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Bacteria and Complement

Edited by Michael Loos

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Preface

Deficiencies in any of the defense mechanisms of the host can lead to severe microbial infections; these are of clinical relevance. Broad up-to-date knowledge in this field allows identification of many unspecific as well as highly specific defense reactions involved in the struggle against infectious diseases. On the other hand, protective structures on the microbial cell surfaces have been adapted and improve the counterpart's chances of survival. In particular, it has been considered that the great diversity of the bacterial envelopes not only determines the anatomical location of the tissue injury but also induces activation of distinct parts of the complex defense system.

The specific defense mechanism, whose most prominent constituent is provided by the antibodies, is in most cases not available when a bacterium successfully enters the host. In this preimmune phase, unspecific defense mechanisms are required. Since the end of the last century, when BUCHNER made the observation that normal serum exerted a bactericidal effect, many investigations have been performed to detect the bacteria-killing principle in serum. Recent findings reveal a strong dependency of the serum bactericidal effect of components of the complement system. Many bacteria are killed by the action of complement; they are designated serum-sensitive. Organisms surviving the nonspecific killing action of serum can be eliminated later on in the development of the specific cellular and humoral response. Until their elimination, the organisms are invasive and cause further tissue-injurious processes which can only be restricted by antibiotic therapy. For that reason it is of eminent interest to detect the mechanism by which complement kills serum-sensitive bacteria.

The editor invited leading scientists to summarize the present status in their fields of research pertaining to bacteria and complement. The main topics concern the interaction mechanisms of gram-negative and gram-positive bacteria with complement with respect to the events triggered on the bacterial envelope. This volume summarizes our knowledge of this unspecific but effective defense mechanism of the infected host.

Mainz, Summer 1985 F. CLAS

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Bacteria and Complement – A Historical Review

M. Loos

In the second half of the nineteenth century, shortly after it became clear from the work of Louis Pasteur (1822–1895) and Robert Koch (1843–1910) that microorganisms cause infectious diseases, extensive studies were untertaken to elucidate the mechanisms of protection from and resistance to infections. This early history of the investigation of immunity is characterized by the dispute between two opposing schools, the protagonists of the "cellular" and the "humoral" theories. Eli Metchnikoff (1845–1919) was the first to recognize the general significance of the phenomenon of phagocytosis in animal tissues. In 1883, he published his first papers in the presentation of the theory of phagocytosis. He "sought especially to develop the idea that the intracellular digestion of microcellular organisms and of many invertebrates had been hereditarily transmitted to the higher animals and retained in them by the ameboid cells of mesodermic origin. These cells, being capable of ingesting and digesting all kinds of histological elements, may apply the same power to the destruction of microorganisms." Despite initial struggle, the phagocytes were soon accepted as the principal, if not the only, defenses of the body against bacterial invasion.

Only a few years later, in 1888, Nutall found that blood of different species had bactericidal activity against *Bacillus anthracis*, *Bacillus megaterium*, *Bacillus subtilis*, and *Staphylococcus aureus*. He observed that destruction took place independently of leukocytes and that bactericidal activity was destroyed by heating to 55° C for 30–60 min. Investigating the effect of certain molds on the clotting of plasma, von Grohmann observed as early as 1884 that these organisms, when treated with plasma, suffered retarded development. Furthermore, he showed that anthrax bacilli had a greatly reduced virulence to guinea pigs after exposure to plasma. Although he was not directly investigating the bactericidal activity of serum, Buchner (1889a) gave him the credit for the first observations in this field.

