SPECIFIC COMPLEX FORMATION BY HUMAN IMMUNOGLOBULINS AND THEIR CORRESPONDING ANTIBODIES

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Specific complex formation by human immunoglobulins of classes M, G, and A with antibodies against class G immunoglobulins was studied by a thermistographic method. Depending on the type of interacting immunoglobulins, the rate and intensity of the process were shown to differ and to be maximal for IgM. Depending on the character of the curves obtained the class of immunoglobulins being synthesized at that given moment could be determined. Disturbance of the internal structure of the macromolecules of the immunoglobulins, as a result of heating, for example, leads to changes in the mechanism of complex formation; the important question in this event is which of the interacting components undergoes denaturation.

KEY WORDS: Immunoglobulins, anti-immunoglobulins; antigen–antibody complex.

In recent years pathological states whose development is due to or accompanied by the appearance of structurally changed, pathological macromolecules in the blood stream are being increasingly frequently observed in patients with immunologic deficiency and in experimental animals. Such macromolecules include, for example, the rheumatoid factor in rheumatoid arthritis, the appearance of which is accompanied by the formation of immune complexes; myeloma immunoglobulins characterized by absolute structural homogeneity and considerably modified functional properties; and "normal" immunoglobulins; but with loss of antibody activity.

However, despite the great practical and theoretical importance of research into the mechanisms of specific interaction between normal and structurally changed immunoglobulins, insufficient attention is still being paid to this question. The object of this investigation was to study the effect of the character of normal and conformationally changed macromolecules on processes of specific complex formation, using the method of thermistography [2, 3].

EXPERIMENTAL METHOD

Standard sets of human immunoglobulins of three classes – M, G, and A – in dilutions of between 1:10 and $1:10^4$ with physiological saline, were used as the antigen. Monospecific rabbit antisera belonging to the IgG class (final dilution in the experiment 1:4000) served as antibody. The monospecific antisera, as well as the standard solutions of immunoglobulins, were provided by the N. F. Gamaleya Institute of Epidemiology and Microbiology.

To induce structural modification of the test immunoglobulins, preliminary heat treatment was carried out by the standard method [5].

Processes of specific complex formation were investigated by means of a highly sensitive semiconductor microthermistor of the MT-54 type [3]. The writers showed previously that this method can be used to assess specific interaction between human immunoglobulin molecules and antibodies against them [2]. The intensity and specificity of the reactions were recorded automatically by the N-36 automatic writer and assessed from the tangent of the angle of inclination ($\tan \alpha$) of the experimental curve relative to the control.

The results of investigation of reactions of specific complex formation in solutions of antisera with immunoglobulins of classes M, G, and A are given in Fig. 1. They show that in low concentrations of immunoglobulin of each class (to 4×10^{-4} for IgM and 5×10^{-3} for IgG and IgA) a significant increase in log tan α is

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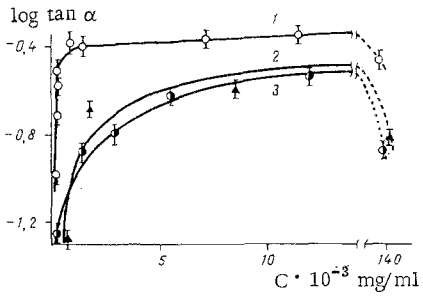

Fig. 1

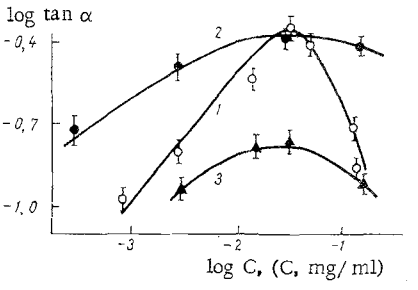

Fig. 2

Fig. 1. Curves of log tan α as a function of antigen concentration (C) for immunoglobulins of classes M (1), A (2), and G (3). Antiserum of class G immunoglobulins diluted with physiological saline 1 : 4000.

Fig. 2. Curves of log tan α as a function of antigen concentration (C) in logarithmic coordinates for class G immunoglobulins. Native protein (1); denatured IgG and native antiserum (2); native IgG and denatured antibodies (3). Antiserum of class G immunoglobulin diluted 1 : 4000 with physiological saline.

observed with an increase in antigen concentration (C). With a further increase in concentration, the curve of log tan α as a function of C flattens out on a plateau, and only at antigen concentration in excess of 140 mg/ml was a significant decrease in log tan α observed. As Fig. 1 shows, in the case of IgM, flattening out on a plateau took place more rapidly and the maximal values of log tan α were significantly higher than those for IgG and IgA. Accordingly, only antibodies of class G immunoglobulins against antigens of classes M, G, and A, of immunoglobulins, which differed only in a small part of the variable region, were used in this investigation, the differences obtained in the curves were determined by the nature of the antigen.

