STUDY OF THE MOLECULAR MECHANISMS OF FORMATION OF COMPLEMENT—IMMUNOGLOBULIN COMPLEXES

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Complement is known to be a multicomponent system [5, 7, 10], consisting of nine serum proteins, and it plays an important role in immunogenesis. The components of complement are biologically inert, but under certain conditions they are activated by the antigen—antibody (Ag—Ab) complex or by aggregated immunoglobulins of antiserum, when they acquire the functions of enzymes and perform a protective or destructive role in immunocytolysis.

In the present investigation the mechanism of binding of complement with Ag—Ab complexes and with aggregated and native immunoglobulins in solution, and also dependence of the degree of binding on the concentration, affinity of the added reagents, and species of complement, were investigated.

EXPERIMENTAL METHOD

Investigation of binding of complement, chiefly of the first component of complement C1, with Ag—Ab complexes and with aggregated and native immunoglobulins was undertaken by the thermistography method [3, 4]. Changes in the effective temperature conductivity of the medium, measured relative to the change in the characteristic resistance of the thermistor with time, was determined by this method.

A monospecific antiserum (sheep) against human IgM (native and aggregated), diluted with physiological saline in the ratio of 1:4000, was used as the antiserum. A standard human blood serum in a dilution of 1:1000 was used as the antigen. Lyophilized and freshly prepared guinea pig sera, and freshly prepared human and rabbit blood sera were used as the source of complement. The complement was inactivated by heating the solution containing complement to 53 ± 0.1°C for 30 min [1]. Immunoglobulins were aggregated by heating to 63 ± 0.1°C for 30 min [6].

EXPERIMENTAL RESULTS

Dependence of the effective temperature conductivity χ on the quantity of added complement in the presence of assigned concentrations of Ag and Ab (dilution of the complement varied from 1 to $1:10^4$) was determined. The region of concentration of complement in which the quantity of bound complement was greatest for all cases tested was observed. This maximal value of complement concentration corresponded to the 1:50 dilution of serum. Later all investigations were conducted with this dilution of complement.

The complement-binding activity of Ag—Ab complexes, and of the separate reagents of this complex, in the native and denatured form, was compared depending on the species of complement and the conditions under which it was obtained (Table 1).

For all types of complement fixation test the addition of inactivated complement either did not change the effective temperature conductivity χ, or led to a very small (not over 7%) change in its value. This indicates that the experimental conditions chosen enable the effect of ballast proteins on the fixation test to be disregarded.

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TABLE 1. Changes in Effective Temperature Conductivity χ of Immunoglobulin Solutions in Complement Fixation Tests

Type of reaction	Serum			
	lyophilized guinea pig	freshly prepared		human blood
		guinea pig	rabbit	
Ab + C	0,044	0,06	0,052	0
Ab* + C	0,21	0,466	0,167	0,24
Ab + C*	0,002	0,008	0,017	0
Ab* + C*	0	0,002	0,005	0
Ag + C	0	0	0,017	0,005
Ag + C*	0,002	0	0	0,002
Ag* + C	0,043	0,123	0,069	0,08
Ag* + C*	0,01	0	0	0,008
At + Ag + C	0,14	0,21	0,11	0,12
At + Ag + C*	0,002	0	0,008	0
At + Ag + C	0,052	0,052	0,034	0,075
At + Ag* + C	0,018	0,048	0,008	0,023
At* + Ag* + C	0,105	0,132	0,076	0,140

*Aggregated Ag and Ab, denatured complement.

For all types of sera tested, maximal fixation was observed with aggregated immunoglobulins of antiserum and Ag–Ab complexes in which the Ag and Ab were native; complement fixation in the first case, moreover, was highest of all. Some fixation of complement was observed during the reaction with native anti-IgM immunoglobulins, as other workers [8, 9] observed previously. It also follows from Table 1 that freshly prepared guinea pig serum was most effective in its complement fixing strength. The results in this table agree with existing views on the mechanism of complement fixation.