In 1889, Buchner confirmed the observations by Nutall and found that cell-free serum has a bactericidal activity against a variety of gram-positive and gram-negative bacteria. Dialysis of fresh serum against water at 0° C for 18–36 h eliminated bactericidal activity, but there was no loss of activity following dialysis against 0.75%–0.80% sodium chloride solution buffered with sodium bicarbonate. Applying the principles of biochemistry to this problem, he concluded that the ability of fresh serum to kill bacteria was due to serum

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proteins with enzymatic activity. The heat-labile, bactericidal activity BUCHNER named "alexine." On 7 May, 1889, BUCHNER (1889b) made the following statement at a lecture in Munich: „Das Vorhandensein bakterienfeindlicher Wirkungen durch flüssige Bestandteile der Körpersäfte läßt die überall nachweisbare Tätigkeit der Phagocyten als weniger ausschlaggebend erkennen." (The presence of bactericidal action in body fluids reveals the overall detectable activity of phagocytes as less decisive.) This statement, by the chief advocate of the humoral theory of resistance to microbial infections, was directed against the cellular theory of immunity proposed by METCHNIKOFF. In his answer to BUCHNER's critique, METCHNIKOFF (1889; English translation 1905) came to the conclusion that based on his own work "the postulates of this theory are often not in accord with the real facts," and that the bactericidal effect of body fluids has nothing in common with the phenomenon of immunity («la propriété bactéricide des humeurs ne correspond nullement aux phénomènes de l'immunité»). However, they "expressed the opinion that a portion at least of the bactericidal power might come from substances that had escaped from the leukocytes during the preparation of the defibrinated blood and of blood serum." Therefore, "alexine is nothing but a leukocytic product."

Another humoral defense was described by VON BEHRING and KITASATO in 1890, who found that the serum of animals which had received a series of injections of nonlethal doses of tetanus toxin had the power to neutralize tetanus toxin specifically and to protect normal animals from otherwise lethal doses of toxin. In addition, PFEIFFER and ISSAEFF (1894) made the observation that when living cholera vibrios were introduced into the peritoneal cavity of guinea pigs previously inoculated with killed cultures or vibrios, they were dissolved, i.e., they underwent bacteriolysis. They found that for a given organism, the bacteriolytic power of serum from animals immunized with that organism was far greater than that from nonimmunized animals.

In 1896, BORDET showed that the lysis of the vibrios was independent of cells. He found that serum from an immunized animal, inactivated by standing or by heat, could be reactivated and restored to its former bactericidal activity by the addition of a small volume of normal serum. BORDET, therefore, suggested that the bactericidal action of serum was due to two factors. One was relatively heat stable and specific for the cholera. This "preventive substance" was incapable of killing bacteria in the absence of the second factor, although it could produce "clumping" of the organisms. The second factor, which he assumed was identical with BUCHNER's alexine, since it was destroyed by heat or standing, was responsible for the actual lysis of the bacteria. The first, not always present in normal serum, appeared in large amounts in the "immune serum" in response to immunization and was later called "sensitizer," "amboceptor," or as it is commonly known today, "antibody." The second factor, alexine, was found to be present in all normal sera and was not increased during immunization and was later called "addiment" or "complement" by PAUL EHRLICH. Based on these observations that bacteria are lysed by antibody and complement, BORDET discovered in 1898 that complement will lyse erythrocytes (E) sensitized with specific antibodies (A) to antigen of the erythrocytes. He immunized animals with red blood corpuscles from another species and produced an antiserum

that lysed these corpuscles. As in bacteriolysis, the lysis was proven to be the result of complement (C) acting on the erythrocytes sensitized by antibodies specific for them. Together with GENGOU, he showed that complement was adsorbed to sensitized bacteria without necessarily causing lysis.

Most of our knowledge about complement action has come from studies using BORDET's EA (immune hemolysis) system. EHRLICH and MORGENROTH (1899a, b) studied the kinetics of the interaction of EA and C and clearly demonstrated the sequential nature of the action of antibody and complement. They found that the antibody served to detect the antigen and to prepare the site for the lytic action of complement upon the target cell. Soon, it became clear that complement is a system composed of several components designated C1, C2, C3, and C4. However, in 1943 the definition of C was essentially the same as that used by BORDET in the late 1800s. Thus, PILLEMER (1943) defined complement as "the portion of fresh blood or plasma which is not increased on immunization and which, when added to certain sensitized cells, continues with the sensitized cells and, under appropriate conditions, results in their destruction or death."

