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Biological Activities of Complement

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Biological Activities of Complement

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With 64 figures and 39 tables

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Preface

The purpose of the International Symposia of the Canadian Society for Immunology is to be informative to immunologists and other interested scientists and to focus on currently important areas of research in the very broad field of immunology. The program of the Fifth Symposium was designed to reflect present trends in complement research in the center of which is the biological and medical significance of the complement system. The Symposium was intended not to be one specialist speaking to another specialist, but rather, the specialist reviewing the current status of research in his specialty and interpreting recent progress to other immunologists and to new research workers interested in entering the field of complement research.

The local committee which organized the Symposium, is grateful for the valuable suggestions for the program outline and possible participants put forward by Drs. E. BECKER; B. CINADER; I. H. LEPOW and M. M. MAYER. The continuous interest and help of Dr. B. CINADER is gratefully acknowledged. I also thank my colleagues Drs. D. A. BARNUM; E. LYNN FOX; M. E. MALCOMSON, P. J. QUINN, I. TIZARD and Miss RUTH SAISON for their help with various aspects of the organization. I wish to express particular appreciation to my secretary Mrs. G. GARGETT for her interest and untiring efforts in numerous organization tasks and in typing duties. The many technical services made available by the administration of the University of Guelph which contributed to the social and scientific success of this Symposium are gratefully acknowledged.

D. G. INGRAM
Guelph, November 1970

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The Complement System

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Components and Reaction Sequence

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The ultimate objective of research in the complement system is the elucidation of the biomedical significance of this system and its reactions. But to understand the biomedical implications of complement, it is necessary to know the biochemistry of the complement reactions and the reactions of complement under physiological and pathophysiological conditions.

The biomedical manifestations of the activities of complement involve biological membranes and there are at least 4 ways in which complement can affect membranes: (l) complement may cause irreversible membrane damage and, in the case of cell membranes, this leads to cell death; (2) complement can cause structural alterations: e.g. to bring about the release of histamine from mast cells, without necessarily killing the cell; (3) complement can induce changes in membranes by membrane activation, as exemplified by enzyme activation on leukocytes to cause a directed migration of polymorphonuclear leukocytes, and (4) complement can induce changes in the surface properties of membranes by attachment of component macromolecules as occurs in immune adherence and phagocytosis.

It seems that complement acts either directly or indirectly on cell membranes to bring about the recognized consequences of activation of this complex system.

The complement system is composed of 9 distinct components, or 11 serum proteins, and these proteins make up approximately 10% of the globulin fraction of human serum [32]. These 11 serum proteins have been purified and identified by function. Several have been characterized chemically. All complement components are macromolecules and each, except Clq, is present in serum or plasma in an inactive form which requires sequential activation.

The Complement Components

The complement system comprises 9 components present in the globulin portion of serum, which are designated C1, C2, C3, C4, C5, C6, C7, C8, and C9. Component C1 consists of 3 serum proteins: C1q, C1r, and C1s. Activated complement complexes which are known to possess enzymatic activity are designated by a bar over the component or components, e.g. $C\bar{1}$.

Some of the characteristics of human complement components are shown in table I [31]. The molecular weight of the various proteins range from approximately 79,000 to 400,000. Most of these proteins migrate electrophoretically as β-globulins but C1s and C9 behave as α-globulins and C1q and C8 as γ-globulins. At least some components contain a carbohydrate moiety, the significance of which is not known.

The amino acid composition of some of the complement components has been reported [4]. Components C3, C4 and C5 have a composition characteristic of serum proteins. On the other hand, C1q is unusual in that it contains 18 or more residues of glycine and 2 or 3 residues of hydroxylysine. The latter amino acid does not otherwise occur in serum proteins. The C1q protein also contains hexosamine and neuraminic acid and a large amount, about 10%, of hexose which consists of equimolar amounts of galactose and glucose. This composition is similar to structural proteins rather than serum proteins indicating that C1q may have evolved from a connective tissue precursor.

Table I. Characteristics of human complement proteins

Complement Protein	Sedimentation coefficient(s)	Molecular weight, X10³	Electrophoretic mobility	Carbohydrate content, %	Serum concentration, µg/ml
C1q	11.1	400	γ2	15	190
C1r	7.0	—	β3	—	—
C1s	4.0	79	α2	—	22
C2	5.5	117	β2	—	20–40
C3	9.5	185	β1	2.7	1,200
C4	10.0	240	β1	14	430
C5	8.7	—	β1	19	75
C6	5–6	—	β2	—	—
C7	5–6	—	β2	—	—
C8	8.0	150	γ1	—	<10
C9	4.5	79	α	—	<10

— Not known.
Adapted from [31].

