Comprehensive Immunology 2

Biological Amplification Systems in Immunology

Edited by NOORBIBI K. DAY
and ROBERT A. GOOD

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Foreword

Interest in complement developed at the end of the nineteenth century from observations on cellular and humoral defense mechanisms against bacteria. It was recognized at that time that there were factors in body fluids of animals and man that were capable of killing and lysing bacteria in the absence of cellular factors. Due to the efforts of two of the founders of immunology, Bordet and Ehrlich, and their colleagues, by 1912 the multicomponent nature of complement action was well recognized, the sequence of reaction of the components in the lysis of erythrocytes was defined, complement fixation as a major tool for studying antibody–antigen interaction was well established, and studies on the physicochemical properties of the components had been started. Yet, with a few notable exceptions, research on complement was largely abandoned by most "mainstream" immunologists for the following two or three decades. When one looks at the contents of the present volume, it is hard to imagine that as recently as 20 years ago, there were probably fewer than ten major laboratories where complement research was the primary theme. The contents attest to the fact that there are today dozens of laboratories on three continents where research on complement is pursued in depth.

It is not easy to point to all the advances that have occurred in complement research during the past few years. The chapters in this book, however, offer a wide selection from the vast subject of complement research representing some of these advances. Our knowledge of complement action was put on a molecular basis by the analysis of the steps leading from the interaction of sheep red cells with antibody to the lysis of the cells due to complement action; from these studies we have progressed so that presently hemolytic activity can be defined on a molecular basis, activation and interaction are interpretable on a biochemical basis, and the physicochemical, biochemical, and biological consequences of these interactions have become amenable to chemical analysis.

It is now understood that the classical complement cascade consists of nine proteins, each existing in serum in a precursor form; the individual components are either activable to a specific enzyme or become part of a multimolecular complex with enzyme activity. The enzymes of the classical pathway are esterases and/or proteases and under certain conditions some can be replaced by trypsin and other common proteases. Thus the activation of a substrate component by the previously activated component in the sequence is usually accompanied by splitting of the substrate into two or more fragments. The various fragments have specific biological properties and most of these properties are discussed in various chapters.

Some of the biological functions link the cellular and humoral compartments of the immune system. For example, a fragment of C5 serves as a positive chemotactic agent for monocytes and granulocytes; monocytes and macrophages have surface receptors for fragments of C3; and there is some evidence that a functional C system in some instances is needed for induction of antibody production.

Ever since it was shown that complement causes injury to cells, investigators have postulated that cells can resist the cytotoxic action of complement. Studies on the cytotoxic effect of complement on nucleated cells now furnish evidence for an active defense mechanism of cells against immune attack. The *in vivo* activation of complement can lead in some instances to self-injury; often *in vivo* activation is accompanied by a drastic reduction in serum levels of certain components. In addition to pathological changes, components may be reduced or absent from serum for genetic reasons. The genetic lack of a component may be associated with pathological changes; however, this is not always the case. Problems concerning the significance of complement in clinical states are being intensively investigated and clarification of many of them is only a matter of time.

The recent revival of the properdin or alternative pathway demonstrates that there are several pathways within the complement system designed to permit activation of the various functions; such backup systems testify to the importance of complement in the preservation of the species; the ubiquity of complement among vertebrates also testifies to the evolutionary significance of complement. Complement seems so important in the individual that, at birth, most animals have a functional complement system. Studies in phylogeny and ontogeny of complement have raised questions of the genetic control and origin of complement. A new and exciting area of research is the genetic mapping of linkages of complement components; such studies may cast some light on the molecular evolution of complement.

During the last decade, we have also learned much about where complement is produced. Not only is the organ site of synthesis known for many of the components, but even the cell types producing complement have been identified in many cases.

On the biochemical side, the molecular structure of several components is being determined. In some instances, polypeptide chains have been identified and amino acid sequencing studies have been initiated. Without doubt such studies will be performed for most if not all components during the next decade.