In the 1940s, ECKER and PILLEMER, and HEIDELBERGER and MAYER began to study not only the formation, but also the structure with the purpose "of elevating complement from the rank of a mysterious and elusive property of serum to the status of a composite of distinct individual chemicals" (PILLEMER 1943). At this time, it was like a dogma that complement activation can only be initiated by the antibody molecule. The antibody had undoubtedly a dominant role, if not the monopoly in host defense. From this point of view it is not surprising that when PILLEMER and his colleagues (1955) described an antibody-independent pathway of complement activation that involved a new serum protein termed "properdin," the "scientific" world doubted, challenged, and ridiculed their observations. PILLEMER and coworkers found that incubation of serum with zymosan (a suspension of boiled yeast cell walls) depleted serum of C3 hemolytic activity with relatively little apparent loss of C1, C4, and C2. This reaction was found to be rather complex: Other serum factors were required for zymosan to fix C3 and when fixed, its biological activity could not be recovered even by using a variety of procedures. After initial reservations, (PILLEMER et al. 1953a, b) the reaction(s) that lead to C3 consumption by zymosan were explained by the announcement of an alternative pathway for complement activation bypassing the early C components C1, C4, and C2 (PILLEMER et al. 1954, 1955, 1956). In addition to zymosan, many other high molecular-weight polysaccharides and bacterial cell walls were found to fix C3 by the properdin-mediated pathway. Therefore, it was thought that this new pathway may play a fundamental role in host defense. However, at this time, antibody research, having reached its first summit, virtually barred propagation of the idea of an antibody-independent host defense mechanism.

More than 10 years later, when the complement sequence had meanwhile been found to be composed of nine distinct proteins (C1–C9), the alternative pathway was again getting attention in studies of the interactions between bacterially derived lipopolysaccharides (LPS) and the complement system, which clearly established that certain substances (e.g., LPS and zymosan), indeed,

could preferentially induce consumption of C3–C9 (the late-acting C components), without affecting the early-acting C components (C1, C2, and C4; BLADEN et al. 1967, GEWURZ et al. 1968). It was found that each of the six terminal C components was consumed in this process with the generation of anaphylatoxin (C3a) and neutrophil chemotactic factors (C5a) (reviewed in GEWURZ 1972). The consumption of C3–C9 was found to occur also in a variety of immunoglobulin-deficient sera, emphasizing the possibility that an alternative pathway analogous to that proposed by PILLEMER and coworkers was involved. Due to the application of advanced protein chemistry it was finally proven by several groups that besides the "classical" C components the alternative pathway consists of several distinct factors (e.g., B, D, P; reviewed in PANGBURN and MÜLLER-EBERHARD 1984).

The rediscovery of the properdin system or the "alternative pathway of complement activation" fully confirmed the earlier observations by PILLEMER and his colleagues. It also made clear that the organism possesses a naturally occurring defense system against microorganisms, which is antibody independent.

In the middle of the 1970s, it also became clear that the classical pathway (C1, C4, and C2) has an important function in host defense during the preimmune phase by direct, antibody-independent binding of C1 to bacterial surfaces (Loos et al. 1974; Loos et al. 1978). This subject is discussed in a subsequent chapter.

Since it was mentioned at the beginning that the early history of the investigation of immunity was characterized by the dispute between the protagonists of the cellular and the humoral theories, it should be noted that today, almost a century later, it is well established that METCHNIKOFF'S macrophages are one of the prominent cell types for serum complement production. However, the individual C components are not merely liberated into the fluid phase as an artefact of in vitro manipulation, as was assumed by METCHNIKOFF. The living macrophages synthesize and secrete most of the C components (BENTLEY et al. 1981; Loos 1983). Furthermore, phagocytosis, especially of microorganisms, requires an intact humoral defense system (i.e., complement alone or antibodies partially cooperating with complement).