The concentrations of complement proteins vary widely in human serum, ranging from less than 10 to at least 1,200 μg per ml (table I). The relative concentrations also vary from one animal species to another. These concentration differences may indicate the quantitative requirements for the individual components necessary for the optimal biological function of complement. The relative importance of the various biological functions, such as cell lysis or phagocytosis, probably varies between species of animals and possibly also between strains within a species. Therefore, certain species or certain strains may have greater requirements than other species or strains for some complement components. For lysis of cells, many more molecules of C3 are needed than of other complement components and the fact that this complement protein is present in largest amount in human serum indicates that in man, cell lysis is an important mechanism of maintaining the integrity of the individual.

The biosynthesis of complement components has attracted the attention of research workers in recent years and improved techniques have made it possible to identify the tissue, and in some cases, the cellular sites of production. C1 is believed to be synthesized by the columnar epithelium of the gastrointestinal tract [9, 10]; C2 [44] and C4 [48] by macrophages; C3 primarily by the liver [1], C6 [43] and possibly C9 [42] also by the liver.

Specific antisera have been prepared against the following complement components from human serum: C1q [30], C1s [15], C2 [20], C3 [23], C4 [39], C5 [36], C8 [27] and guinea pig components C2 [29], C3 [26], C4 [5]. These antisera form precipitin lines in agar gels and are capable of inhibiting the respective components in immune hemolysis. Each complement protein appears to be antigenically distinct since antisera to the individual components do not cross-react with antibodies to other components. However, antibodies to C components cross-react with homologous components in different species, e.g. anti-human C3 reacts with human C3 and with guinea pig C3.

The Mechanism of Immune Cytolysis

Cell lysis by complement usually requires the entire complement reaction sequence and is considered the prototype of complement activity. One molecule of the IgM type of antibody or 2 molecules of IgG, the types which are capable of fixing complement, combine with cell surface antigens to form a site at which the C system can be activated. C1 combines with the membrane-bound antibody and becomes activated to the enzyme $C\bar{1}$. This enzyme catalyses the

activation of C4 which then becomes attached to the membrane (or to the $C\overline{1}$-antibody complex). $C\overline{1}$ also activates C2 which links with C4 to form the enzymically active $C\overline{42}$ complex. $C\overline{42}$ acts on its natural substrate, C3, to catalyze the attachment of C3 to the membrane and generate the new peptidase $C\overline{423}$. The $C\overline{423}$ enzyme reacts with C5. In the presence of C6 and C7, the activity of $C\overline{423}$ on C5 is much more effective in proceeding to the ultimate goal of cell lysis and it appears that C567 may act in a physically associated form as a complex. The activated C5 becomes attached to the membrane and the C567 complex allows C8 to be physically taken up to the cell membrane. Erythrocyte membranes which have been acted upon by C8 show protracted but persistent low-grade hemolysis in the absence of C9. The lytic activity of C8 is greatly enhanced by C9 to bring about rapid and irreversible damage as exemplified by the release of hemoglobin and other intra-cellular constituents from erythrocytes which undergo immune hemolysis.

The complement reaction sequence can be classified into 4 stages: (a) the generation of $C\overline{1}$; (b) the development of $C\overline{423}$; (c) the C567 stage and (d) the reactions with C8 and C9. The first two stages have been characterized as the activation mechanism and the last 2 stages, the attack mechanism [31].

Generation of $C\overline{1}$. The 3 subcomponents C1q, C1r and C1s occur in serum as a calcium-dependent complex which has a sedimentation coefficient of 18 S [34]. On addition of a suitable chelating agent, such as EDTA, the complex disassociates into the subunits. This trimolecular complex can interact with aggregated immunoglobulin or antigen-antibody complexes through C1q which has a valency of approximately 6 for IgG [31]. C1q reacts with IgG1, IgG2, IgG3 or IgM. However, IgG4, IgA, IgD and IgE do not have the specificity to react with C1q [31]. These immunoglobulins are not able to fix complement, presumably because they lack a site for the attachment of the first sub-component of, C1q. The portion of IgG with which C1q interacts is located on the Fc portion of the molecule. Although C1q can react with free immunoglobulin molecules, the conformational changes associated with attachment of antibody to antigen or with aggregation of γ-globulin enhances this reaction and permits a firmer binding of C1q with immunoglobulin [31]. The stable C1q, since it can combine with immune complexes directly, represents an exception to the general rule that complement components require activation.