During aggregation of immunoglobulins or the formation of Ag–Ab complexes structural changes take place in the Fc-fragment of immunoglobulins, during which additional C1 binding sites, previously blocked, become exposed, with the result that their complement-fixing capacity is increased. In the case of fixation of C1 by the native immunoglobulin molecule, this bond as a rule is weak, for the complement-fixing sites are screened. The values given in Table 1 are therefore maximal for fixation of complement both with Ag–Ab complexes and with aggregated anti-IgM.

A comparative study was made of the complement-fixing activity of complexes in the following cases: 1) Ag and Ab — native; 2) Ag and Ab — aggregated; 3) either Ag or Ab aggregated (Table 1). Maximal fixation with complement was observed in the first two cases, and the change in the value of χ was greater in the second case. These findings can be explained in the same way.

It was shown previously [2] for immunoglobulins of IgG class that if precipitation reactions in cases 1-3 are compared, the specific precipitation reaction is observed most intensively during interaction between native Ag and Ab. The components of complement are known to be α-, β-, and γ-proteins with high molecular weight; for example, the components of C1 (C1q, C1r, C1s) have molecular weights of 400,000, 168,000, and 678,000, respectively; addition of even the first component of complement C1 to the complexes thus makes them significantly larger. Moreover, the more specific the interaction between Ag and Ab, the firmer the binding with complement. Consequently, addition of the same quantity of complement ought to lead to maximal changes in case 1 compared with cases 2 and 3, but this was not observed experimentally. The reason is that, despite the lost specificity of the aggregated macromolecules of antiserum (on account of a change in the conformation of the variable regions of Fab-fragments), no specific precipitation was observed and the addition of complement led to its fixation, not with the Ag–Ab complex as in case 1, but with aggregated immunoglobulins of the antiserum (case 2).

In case 3 preliminary mixing of the native antiserum and denatured antigen led to the formation of precipitating complexes, but the specificity and intensity of this process were much less than in case 1 [2]. The addition of complement therefore leads to its weak fixation with nonspecific Ag–Ab complexes.

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ANTIGENIC KINSHIP OF POLYPEPTIDE STIMULATORS OF IMMUNOGENESIS FROM THE THYMUS AND CEREBRAL CORTEX

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The writer showed previously [4] that injection of a low-molecular-weight polypeptide from the cerebral cortex into mice has a marked stimulating action on immunogenesis, similar to the effect of a polypeptide prepared from the thymus by the same method [3]. The low-molecular-weight polypeptide isolated in this way from the white matter of the brain had no such effect [4]. It was therefore interesting to study whether the stimulators of immunogenesis from the thymus and cerebral cortex are antigenically related, or whether the cortex, where the θ-antigen is located [2, 10], contains an active substance which resembles the polypeptide from the thymus only functionally.

The object of this investigation was to make a serologic analysis of low-molecular-weight polypeptides from the thymus, cerebral cortex, and white matter of the brain in two tests: the complement fixation test and the complement-dependent cytotoxic test.

EXPERIMENTAL METHOD

Preparations were obtained from the thymus glands and gray and white matter of the brain of calves by acetic acid extraction as described previously [7]. The lyophilized preparations had a molecular weight of under 10,000. It was shown by methods of ascending paper chromatography, ion-exchange chromatography, and electrophoresis on paper and in polyacrylamide gel that the isolated preparations were complexes of polypeptide fractions. The complex of polypeptide fractions from the thymus has been called "thymarin" [3], and the polypeptide from the cerebral cortex was given the working name of "cortexin." A preparation of thymosin with a molecular weight of 12,500, prepared from calf thymus glands by Goldstein's original method [12] in the Department of Virology, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, and generously sent to the present writer for investigation, also was studied.

To determine the degree of antigenic kinship between thymarin, cortexin, and polypeptide from brain white matter, antisera were prepared against them and these were cross-absorbed

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