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The Complement System: Activation and Control

M. Loos

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1 Introduction

One of the hallmarks of immunology has been analysis and characterization of the C system in biological fluids. It is composed of 11 proteins of the "classical" pathway:[1] C1q, C1r, C1s, C4, C2, C3, C5, C6, C7, C8, and C9. There are three proteins of the "alternative" pathway (IUIS-WHO Nomenclature Committee 1981) B, D, and P. Finally, there are four control proteins: $C\bar{1}$ inhibitor ($C\bar{1}$ INH) and C4b binding protein (C4b-bp) for the classical pathway, I (C3b inactivator or C3b INA) and H (β_1H or C3b INA accelerator) for the alternative pathway, and anaphylatoxin inactivator. Due to the dramatic advances in protein chemistry, these 19 distinct serum proteins have been highly purified and characterized physiochemically (Table 1).

For some of these proteins the amino acid compositions and sequences are partially completed (for further details see Cooper 1985, Fothergill and Anderson 1980, Müller-Eberhard 1975, Müller-Eberhard and Schreiber 1980, and Porter and Reid 1979). The partial understanding of the structure

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[1] The nomenclature used for the proteins of the classical complement pathway conforms with that agreed upon by the World Health Organization (Bull. Wld. Hlth. Org. 39:935–938, 1968): sheep erythrocytes (E) sensitized with rabbit antibody (A) containing complement (C) components $EAC\bar{1}$, EAC4, $EAC\bar{1}4$. A component in the activated state, such as $C\bar{1}$, is designated with a bar over the number, $C\bar{1}$; the activated forms of two subcomponents of native C1, C1r, and C1s, are written with a bar over the letter, $C1\bar{r}$ and $C1\bar{s}$.

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Table 1. Physiochemical properties of the components and regulatory proteins of the complement system

Component	Mol. wt.	Serum concentration (μg/ml)	Sedimentation coefficient	Electrophoretic mobility	Polypeptide chains number, mol.wt.	Activation products	Polypeptide chains (number, mol.wt.)
Classical pathway							
C1	900000	200–300	19				
C1q	410000	150–180	11	γ_2	18:6A: 24000 18:6B: 23000 18:6C: 22000	–	–
C1r	85000	50	7	β	1: 85000	$C1\bar{r}$	1a: 56000 1b: 27000
C1s	85000	100	4	β_1	1	$C1\bar{s}$	1a: 56000 1b: 27000
C4	210000	400–450	10	β_1	3: 90000 3: 78000 3: 33000	C4a C4b	1: 10000 3: 85000 3: 78000 3: 33000
C2	110000	30	6	β_1	1	C2a C2b	1: 75000 1: 35000
Alternative activating pathway of complement							
B	93000	200–225	6	β_2	1	Ba Bb	1: 30000 1: 63000
D	25000	1.5		γ	1	$\bar{D}$	1: 22000
P	220000	10–25	5.2	β	4: 56000		
Common pathway							
C3	195000	1200–1300	10	β_2	2:110000 2: 85000	C3a C3b	1: 9000 2:110000 2: 75000
C5	205000	80	8.7	β_1	2:120000 2: 85000	C5a C5b	1: 11000 2:110000 2: 85000
C6	128000	75	6	β_2	1		
C7	121000	55	6	β_2	1		
C8	155000	80	8	γ_1	3: 77000 3: 63000 3: 14000		
C9	79000	200	4.5	α	1		
Regulatory proteins							
$C\bar{1}$-INA	109000	180	4	α_2	1		
C4b-bp	540000		10.7	β/γ	6–8: 70000		
H(β_1H)	150000	500–520	6.4	β	1		
I(C3b-INA)	93000	25–35	4.5	β	2: 55000		
Anaphylatoxin inactivator (AI)	310000	30	9.5	α_2	8: 36000		