When C1q combines with antigen-antibody complexes in the presence of Ca^{++}, C1r is activated, and it in turn activates the proenzyme C1s.

$$EA \underset{Ca^{++}}{\overset{C1q,r,s}{\rightleftharpoons}} EAC\overline{1}$$

This activated component, $C\bar{1}$, has esterolytic activity which resides in C1s [2]. Normal serum contains an inhibitor of $C\bar{1}$ which interferes with its action on synthetic amino acid esters [25]. Treatment with plasmin or trypsin also converts C1 to $C\bar{1}$ [40]. Binding of C1 is reversible and following activation, $C\bar{1}$ may transfer to other binding sites so that it may be present both in the free and cell-bound form [3].

Development of $\overline{C423}$. Activated C1 ($C\bar{1}$) catalyzes binding of C4 and C2 to the cell surface to yield C3 convertase. As a result of the action of $C\bar{1}$, C4 is cleaved into 2 fragments designated C4a which has a molecular weight of 15,000, and C4b with a molecular weight of about 230,000. The latter may bind to membrane receptors or to antibody [53]. Only a small percentage of C4b becomes firmly bound to cell surfaces, the rest remains in the fluid phase due to rapid decay of the binding capacity to become (C4b)i.

$$EAC\bar{1} \xrightarrow{C4 \quad C4a + (C4b)i} EAC\bar{1},4b$$

The cell-bound fragment of C4 may react with some immunoconglutinins to bring about conglutination of the EAC1,4 complex [21]. A natural inhibitor of native C4 has been found in the serum of several species [19].

The enzyme $C\bar{1}$ also cleaves C2 into 2 fragments C2a, molecular weight 85,000, and C2b. C2a, in the presence of Mg++, combines with cell-bound C4b to form the enzymatically active complex $\overline{C4b,2a}$ referred to as C3 convertase. Also produced in this reaction is an inert fragment C2b, and (C2a)i, a fragment which has lost its combining activity. Bound C2a if released, forms the inactive derivative $C2a^d$ [28].

$$EAC\bar{1},4b \xrightarrow[Mg^{++}]{C2 \quad C2b + (C2a)i} EAC\,1,\overline{4b,2a} \longrightarrow C2a^d + EAC1,4b$$

With the formation of C3 convertase, $C\bar{1}$ may be removed without interfering with the subsequent reaction sequence. $\overline{C4b,2a}$ acts enzymatically on C3 cleaving it into 2 fragments, C3a and C3b. C3a, with a molecular weight of about 7,000, has anaphylatoxic and chemotactic activities [46, 52]. A small portion of C3b becomes bound either to a $\overline{C4b,2a}$ site, antibody, or on the cell membrane near a $\overline{C4b,2a}$ site:

$$EAC\bar{1},\overline{4b,2a} \xrightarrow{C3 \quad C3a + (C3b)i} EAC1,\overline{4b,2a,3b}$$

The $\overline{C4b,2a,3b}$ site is probably identical with so-called C3-dependent peptidase [11].

A natural inhibitor of cell-bound C3 inactivates the fixed component but does not affect native C3 in plasma. This inactivator is identical with the conglutinogen-activating factor [22] which is essential for the preparation of C1,4,2,3 cells, prepared with purified human C components, to react with bovine conglutinin.

The C3b fragment fixed to immune complexes is responsible for immune adherence [35], reaction with conglutinin and immunoconglutinin [21], enhancement of phagocytosis [13] and the complement dependent adherence of antigen-antibody complexes to leukocytes and other cells [16].

The C567 Stage. The next step in the complement sequence is the reaction of $C\overline{423}$ with the C5 molecule, yielding 2 fragments C5a with a molecular weight of 15,000 and 10,000 for guinea pig [45] and human [8] sera, respectively, and C5b, molecular weight about 170,000 [28]. The C5b fragment becomes bound to the cell membrane and forms the base for the later acting complement components.

$$C\overline{4,2,3} \xrightarrow{C5 \rightarrow C5a} \overline{C4,2,3,5b}$$

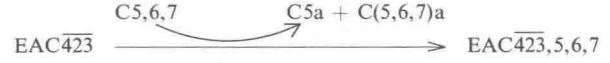
The smaller fragment, C5a, is capable of causing contraction of the guinea pig ileum. The anaphylatoxic activities of C5a and C3a differ in that no cross-desensitization (tachyphylaxis) can be demonstrated. The C5a fragment also has the capacity to attract rabbit polymorphonuclear leukocytes [45]. This chemotactic action represents an important activity of the complement system in inflammation. When guinea pig serum is treated with endotoxin, the C5a fragment which is generated, appears to have both anaphylatoxic and chemotactic activities [47, 51].

