COMPLEMENT-FIXING ACTIVITY OF IMMUNE COMPLEXES FROM NORMAL SUBJECTS AND MYELOMA PATIENTS

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There is now considerable experimental evidence of the pathogenetic role of immune complexes (IC) in several serious so-called immunocomplex diseases, such as systemic lupus erythematosus, rheumatoid arthritis, vasculitis, and glomerulonephritis [6, 7, 11, 12]. In addition, investigations have shown a marked rise of IC levels in lymphoproliferative diseases and, in particular, in multiple myeloma [1, 6]. The role of IC in these diseases is not clear, although it has been suggested [6] that IC have a blocking action on receptors of T killer cells, or they can form lattices on the surface of target cells, masking their antigenic determinants.

The fate of circulating IC depends essentially on the physicochemical properties of the antigen and antibodies contained in them. The prolonged circulation of IC in the bloodstream and their ability to be deposited in the vessel walls and tissues are largely determined by the size of the IC, and by the fact that they can fix complement [2, 12].

Our previous investigation [5], aimed at studying the effect of the physicochemical properties of IC on their complement-fixing activity was carried out mainly on model IC with different molecular weights. The model of IC consisted of aggregated IgG, isolated from human blood serum.

The complement-fixing activity of IC from normal subjects and from patients with myeloma was compared.

EXPERIMENTAL METHOD

Human IgG isolated from blood obtained from healthy blood donors was used [3]. Myeloma IgG_1 ($\varkappa$) was isolated in the same way from the serum of a patient with multiple myeloma. Myeloma proteins have enhanced aggregating powers [8], and for that reason, when required for quantitative measurements and for comparison of heat-induced aggregates from normal and myeloma proteins, the latter were used on the 2nd day after isolation, when the presence of aggregates in the original solutions could not be recorded nephelometrically. A freshly prepared solution of standard lyophilized guinea pig serum, obtained from the I. I. Mechnikov Moscow Research Institute of Vaccines and Sera, was used as the complement. Model IC were prepared by aggregation of the immunoglobulins by heat at 63°C for 20 min. To obtain complexes with different molecular weights, aggregation was carried out with initial concentrations of IgG solutions of between 1 and 11.6 mg/ml. The molecular weights of the aggregates were estimated nephelometrically [9]. Later the model IC obtained from blood from myeloma patients and normal donors, will be described as IC_1 and IC_2, respectively.

The complement-fixing activity of IC_1 and IC_2 was determined by thermistography [4] and by laser nephelometry [9]. In the first case the intensity of the reaction was evaluated by determining the change in the effective thermal conductivity of the solution $\varkappa$ during binding of IC and complement. In the second case the complement-fixing activity of IC was estimated as the increase in the intensity of scattering of light after addition of complement to the solution of aggregated IgG. Reactions of IC with inactivated complement and of native complement with an unaggregated solution of IgG in the initial concentrations served as the control.

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TABLE 1. Characteristics of Model IC Obtained by Heat-Induced Aggregation of Normal and Myeloma Immunoglubulins

Initial IgG concentration in solution, mg/ml	Mean molecular weight of complex, IgG units		Weight of unit of aggregate ($\times 10^{-16}$), mg		Complement concentration in solution ($\times 10^{-15}$)		Ratio of concentrations of myeloma aggregates to normal
	IC_2	IC_1	IC_2	IC_1	IC_2	IC_1	
1	10	4.3	25	10.8	8.0	37	4.6
2	15	8	37	20.0	10.8	40	3.7
3.5	20	14	50	35.0	14.0	39	2.8
6	28	18	70.0	45.0	17.1	53	3.0
8.3	33	23	82.5	56.0	19.3	60	3.0
11.6	—	34	—	85.0	—	55	—

EXPERIMENTAL RESULTS

After heat-induced aggregation of normal and myeloma IgG, IC with a much lower molecular weight than normal IgG at any protein concentration were formed from solutions containing myeloma IgG with the assigned protein concentration (Table 1). For instance, with a change in concentration of normal IgG from 1 to 8.3 mg/ml, IC with molecular weights from 10 to 33 IgG were obtained. Meanwhile, for myeloma IgG the molecular weight of IC_1 changed from 4.3 to 23 IgG, respectively. Estimation of the fraction of aggregated protein in the solution by the method of 10% precipitation with PEG (mol. wt. 6000) [10] showed that with all the initial protein concentrations, if normal IgG was used about 20% of the total protein concentration in the solution aggregated, compared with 40% in the case of myeloma IgG. This indicates increased powers of aggregation of the myeloma IgG compared with normal.