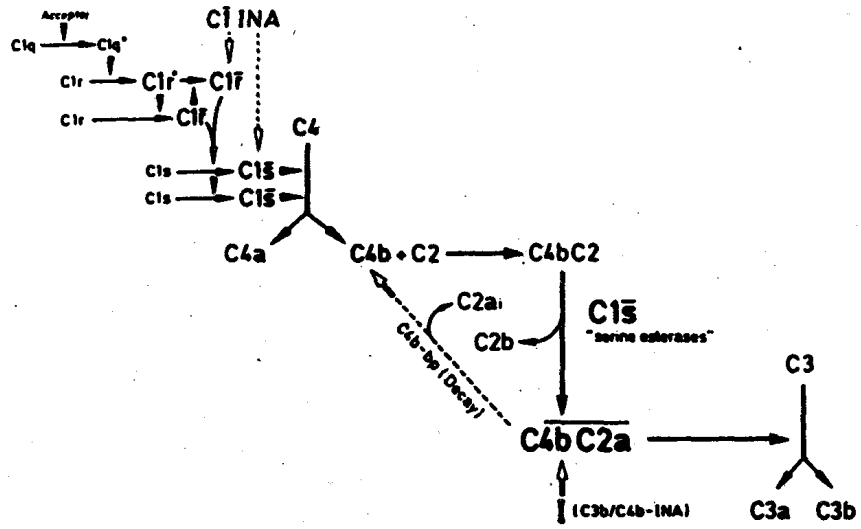

Fig. 1. Schematic diagram of the activation cascade of the classical complement pathway

of the individual C proteins led to an increased understanding of their functions. It became clear that complement is not only involved in antibody-mediated reactions such as lysis of bacteria, viruses, or antibody-coated erythrocytes or other cells. Complement probably has even more important functions in inflammation, chemotaxis, and phagocytosis by generation of small peptides (C3a, C4a, C5a) during the complement activation sequence that are themselves chemotactic and vasoactive as well as having the capacity to cause degranulation and activation of granulocytes and macrophages.

The third component, C3, which probably is the most important C component in terms of host defense, can be activated by two different pathways: the classical pathway starting with C1, C4, and C2, and the alternative pathway via a cleavage product of native C3, namely C3b, together with B, D, and P (Fig. 1). Within the past few years, the sequences of both pathways leading to C3 activation were analyzed.

2 The Classical Pathway

The activation of the complement system via the classical as well as via the alternative pathway represents sequentially occurring multistep activation cascades.

The classical pathway (Loos 1982a) can be activated by antigen-antibody complexes and involves the C components C1, C4, and C2 (Fig. 1). C1 by itself is a macromolecule composed of C1q and doublets of C1r and C1s which are linked by Ca^{++} ions ($C1r_2 \cdot Ca^{++} \cdot C1s_2$). The standard system for measurement of classical pathway activity has been sheep erythrocytes (E) sensitized

with specific antibody (A) of the IgG or IgM class. Both antibodies, IgM and IgG, have a binding site for the C1q subcomponent of macromolecular C1. The binding sites are located in the Fc portion of the antibody molecules (IgM, $C\mu4$ (Fc) 5 μ; IgG, C_H2). The human IgG subclasses, however, have different binding affinities for C1q and C1. The subclass IgG3 is the most efficient followed by IgG1, and to a lesser extent IgG2. In contrast, IgG4 is similar to Fab or $F(ab')_2$ fragments, and does not bind C1 or C1q. A single molecule of anti-Forssman antigen IgM on the surface of a sheep red blood cell is capable of binding one C1 molecule and initiating the C cascade. In contrast, at least two IgG molecules in close proximity ("doublet") on the cell surface are required to fix a single C1 molecule. In addition to the immunoglobulins IgM and different subclasses of IgG, a variety of other substances interact directly with C1 and C1q (Loos 1982b). A number of polyanionic substances because of their negative charges form a complex with C1q by reacting with the cationic C1q, the most basic serum protein with an isoelectric point of about pH 9.2. A direct binding of C1q was also shown for polynucleotides such as polyiniosinic acid, DNA carageenan, heparin, dextran sulfate, polyvinyl sulfate, polyanethol sulfonate (Liquoid), and chondroitin sulfate. Certain bacterially derived lipopolysaccharide (LPS) preparations bind directly to isolated C1 via C1q. This C1q-LPS interaction appears to be influenced by the core and the O-specific sugar portion of LPS because the lipid A portion, which is identical in all LPS, has a strong C1q binding capacity. Dinitrophenylated human serum albumin (DNP-HSA) and trinitrophenylated erythrocytes (E-TNP) were also shown to bind directly to C1q independently of antibody. The C1-binding capacity was proportional to the DNP substitution rate. The envelopes of some RNA viruses, including Maloney leukemia virus and vesicular stomatitis virus, also have receptors for C1. The C-reactive protein (CRP), an acute phase serum protein, binds directly to C1q and C1. In this case the complex behaves remarkably similar to C1q and C1 bound to immunoglobulins. A polysaccharide (PS) isolated from the venom of the tropical, arboreal ant *Pseudomyrmex* sp. reacted with C1q only when a certain site of C1q was available to PS.