It should be evident to the reader that even a book of this type cannot cover all that is known about complement. The treatment of the subject is necessarily weighted according to the points of view and special concerns of the individual authors. Thus omissions may have occurred. All in all, however, the editors have gathered together much new material in easily readable chapters that should make an exciting and adventurous journey through the complement system.

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Biochemistry and Biology of Complement Activation

W. OPFERKUCH and M. SEGERLING

1. Introduction

The immunological response of the organism to a foreign antigen involves both the humoral and cellular immune system. When antigen is recognized by humoral antibodies, this recognition step is the trigger of a biological reaction mediated by the complement system. The complement system, therefore, may be considered as the effector and amplification system of the humoral immune reaction.

The complement system consists of eleven distinct components, and thus far, seven inhibitors of single components have been described (Austen, 1974; Lepow, 1971; Mayer, 1973; Müller-Eberhard, 1974; Nelson, 1974; Rapp and Borsos, 1970) (see Section 3).

The single components exist in serum in an inactive precursor form. When activated, they react with each other in a certain sequence, during which various biological activities are generated. It is now well established that the complement system not only represents an important part of the host's defense mechanisms, but is also involved in various pathological processes.

Most of the biochemical events in each individual activation step and the interactions of the single components have been elucidated. The study of the immune hemolysis by antibody and complement has proved to be a fruitful model in these investigations. However, although observations made *in vitro* probably reflect what is happening biologically *in vivo*, one should be aware that *in vitro* observations may represent only a narrow section of the total complement activity *in vivo*. The latter is of complex nature and comprises not only the interactions of the eleven components, but also the influence of the inhibitory mechanisms by

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which it is balanced and moderated. In addition, the complement system is also connected in a poorly understood manner with other serum protein systems. e.g., with the blood-clotting, kinin, and plasmin systems (Kaplan and Vogt, 1974). Therefore, complement activity *in vivo* does not necessarily imply that the final step, the lysis of an invading parasite or other target cells, will be reached. Already, early events during the activation of complement in connection with the other serum protein systems cause considerable biological activities, amplifying the inflammatory processes in a beneficial or deleterious way.

This chapter will discuss the action and biochemistry of complement, obtained from *in vitro* experiments, in regard to possible concepts and aspects of its mode of action *in vivo*.

2. Biochemistry of Complement Activation

Biochemical data and the concentrations in human serum of each of the eleven known complement components are listed in Table 1. If the reaction sequence is started by immune complexes, it is called the *classical pathway* of complement activation. In addition to this pathway, a so-called alternative pathway is known, in which activation of the complement sequence starts when the third component becomes activated by the properdin system (Pillemer *et al.*, 1954) (see Chapter 2). Once activation is initiated, the components react sequentially in a cascade-like manner, by which some of them gain enzymatic activity (see Figure 1). Immune complexes normally initiate the activation of the complement sequence by binding to, and thus activating, the first component. In general, immune complexes consisting of IgG or IgM antibodies are effective in binding the first component of complement (Ishizaka *et al.*, 1966). Of sublasses of human IgG, γ_1 and γ_2 bind and activate C1 readily, γ_3 binds poorly (Ishizaka *et al.*, 1967), and γ_4 cannot bind at all (Augener *et al.*, 1971). IgA, IgD, and IgE have no complement-binding capacity (Ishizaka *et al.*, 1970). The chemical structure of the antibody molecule reacting with C1q is located on the Fc portion (Kehoe and Fougereau, 1969). This part of the molecule is exposed when the antibody has reacted with its corresponding antigen (Valentine and Green, 1967).