C6 and C7 interact with bound C5 in an unknown manner. The EAC1,4,2,3,5 complex decays at 37 °C with a half-life of 2 min. [12]. Similarly, the EAC1,4,2,3,5,6 complex is labile whereas the intermediate EAC1,4,2,3,5,6,7 is relatively stable [18]. The uptake of C5 and the reaction with C6 and C7 is greatly enhanced when all 3 components are present in the reaction mixture. It has been suggested that C5, C6 and C7 exhibit an affinity for each other and tend to occur as a complex in serum [37]. The fact that the C5,6,7 complex may act as a unit greatly increases the uptake of C5 onto the cell membrane.

An inactivator of bound C6 has been detected in serum [50].

The reaction of C5,6,7 with EAC$\overline{423}$ also gives rise to an activated trimolecular complex (C5,6,7)a which has a molecular weight more than 200,000 [52]. This high molecular weight factor is capable of inducing chemotactic activity of polymorphonuclear leukocytes.

The complex reaction of C5,6,7 may be illustrated as:



The generation of the C567-activated complex by a serum substance has recently been described [24]. The activated C567, which has a short half-life, can attach to normal red blood cell membranes, conferring on them the capacity to be lysed by C8 and C9. These cells have neither antibody nor components C1,4,2,3 on their surface. This phenomenon has been designated reactive lysis [24].

The reactions with C8 and C9. Following attachment of C5,6,7 the C8 component reacts and becomes physically taken up by the cell membrane [49]. Cells which have been acted upon by C8 undergo a low-grade, persistent lysis [31] and the C8 component may induce the functional lesion [49].

C9 becomes physically bound and functions as an enhancing factor to accelerate cell lysis [31]. The C9 component may be substituted by organic compounds which have chelating ability. Lysis of EAC1,4,2,3,5,6,7,8 cells, which resembles lysis by C9, can be produced with 1, 10-phenanthroline or 2, 2′-bipyridine and this lysis depends on their chelating function.

The sequence of events which follows the action of C8 and C9 on the cell surface is unknown. By electron microscopy, using negative staining techniques, membrane lesions may be seen which appear as holes which measure 80 to 100 Å in diameter. These lesions are characteristic of membrane damage by complement. Lysis begins with loss of intracellular small molecules, potassium, into the supporting medium, and the subsequent influx of extracellular water due to intracellular osmotic pressure [14] results in swelling of the cell and eventual rupture of the membrane.

Biological Activities of Complement

Recent progress in complement research has been most active in the biochemistry of the complement components and their reactions. These ad-

vances have been greatly facilitated by modern developments of techniques in protein purification and by the recognition of the sequential enzyme activation of the complement system. Some 'spin-off' of this biochemical research has resulted in improving our understanding of the biological activities of complement.

A summary of the biological activities of some of the complement intermediates and reaction products are presented in table II. In a number of instances, it is now possible to show that particular complement components, intermediate complexes, or activated component fragments are involved in particular biological functions. Further developments in this field will be evident throughout this symposium.

Table II. Biological activities of complement components

Complement complex	Component fragment	Function
C1,4		immunoconglutination [21]
C1,4,2,3		adherence to leucocytes [16] conglutination [21] histamine release [17] immune adherence [35] immunoconglutination [21] opsonization [13]
	C3a	anaphylatoxin [46] chemotaxin [52]
	C5a	anaphylatoxin [47] chemotaxin [45]
C1,4,2,3,5,6		arthus reaction [6,7] histamine release [17]
	C(567)a	chemotaxin [52]
C1,4,2,3,5,6,7,8		slow hemolysis [49]
C1,4,2,3,5,6,7,8,9		bacteriolysis [33] erythrocyte lysis [28] virolysis [38]

The recognition of families or the development of strains of animals which lack, totally or partially, specific complement components or inhibitors, allows the design of scientific experiments which will answer many questions regarding the purpose and function of the complement system.

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