COMPARISON OF THE PHYSICOCHEMICAL PROPERTIES OF MODEL IMMUNE COMPLEXES OF NORMAL AND MYELOMA PROTEINS

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There is convincing experimental and clinical evidence that in serious diseases such as rheumatoid arthritis, autoimmune thyroiditis, systemic lupus erythematosus, and psoriasis, which belong to the group of immunocomplex diseases, circulating immune complexes (CIC) undoubtedly play a role. The part played by immune complexes (IC) in the development of malignant neoplasms also is currently under discussion. A raised CIC level is observed in many human tumors (sarcoma, melanoma, carcinoma of the colon, lungs, ovary, and so on) [7, 8].

Normalization of the CIC level in the postoperative period in patients with these diseases correlates with a more favorable prognosis. In the case of an unfavorable outcome (the presence of metastases), the CIC level remains high after the operation and may actually continue to rise. We know that changes in the specific cellular immune response are observed in the sera of patients with tumors in various situations and in experimental animals; in particular, increased activity of suppressor T cells and changes in the proliferative activity of the lymphocytes are observed [1]. These changes, according to the available data [9], may be connected with the blocking action of tumor-specific IC. The question accordingly arises: why, in malignant neoplasms, do the CIC possess affinity mainly for receptors of immunocompetent cells, and why are they much less able to be deposited in target organs. It likewise is not yet clear how the physicochemical structural features of these complexes differ from those of complexes circulating under normal conditions, or how strong is the complement-fixing activity (CFA) of these complexes, which ultimately determines the clearance of IC and the development of inflammatory reactions at the sites of their deposition.

In the investigation described below complex formation by normal and myeloma immunoglobulins was studied during heat aggregation and the CFA of the heat aggregates was determined in relation to their molecular weight.

EXPERIMENTAL METHOD

Human IgG and IgG ($\varkappa$), isolated from blood serum obtained from healthy individuals and a patient with multiple myeloma, respectively, were used [2]. 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 complement. Heat aggregates, for use as models of IC [4], were prepared by heat aggregation of immunoglobulins at 63°C for 20 min. To obtain aggregates with different molecular weights, aggregation was carried out with solutions of immunoglobulins in initial concentations of 1 to 11.6 mg/ml. The size of the aggregates was estimated by laser nephelometry [5].

CFA of the model IC, obtained from blood from the myeloma patient (IC_1) and a normal blood donor (IC_2) was determined by thermistography [3] and laser nephelometry [5]. In the first case the reaction was read as the change in effective heat conductivity of the solutions $\varkappa$, whereas in the second case, the reading was based on 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 unaggregated IgG in initial concentrations served as the controls.

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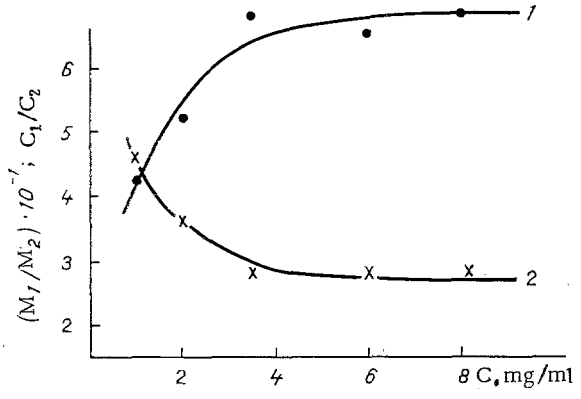

Fig. 1

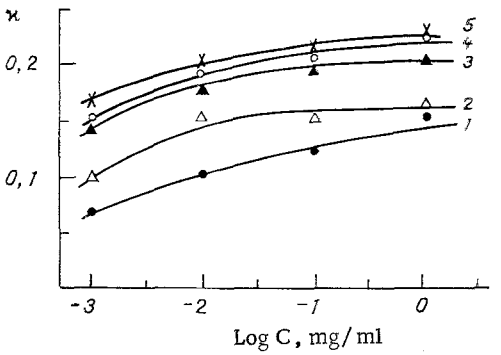

Fig. 2

Fig. 1. Dependence of M_1/M_2 (1) and C_1/C_2 (2) on protein concentration in solution. M_1, M_2) Average molecular weights; C_1, C_2) concentrations of single aggregates formed by myeloma (M_1, C_1) and normal (M_2, C_2) IgG.

Fig. 2. Dependence of the effective heat conductivity $\varkappa'$ on log C. C) concentration of myeloma IgG in a solution containing aggregates of immunoglobulin with different molecular masses: 1) 4.3 IgG; 2) 8.0 IgG; 4) 1.8 IgG; 5) 23 IGG with addition of 0.02 mg complement in dilution of 1:5.

EXPERIMENTAL RESULTS

Heat aggregation of solutions of normal and myeloma IgG with identical initial protein concentration took place at different rates and intensities. As a result of aggregation of normal and myeloma immunoglobulins complexes differing in average molecular weight and concentration were formed. With low initial protein concentrations (up to 4 mg/ml) large quantities of aggregates with a smaller molecular weight were formed from the myeloma immunoglobulins compared with normal, whereas with both types of immunoglobulins in concentrations of over 4 mg/ml the rate of formation of the individual aggregates and their quantity were equal (Fig. 1).