TABLE 1. Proteins of the Classical Human Complement System[a]

Protein	Serum concentration (μg/ml)	Sedimentation coefficient (S)	Molecular weight	Relative electrophoretic mobility	Number of chains
C1q	180	11.1	400,000	γ_2	18
C1r	—	7.5	180,000	β	2
C1s	110	4.5	86,000	α	1
C2	25	4.5	117,000	β_1	—
C3	1600	9.5	180,000	β_2	2
C4	640	10.0	206,000	β_1	3
C5	80	8.7	180,000	β_1	2
C6	75	5.5	95,000	β_2	1
C7	55	6.0	110,000	β_2	1
C8	80	8.0	163,000	γ_1	3
C9	230	4.5	79,000	α	—

[a]Reprinted from Müller-Eberhard, H. J., 1975, *Ann. Rev. Biochem.* **44**:697.

Dose–response experiments have shown that a single IgM molecule can fix C1, whereas two closely spaced IgG molecules (doublet) are required (Borsos and Rapp, 1965).

C1 is a macromolecule consisting of three distinct subunits: C1q, C1r, and C1s (Lepow *et al.*, 1963). Their molecular weights are listed in Table 1. The integrity of the molecule is calcium ion–dependent, and the subunits can be dissociated by chelating agents (Lepow *et al.*, 1963) and high ionic strength (Colten *et al.*, 1968). Both processes are reversible. Present evidence suggests that the activation of C1 is an internal step within the macromolecule, which is dependent on time and temperature. The subunit C1q is attached to the antibody of the immune complex (Calcott and Müller-Eberhard, 1972) and after it has been fixed, it converts C1r into a peptidase-like enzyme ($C\overline{1r}$) (Valet and Cooper, 1974a). The biochemical event leading to C1r activation is still unknown. C1s is a proesterase and is activated by $C\overline{1r}$ to become an active esterase (Naff and Ratnoff, 1968; de Bracco and Stroud, 1971; Valet and Cooper, 1974b). The activation of C1s results in the cleavage of its polypeptide chain (Sakai and Stroud, 1973).

Studies of the behavior of the C1 molecule by different chemical treatments and various purification methods suggested that C1q may consist of at least three different polypeptide chains (Opferkuch, 1967). This view was supported by the finding that C1 activity after ultracentrifugation at high ionic strength was recovered in the same region as serum albumin (Colten *et al.*, 1968), revealing that the C1q molecule consists of different noncovalently linked polypeptide chains. Structural

