	18,5	5

a function of C', where C' is the concentration of added complement, would have a plateau region; moreover, the greater the M of the model IC, the higher the concentrations of complement with which saturation would be expected. This was indeed observed experimentally (Fig. 3). As Fig. 3 shows, curves of tan α as a function of C, where C is the degree of dilution of added complement, have a plateau region both for solutions of aggregates with M = 10 IgG and for solutions of aggregates with M = 15 IgG, and the transition to the region of saturation for M = 10 IgG and M = 15 IgG corresponds to dilutions of complement of 1:12 and 1:5. A thousandfold increase in the concentration of aggregated immunoglobulins with an assigned M leads to the same increase in complement-fixing activity as only a twofold increase in molecular weight of the complex (Figs. 1 and 2). This indicates that the decisive parameter changing the complement-fixing activity of model IC is the size of the complex and not their number.

Consequently, the complement-fixing activity of aggregated immunoglobulins depends essentially on their M. With an increase in M of the complex its ability to fix complement increases. The quantity of complement able to bind itself to aggregated immunoglobulins rises as a nonlinear function with an increase both in the number of complexes and in the concentration of added complement. The decisive parameter changing the complement-fixing activity of aggregated immunoglobulins is the size of the complex and not their concentration.

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