Dependence of the ratio between molecular weights of the aggregated complexes (M_1/M_2, where M_1 and M_2 denote mean molecular weights of aggregates formed by myeloma and normal IgG, respectively) on protein concentration in the solution is shown. This dependence is exponential in character. With low initial IgG concentrations (under 3 mg/ml) an increase in the ratio M_1/M_2 with an increase in protein concentration is observed, but with a further increase in concentration the M_1/M_2 ratio remained virtually unchanged. Hence it follows that the rate of aggregate formation by myeloma and normal proteins depends essentially on their concentration in solution.

As a result of aggregation, and in the presence of a low protein concentration, myeloma IgG thus forms aggregates in larger quantities, but with a smaller molecular weight, than normal IgG.

We also studied the complementary activity of model complexes of myeloma IgG and compared it with that of complexes from normal IgG. Dependences of effective thermal conductivity $\varkappa$ on protein concentration K are shown for IC_1 with different molecular weights (from 4.3 to 23 IgG) on the addition of 0.02 ml of standard complement in a dilution of 1:5. Similar dependences were obtained on the addition of a smaller quantity of complement in a dilution of 1:50. In the latter case these dependences had only smaller absolute values, and the character of the curves was unchanged.

Aggregation of myeloma IgG led to the formation of aggregates which, in the assigned protein concentration, had different molecular weights and concentrations. In order to interpret the data, we therefore recalculated them [4], to allow for an equal number of interacting complexes (Table 1). It will be clear from Table 1 that with an increase in protein concentration from 1 to 8.2 mg/ml the mean molecular weight of the complex of normal protein was increased by 3.3 times, during an increase in concentration of IC_1 by 2.4 times. With myeloma IgG under analogous conditions of aggregation, a greater increase in molecular weight (by 5.3 times) was observed; the concentration of complexes formed under these circumstances was increased by only 1.6 times.

After recalculation of the data, and allowing for an equal concentration of IC_1 in the solution, similar curves were obtained. Consequently, the complement-fixing activity of myeloma complexes, just as that of normal IC_2, depends only a little on the concentration of the complexes, but greatly on their molecular weight.

For comparison, the similar dependence is given for normal IC_2. The data show that the complement-fixing activity of IC_1 is higher than that of IC_2. The character of dependence of

$\varkappa$ on M/M_o (where M_o is the weight of one IgG molecule) differs for myeloma and normal IC. For myeloma complexes, with an increase in molecular weight at first there was an increase in complement-fixing activity, but later, when M/M_o was over 23, it either was unchanged or decreased a little depending on the IC concentration. With an increase in concentration of the complexes, the effect of a rise and fall of complementary activity reached a maximum. All curves showing changes in $\varkappa$ depending on M/M_o of aggregates of myeloma IgG appeared stretched along the M/M_o axis. This indicates considerable complement-fixing activity of myeloma IC_1 (both small and large), and this distinguishes myeloma IC_1 from normal IC_2.

For myeloma IC_1 and normal IC_2, with an increase in molecular weight of the aggregates (M/M_o not more than 23) the number of complement-fixing sites of the single complex evidently increases. At high values of M/M_o, despite the increasing number of IgG molecules included in the aggregate and, correspondingly, the increasing number of binding sites with complement, larger complexes become structurally unstable. The addition of complement therefore leads to destruction of these aggregates. Investigations on the laser nephelometer, to determine the size of the myeloma complex IC_1 before and after addition of standard complement to it confirm this hypothesis. In addition, the structure of the myeloma complex is much more stable than that of the normal complex. This is evidently due to structural features of the myeloma proteins, to the different carbon composition of the polypeptide chain, and to the considerable increase, compared with normal immunoglobulins, in the content of charged and ionized groups on the surface of the Fab-fragments, and the increased hydrophobicity of the F_c-fragments [6, 8], which taken together, change the overall hydrophilic-lipophilic balance of the surface of the protein globule and its complement-fixing activity.

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