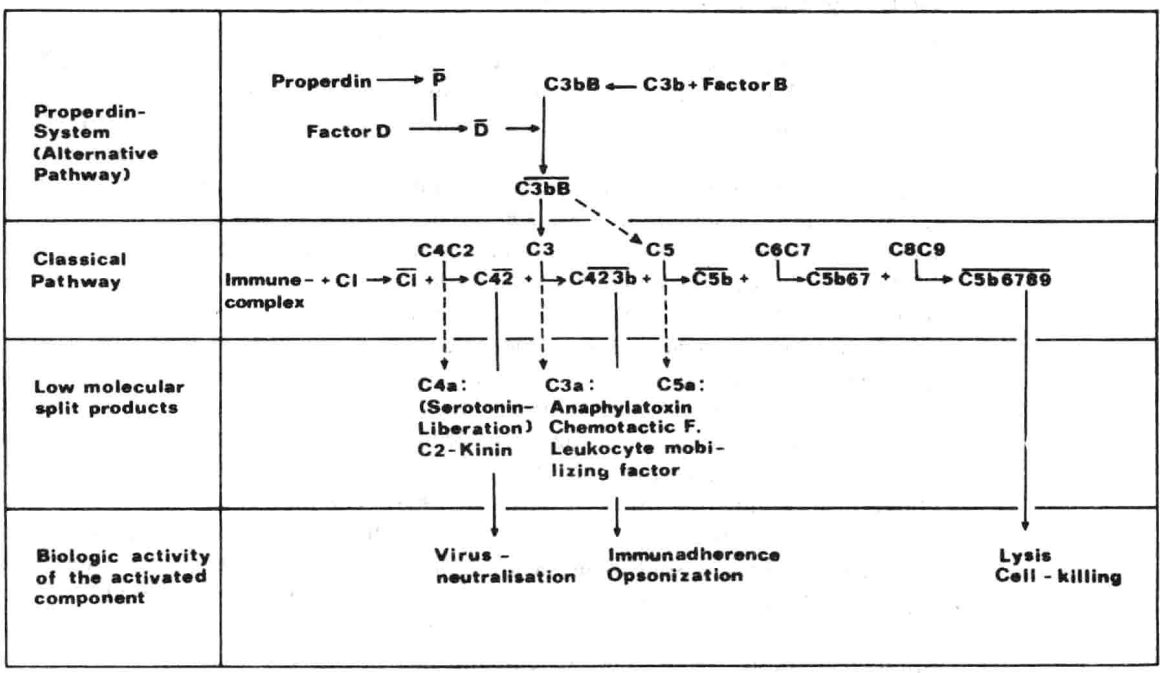

Figure 1. Diagrammatic representation of the classical and alternative pathways of complement activation.

analysis and electron microscopy of purified C1q led to the development of two models. The first one proposes that C1q is composed of six identical noncovalently bound subunits, and the second one assumes the existence of two different polypeptide chains (Shelton *et al.*, 1972; Calcott and Müller-Eberhard, 1972). The nonlinear dose–response curve of serum C1 indicates the existence of a hitherto unrecognized factor, which influences the activation of the C1 molecule (Loos *et al.*, 1973). A similar observation was made when a kinin fragment was added during C1 titration (Gigli *et al.*, 1971). Recently, Assimeh and Painter (1975) have presented evidence for the existence of a fourth subcomponent of C1, C1t.

2.2. Generation of the $\overline{42}$ Enzyme (C3-Convertase)

The natural substrates of the C1-esterase are the components C4 and C2 (Becker, 1956; Lepow *et al.*, 1956a; Lepow *et al.*, 1956b). Both are split into two fragments: a major one, which represents the activated component, and a small polypeptide fragment, which can be detected in the fluid phase. The C4 molecule consists of three polypeptide chains (α, β, and γ) that are linked by disulfide bonds (Schreiber and Müller-Eberhard, 1974). During activation of C4, a small polypeptide fragment called C4a (6000–7000 daltons mol. wt.), is cleaved from the α chain by C1s (Patrick *et al.*, 1970; Budzko and Müller-Eberhard, 1970), thus uncovering the binding site of activated C4, C4b. Simultaneously, a second functional area is exposed, which represents a very stable binding site for C2 (Müller-Eberhard *et al.*, 1967). Recent studies (Cooper, 1975) showed that this binding site might be located on the α chain. C4b molecules, bearing oxidized human C2, were not accessible to a C4b inhibitor that splits the α chain into the α_2 and α_3 fragments (Cooper, 1975). The combining site of the C4 molecule has a very short half-life and undergoes rapid decay unless it becomes bound to its corresponding receptor.

Due to the short half-life of its membrane binding capacity, only about 10% of activated C4b is actually bound to the cell membrane, whereas 90% can be detected in a hemolytically inactive state in the supernatant (Polley and Müller-Eberhard, 1966). Nevertheless, the activation of C4 has an amplifying effect on the further complement reaction. The high serum level of C4 enables a single activated, bound C1 molecule to assemble at least 200 molecules of C4b around its hemolytic site, which could be shown by the uptake of radioactively labeled C4 (Cooper and Müller-Eberhard, 1968) and by hemolytic analysis of the SAC$\overline{42}$ intermediate (Opferkuch *et al.*, 1971a; Borsos and Opferkuch, 1970).