2.1 The Internal Activation of C1

The activation of C1 by antigen-antibody complexes revealed that different steps are involved which lead to the internal activation of native C1 (Loos 1982a):

Binding of C1 to antigen-antibody complexes via C1, the recognition unit of C1, is temperature and time independent. The conversion of bound C1q to C1q* is dependent on temperature and represents a conformational change which occurs within the C1q molecule as shown by using monoclonal anti-C1q antibodies. It could be demonstrated that the binding of C1q to immune complexes exposes new antigenic determinants, whereas other determinants present on fluid phase C1q are no longer detectable on C1q bound to EA. The appearance of C1q* induces a further conformational change in C1r leading reversibly

to the exposure of the enzymatic site of one C1r monomer, designated C1r*. C1r* cleaves the second monomer of C1r to $C1\bar{r}$ that is present in macromolecular C1. $C1\bar{r}$ cleaves now the single chain C1r* molecule which activated it initially and a second two-chain $C1\bar{r}$ molecule is generated. The last step of the internal activation is the cleavage of C1s to $C1\bar{s}$ by $C1\bar{r}$, leading to the C1 molecule described as activated C1, namely $C\bar{1}$.

There are a number of similarities between the two subcomponents $C1\bar{r}$ and $C1\bar{s}$: both are serine esterases which are inhibited by $C\bar{1}$ INA, DFP, and NPGB. Both precursor forms are single-chain molecules which are cleaved upon activation into a larger "a" and a smaller "b" chain linked by disulfide bonds. The identities of the molecular weights of precursor chains and chain fragments were confirmed by showing a high similarity in their amino acid composition, especially of the esterolytic center. However, the two subcomponents differ in their substrate specificities: $C1\bar{r}$ can activate only C1s, but not C4 and C2; $C1\bar{s}$ can not activate C1r, but it does cleave C4 and C2. C1s does not cleave C4 and C2. Furthermore, C1s does not bind to C1q in the absence of C1r, but ^{125}I-C1r is taken up by EA provided that C1q is present.

2.2 The $C\bar{1}$ Inactivator, a Naturally Occurring Control Protein for $C\bar{1}$

The $C\bar{1}$ INA is a naturally occurring protein in serum (ZICCARDI 1983). It inhibits not only $C1\bar{r}$ and $C1\bar{s}$, but also plasma kallikrein, plasmin, trypsin, chymotrypsin, Hageman factor (factor XIIa), and activated thromboplastin antecedent (XIa). The inhibitor is a heat- and acid-labile α_2-neuraminoglycoprotein which binds stoichiometrically with $C\bar{1}$. Although functionally identical, the $C\bar{1}$ INA of guinea pig serum differs from human $C\bar{1}$ INH in its molecular weight and sedimentation rate: human $C\bar{1}$ INH has a mol.wt. of 90000 to 110000 and a sedimentation rate of 3.0–4.2 S, whereas guinea pig $C\bar{1}$ INH has a mol.wt. of 170000 and a sedimentation rate of 6.1 S, twice that of human $C\bar{1}$ INA.