It will be clear from Fig. 1 that with low initial protein concentrations the formation of a single aggregate from normal immunoglobulins took place with a higher intensity but in smaller amounts than from myeloma IgG. With initial concentrations of over 4 mg/ml the rate of formation of new aggregates and the change in their molecular weights were the same in IC_1 and IC_2 complexes. These results are in agreement with those obtained by other workers [6], who found increased aggregation powers of myeloma proteins.

FCA of myeloma and normal model IC was compared (Fig. 2). With complement in a dilution of 1:50 the curves showed similar patterns of dependence but the absolute values of $\varkappa$ were smaller. It will be clear from Fig. 2 that with an increase in the molecular weight of the complex, its CFA activity also increased. The same relationship for CFA was obtained by the writer previously [4] for complexes of normal IgG. In myeloma model IC, just as with normal IC, dependence of CFA on protein concentration was weak (Fig. 2).

During aggregation of immunoglobulins with an assigned concentration complexes of different concentrations and different molecular weight were formed depending on the initial protein concentration in solution. To compare CFA of complexes with different molecular weights, it was therefore necessary to reduce the concentration of the complexes to the same values. Dependence of CFA of the model IC on their molecular weight with different concentrations of complexes is shown in Fig. 3. Dependences of $\varkappa'$ on M/M_0, where M and M_0 are the mean molecular weights of the aggregates and of native IgG, differed for normal and myeloma IC (Fig. 3). The graphs showing $\varkappa'$ as a function of M/M_0 for myeloma IC extended further along the abscissa and the maximum of the curve was indistinct, especially with low concentrations of the complex. In the region of low molecular weights of the aggregates, CFA of the myeloma IC was manifested most clearly.

The fact that dependence of CFA on the molecular weight of the myeloma complexes was of this nature indicates that myeloma complexes, both small and large, have greater CFA than nor-

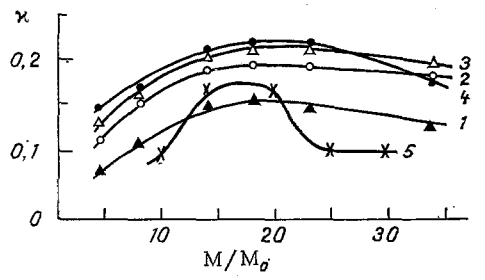

Fig. 3. Dependence of CFA of myeloma (1-4) and normal (5) model IC on relative molecular weight of the complex M/M_0, where M_0 is the molecular weight of IgG on addition of 0.02 ml of complement in a dilution of 1:5. Concentration of IC in solution: 1) $9 \cdot 10^{10}$; 2) $9 \cdot 10^{11}$; 3) $9 \cdot 10^{12}$; 4) $9 \cdot 10^{13}$; 5) $1.6 \cdot 10^{12}$.

mal complexes and they are more stable in structure. Solution of model IC formed from normal IgG with mol. wt. of over 20, observed experimentally (laser nephelometry) under the influence of added complement, did not take place or did so only very weakly for the same complexes formed from myeloma immunoglobulins. An increase in CFA was observed for complexes of both types with an increase in molecular weight of the aggregates to not more than 20 IgG, and this was evidently connected with an increase in the number of complement-binding sites of the single complex under these circumstances. Large complexes are unstable in their structure [9], and for that reason the addition of complement causes their dissociation. With an increase in concentration of the complexes the effect of a rise (for the region of low values of M/M_0 up to 10 IgG) and fall of CFA were most distinctly observed. It will be clear from Figs. 2 and 3 that CFA of myeloma and normal model IC depends primarily on their molecular weight and to only a slight extent on concentration.

CFA of myeloma and normal IC changes as a nonlinear function of molecular weight of the complex, and is much higher for complexes formed from myeloma immunoglobulins. The distinguishing feature of complex formation by complement with the myeloma complex is due to the structural features of the myeloma proteins, the increased carbohydrate composition of the polypeptide chain, the considerable number of charged and ionized groups on the surface of the Fab-fragments, and increased hydrophobicity of the Fc-fragments. This ultimately changes the secondary, tertiary, and quaternary structures of the macromolecule and leads to various conformational changes during heat denaturation of myeloma and normal immunoglobulins.

LITERATURE CITED

1. I. L. Weissman, L. E. Hood, and W. B. Wood, Introduction to Immunology [Russian translation], Moscow (1983), pp. 133-136.
2. A. E. Gurvich and O. B. Kuzovleva, Immunochemical Analysis [in Russian], Moscow (1968), pp. 54-67.
3. N. A. Konstantinova, V. V. Lavrent'ev, G. E. Anikina, et al., Lab. Delo, No. 8, 125 (1979).
4. N. A. Konstantinova, I. A. Tumanova, V. V. Lavrent'ev, and R. V. Petrov, Byull. Éksp. Biol. Med., No. 12, 72 (1982); No. 5, 583 (1984).
5. K. K. Ostreiko, I. A. Tumanova, and V. V. Lavrent'ev, Zh. Fiz. Khim., 56, No. 5, 1322 (1982).
6. L. V. Chasovnikova, N. A. Matveeva, D. V. Stefani, and V. V. Lavrent'ev, Mol. Genet., No. 3, 39 (1983).
7. K. Hoffken, I. D. Meredith, R. A. Robins, et al., Lancet, 1, 672 (1978).
8. T. A. Poulton, M. E. Crowther, F. C. Hay, et al., Lancet, 2, 72 (1978).
9. M. Takahashi, S. Takahashi, and S. Hirose, Prog. Allergy, 27, 134 (1980).