It has been reported that in the presence of magnesium ions, the native C2 molecule and activated C4 can form a loose inactive complex either in the fluid phase or in a cell-bound state (Sitomer *et al.*, 1966). Interaction of the $\overline{C1s}$ esterase with C2 results in splitting of the C2 molecule into two fragments. The larger one, C2a, remains bound to the C4b, thus representing the activated $\overline{42}$ enzyme or C3-convertase (Polley and Müller-Eberhard, 1968). The molecular weight of activated C2 (C2a) is about 33,000 daltons less than that of the native molecule. The polypeptide which is split from the C2 molecule could not be isolated, and little is known as to whether the C2b fragments consist of single polypeptide chains or whether they are even split further into more pieces (Mayer *et al.*, 1967; Polley and Müller-Eberhard, 1968). The $\overline{42}$ enzyme has proteolytic activity and its natural substrates are C3 and C5 (Müller-Eberhard *et al.*, 1967; Shin and Mayer, 1968; Shin *et al.*, 1968).

Treatment of human C2 with critical amounts of iodine results in a marked increase of hemolytic activity and a prolonged half-life stability of the activated $C\overline{42}$ enzyme (Polley and Müller-Eberhard, 1967). It is assumed that the C2 molecule undergoes a chemical modification after iodine treatment, which is supported by the following observations: Inactivation of C2 by binding of *p*-chloromercuribenzoate (*p*-CMB) indicates the presence of free, reactive SH groups (Leon, 1965). Upon iodination, the SH groups are oxidized and form intramolecular disulfide bonds, and the C2 molecule is no longer accessible to the binding and inactivation of *p*-CMB. However, after mild chemical reduction of the iodinated C2, both increased hemolytic activity and prolonged enzymatic stability return to values of native, untreated C2, and hemolytic activity can be inactivated by *p*-CMB (Polley and Müller-Eberhard, 1967).

The $C\overline{42}$ enzyme is unstable and undergoes a time- and temperature-dependent inactivation (Mayer *et al.*, 1964; Borsos *et al.*, 1961b). During this process, activated C2a is converted into the inactive form C2d (Stroud *et al.*, 1966), which is released from the C4b molecule into the fluid phase. The activity of the $C\overline{42}$ enzyme can be restored when another activated C2a molecule is bound to its receptor on the C4b molecule. Recently it could be shown that a cell-membrane (Hoffmann, 1969a,b) and a serum-associated factor (Opferkuch *et al.*, 1971a) accelerate the natural inactivation of C2a. These factors are probably the reason for the natural decay.

2.3. Activation of the C3 Molecule and Its Antigenic Properties

The C3 molecule consists of two polypeptides (α and β chain), which are linked by disulfide bonds (Nilsson *et al.*, 1975). When C3 is activated by the $\overline{42}$ enzyme (C3-convertase), a small fragment, C3a (about 9000 daltons mol. wt.) (Bokisch *et al.*, 1969), is split off the *N*-terminal part of the α chain (Nilsson and Mapes, 1973). The larger fragment, C3b, represents the activated component, which in its nascent state forms a triple complex with the C3-convertase, the $\overline{423b}$ enzyme; and C3b becomes firmly bound to the cell membrane (Müller-Eberhard *et al.*, 1966).

Once C3b is activated and bound to the cell membrane, its hemolytic activity is very stable. C3b fulfills important biological functions in phagocytosis and possibly in the humoral immune response (see Section 4.3).

C3b can be enzymatically degraded by a naturally occurring inactivator (C3b inactivator), as will be discussed later. The resulting fragments are C3c and C3d (Ruddy and Austen, 1971). C3d remains bound to the cell surface and consists of polypeptides that were originally part of the α chain. The second split product, C3c, can be recovered from the supernatant and is composed of the β chain and parts of the α chain. All these split products—C3a, C3b, C3c, and C3d—bear distinct antigenic determinants, against which specific antisera have been raised (Pondman and Rother, 1972; Molenaar *et al.*, 1974).

2.4. The Role of Activated C5 and the Formation of a C5b6789 Complex

Similar to the C3 molecule, C5 is composed of two polypeptide chains (α and β), which are linked by disulfide bonds (Nilsson *et al.*, 1975). The $\overline{C423b}$ enzyme splits a polypeptide of molecular weight of between 9000–15,000 daltons from the *N*-terminal part of the α chain (Shin *et al.*, 1968; Cochrane and Müller-Eberhard,