It has been reported that human $C\bar{1}$ INA interacts with $C1\bar{r}$ and $C1\bar{s}$ in macromolecular $C\bar{1}$, resulting in a stable $C1\bar{r}\cdot C1\bar{s}\cdot C\bar{1}$ INA complex. The latter was demonstrated by antigen-antibody crossed immunoelectrophoresis to migrate in the α_2 position. Under physiological conditions, the interaction of human $C\bar{1}$ INA with $C1\bar{r}$ and $C1\bar{s}$ in a macromolecular human $C\bar{1}$ caused the release of these subcomponents from immune complex-bound $C\bar{1}$, showing that binding of $C\bar{1}$ INA decreased the inter-subcomponent binding forces in the $C\bar{1}$ molecule; the subcomponent C1q remained bound to the immune complexes. The observation that the $C\bar{1}$ INA binds to both $C1\bar{r}$ molecules is also in agreement with the second-order reaction observed for guinea pig $C\bar{1}$ INA and $C\bar{1}$. It should be pointed out that, in regard to activation of macromolecular C1 to $C\bar{1}$, the subcomponent $C1\bar{r}$ is the first to be acted on by the $C\bar{1}$ INA, emphasizing that C1 activation may be inhibited at a state where C1s remains a zymogen. Furthermore, $C\bar{1}$ INA is the regulatory protein in serum controlling spontaneous activation of C1 in serum.

2.3 Formation of the C3 Convertase, $\overline{C4b2a}$

The natural substrates of $\overline{C1s}$ in serum are C4 and C2, the next two components in the C cascade of the classical pathway. Cleavage of C4 and C2 on cell surfaces by $\overline{C1s}$ leads to the formation of the classical pathway C3 convertase, $\overline{C4b2a}$ (Loos and Heinz 1984). The component C4 is composed of three polypeptide chains, α, β, γ with mol.wts. of 90000, 70000, and 33000. C4 is a very sensitive substrate of $\overline{C1s}$. When subjected to the action of $\overline{C1s}$ from the α-chain a 6000-dalton peptide (C4a) is cleaved and on the larger fragment of C4 (C4b) a highly labile binding site is exposed. Only when the cleavage of C4 takes place in the presence of acceptor sites for C4b, such as $EA\overline{C1}$, C4b sites are generated, $EA\overline{C1}4b$, for the further participation of C4 in the cascade. Otherwise, in the absence of acceptor sites for C4b the labile binding site on C4b is no longer available, and C4 becomes C4bi incapable of furthering the C cascade.

The formation of a hemolytically active C4b site represents the binding site for C2, the second natural substrate of $\overline{C1s}$. The generation of the classical C3 convertase, the third enzyme besides $\overline{C1r}$ and $\overline{C1s}$ of the classical pathway, is at least a two-step reaction. First, binding of native C2 to bound C4b, i.e., $EA\overline{C1}4b$, is dependent on the presence of Mg^{++} ions. The uptake occurs even at 0° C and is completed within 5–10 min. The conversion of the C4b2 complex into the C3 convertase is dependent on the enzymatic cleavage of C2 in C2b and C2a by $\overline{C1s}$ or other proteolytic enzymes like trypsin, chymotrypsin, etc. The larger fragment C2a remains bound to C4b and the formed $\overline{C4b2a}$ complex now becomes able to cleave the next component of the cascade, C3. In contrast to the C4b2 complex, the newly formed $\overline{C4b2a}$ is no longer dependent upon Mg^{++}, indicating a Mg^{++} independent arrangement of C2a with C4b. Thus, $\overline{C4b2a}$ is no longer sensitive to chelating agents like EDTA or EGTA. The enzymatic site of the C3 convertase is located in the C2a molecule and has substrate specificity for C3 and after reassociation with the major split product C3b ($\overline{C4b2a3b}$) enzymatic activity against C5. Therefore, C2 is the zymogen carrying the proteolytic site for C3 and C5 in the C3 and in the C5 classical pathway convertases. $\overline{C4b2a}$ is unstable and undergoes a time- and temperature-dependent decay unless there is a sufficient quantity of C3 in the vicinity of the cell-bound complex to mediate the next site in the sequence. During this decay, the C2a fragment is released in a functionally inactive form, C2ai. The remaining C4b site is now able to take up new native C2 and a new $\overline{C4b2a}$ enzyme can be generated.