It is known that the IgM macromolecule has a molecular weight of 900,000 and is a pentamer, whereas serum immunoglobulins of the G and A classes are monomers with a molecular weight of 150,000 and 170,000 respectively. The number of antigenic determinant groups on the IgM molecule also is greater than that of IgG and IgA. An equal number of interacting macromolecules of immunoglobulins of different classes therefore forms complexes which differ substantially in size. For those reasons the absolute values and rate of change of log tan α with an increase in antigen concentration were greater for interacting macromolecules of the IgM class.

The sensitivity of the thermistographic method is thus such that, when monospecific antisera are used, the class of immunoglobulins being synthesized at a given moment can be determined from the character of the curves obtained.

The next stage of the work was to study the effect of structural conformational changes in the macromolecules, with particular reference to class G immunoglobulins, on the process of specific complex formation (Fig. 2). For this purpose interaction between native class G immunoglobulins and the corresponding monospecific antiserum, between denatured IgG and native antiserum, and also between native IgG and denatured antibodies was investigated. As Fig. 2 shows, the dependence of log tan α on log C was extremal in character for all three cases during a change in antigen concentration from 0.14 to 0.14×10^{-3} mg/ml.

In the case of interaction between native macromolecules, the maximum was narrow and could be clearly distinguished, whereas after denaturation of either of the interacting components the maximum was widened. It is interesting to note that, when native IgG antigen and denatured antiserum were used in the reaction, the dependence of log tan α on log C was least clearly defined of all.

The results illustrated in Fig. 2 can be explained by a change in the structure of the immunoglobulin macromolecules during heat treatment. Heating protein to 63°C for 20 min is known [1] to lead to rupture of intramolecular hydrogen and hydrophobic bonds, as a result of which the packing of the immunoglobulin molecules become loose; under these circumstances additional determinant groups, previously hidden inside the macromolecule, come out on to its surface. The native macromolecule is thereby converted into an activated molecule, and this leads to an increase in complex formation and to widening of the zone of specific interaction. In the case of heat treatment of the antiserum, loosening of the structure of the immunoglobulin macromolecules also takes place but, in addition, there is partial loss of specificity of the antibody molecules on heating [4], and this leads to a sharp decrease in the quantity of precipitate formed. As a result the maximum (see curve 3 in Fig. 2) is indistinct and the values of log tan α lie at a lower level than those of curves 1 and 2.

Hence, depending on the type of interacting immunoglobulins, the process of specific complex formation takes place at different speeds and intensities, and it is most evident for IgM. Consequently, depending on the character of the curves obtained, the class of immunoglobulins being synthesized at a given moment can be determined. Disturbance of the internal structure of the immunoglobulin macromolecules (in particular by heating) leads to a change in the mechanism of complex formation; the important question here is which of the interacting components undergoes denaturation. The sharpest decrease in complex formation is observed in the case of thermal denaturation of the antiserum, because of partial loss of specificity of the antibody macromolecules on heating.

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EVALUATION OF THE IMMUNOHISTOLOGICAL STATE OF LYMPH NODES REGIONAL WITH RESPECT TO TUMORS

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The immunoreactivity of lymph nodes regional with respect to the tumor was studied in 22 patients with melanoma. Blast-transformation, rosette-formation, and immunofluorescence tests were used to detect T- and B-lymphocytes. Lymphocytes were isolated from one half of the lymph node, the other half was used for histology. The results showed that function of the regional lymph nodes correlates well with morphology.

KEY WORDS: immunreactivity; lymphocytes; lymph node.

The study of the efficacy of the antitumor barrier of the various functional systems in man is a very urgent problem to which many investigations have been devoted [1, 2, 4, 6, 9, 11, 12].

The present writer [8] has shown that the immunologic activity of T- and B-lymphocytes from lymph nodes with tumor metastases is lower than that of lymphocytes from lymph nodes without metastases. These results naturally have posed fresh problems: What morphological changes are observed in the lymph nodes besides signs of metastasization, to correspond to these different immunologic backgrounds? The work of Tsakraklides et al., [11, 12] confirmed the value of studying immunomorphological changes in lymph nodes from cancer patients. The investigation described below was devoted to this purpose. The results of immunologic tests to study T- and B-lymphocytes in vitro were used as criteria of immunoreactivity.

The state and the cell composition of the cortical and paracortical zones, the medullary layer, and the sinuses of the lymph nodes served as steps in the histological analysis.

The results were accordingly combined into three groups: severe, moderately severe, and mild reactive changes, which can also be represented as marked hyperplasia, moderate hyperplasia, and hypoplasia of the lymphoid tissue of the lymph nodes.

The investigations were based on the recommendations of Cottier et al., [3] on standardization of morphological signs of immunologic activity of lymph nodes.

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