2.4 C4b-Binding Protein

Serum contains a glycoprotein with a specific binding affinity for C4b which is thus termed C4 (or C4b) binding protein (C4-bp or C4b-bp; Nussenzweig and Melton 1981). C4-bp is the control protein of the classical C3 convertase, $\overline{C4b2a}$. It binds specifically to C4b and controls the formation and function of $\overline{C4b2a}$ in two ways. First, C4b-bp by itself prevents the assembly of $\overline{C4b2a}$

and accelerates the release of C2a from C4b. This effect is obviously caused by a competition between C4b-bp and C2a for binding to C4b. Secondly, together with the serum enzyme C3b/C4b inactivator, C4b-bp cleaves the α'-chain of C4b, leading to an irreversible loss of the ability of C4b to participate in the formation of C3 convertase. In both cases, the consumption of C3 is diminished as a result of the action of C4b-bp on C4b.

3 The Alternative Pathway of Complement

The alternative pathway of complement leads (Fig. 2) under the participation of C3b, the major split product of C3, factors D and B, as well as factor P (properdin) to the generation of C3 convertases (PANGBURN and MÜLLER-EBERHARD 1984). A variety of activators of this pathway have been described such as bacterial (LPS), yeast (zymosan), or plant (inulin) polysaccharides, polyanionic substrates like dextran sulfate, a cobra venom factor (CVF), the Fab portions of immunoglobulins, e.g., IgA or IgE, etc. The mechanisms by which these heterogenous groups of substances can initiate the activation of this pathway are only partially understood. Since the activation of this pathway requires C3b as acceptor for factor B, one postulates that the binding site for B on C3 is generated by a nucleophilic modification and scission of the thioester bond in C3 resulting in formation of a molecule C3(H_2O) possessing all of the functional properties of C3b. The generated C3b-like C3 molecule, serves as the receptor for factor B which in turn provides the enzymatic site of the C3 convertase of the alternative pathway. Under the usual circumstances, the fluid phase interaction of C3bB, -D, and -P would be prevented by the action of the naturally ocurring regulating proteins, C3b-inactivator (I) and factor H, the C3b inactivator accelerator. Binding of the generated C3b to microbial and mammalian cell surfaces which activate the alternative pathway protect C3b from inactivation by I in the presence of H. Factor B binds in a Mg^{++}-dependent reversible association with C3b. Upon cleavage of bound B by $\overline{D}$, a C3-converting activity, C3bBb, is formed. This C3 convertase generates more C3b, the acceptor for B and amplifies the generation of alternative pathway C3 convertase. The bimolecular amplification convertase C3bBb undergoes an

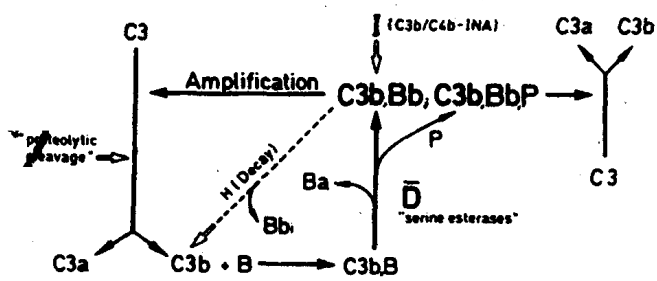

Fig. 2. Schematic diagram of the alternative pathway of complement activation