Methods in Complement for Clinical Immunologists

EDITED BY

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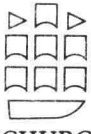

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Preface

Investigation of the complement system is one of the more frequently requested services which are offered by the clinical immunology laboratory. This service serves two distinct functions: a) the monitoring of complement activation both as a diagnostic aid and as a guide to disease activity, and b) the detection and diagnosis of complement deficiency syndromes.

The expansion of the complement service provided by clinical immunology laboratories has resulted primarily from the advent of newer and more effective techniques for the isolation of individual complement components, and from the availability of monospecific antisera. These reagents form the foundation upon which our ability to measure the levels of individual components by functional and by immunochemical assays is based. As static measurements of levels of individual components cannot give accurate measurement of turnover of the complement system, assays which measure the plasma concentrations of cleavage products of C3 and factor B have been devised. Because antigen-antibody complexes activate the classical pathway of complement, the measurement of their serum concentrations may provide useful supplementary information. The detection of immune complexes and complement components in tissues, which is so dependent upon the availability of monospecific antisera, has revolutionized the diagnosis of patients with skin and renal diseases. The production of immunoconglutinins, antibodies to activated complement components, reflects continued complement activation. It is to be hoped that the newer and more quantitative techniques will define more clearly the value of measuring immunoconglutinin levels.

Although the endpoint used in the functional assays of components is lysis of erythrocytes, it is a mistake to assume that cytolysis is the only biological effect of complement activation. Activation products influence many functions including vascular permeability, chemotaxis and the phagocytosis and killing of bacteria. Unfortunately only chemotaxis assays are available in most laboratories; it is hoped that assays of other complement-dependent activities will be developed. Investigation of these activities in patients with complement deficiency syndromes will undoubtedly shed new light on the functions of the complement system.

Receptors for C3 cleavage products (C3b, C3bi and C3d) are present on many cell types. Observations which suggest that deficiency of C3b receptors may

occur in patients with systemic lupus erythematosus, emphasises the need to measure these receptors, not only to help characterize leukocyte populations, but also to understand the pathogenesis of immune complex diseases.

The *in vitro* culture of cells and tissues which synthesise complement components has shown that most of these components are synthesised as single chain precursor molecules, which undergo proteolytic cleavage to reach their biologically active plasma form. Using the same tissue culture methods, the transmembrane signals which regulate the rates of synthesis of complement components are being defined. Further use of the tissue culture techniques will no doubt further our understanding of the molecular basis of the changes in the complement system which occur in disease states.

Genetic polymorphism has been observed for most of the human serum and red cell enzyme systems. The study of the polymorphism of complement components has become more widespread following the recognition that three components (C2, Bf and C4) are encoded by genes within the major histocompatibility complex. Thus the study of the polymorphism of these components should provide genetic markers for the histocompatibility region which may be helpful in recipient-donor matching in tissue transplantation. More recently the observations that certain polymorphic variants of complement components are associated with particular diseases, and that certain variants lack functional activity has provided further impetus to study this aspect of the complement system.

The techniques required for the development of a complete complement service are diverse and, at the present time, available in only a few laboratories. I hope this book will provide sufficient background knowledge and technical information to enable more laboratories to develop these methods.

The responsibility of the clinical immunologist is not confined to the running of the laboratory. He must be able to advise on the management or even assume the responsibility for the management of particular patients. For this reason, a chapter dealing with the diagnosis and management of the complement deficiency syndromes has been included.

By way of acknowledgement, I wish to thank Mrs Jean Veitch, Mr Allister Hamilton, Dr Lindsay Morrison and Mr Peter Kerrigan for the preparation of the photographs used in Chapters 2 and 3. I am also grateful to Mrs Maureen Ralston who typed the manuscript, and to Drs Ronald Thompson and Michael Kerr for their valuable criticisms and suggestions during the preparation of this book.

Glasgow, 1985 K. W.

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Contents

An introduction to the complement system

INTRODUCTION

The purpose of this book is to provide technical descriptions of methods used in laboratories engaged in complement work. However, with such a complex biological system, the principles involved in the assay of individual components and the changes which occur in the system in disease can only be appreciated if the reader has a basic understanding of the molecular aspects of complement activation. This chapter will hopefully provide that understanding. It is intended to be an introduction to the complement system rather than a comprehensive review. At the end of the book a number of comprehensive reviews are listed.

The complement system is a group of self-assembling plasma proteins which constitutes the principal humoral mediator of antigen-antibody reactions. In 1884 Grohmann (Grohmann, 1884) showed that blood plasma was capable of killing bacteria, and later Buchner (Buchner, 1889a, b) showed that fresh serum was bactericidal. Buchner termed this activity alexin and noted that it was sensitive to heat (55°C for 30 min) and dialysis against distilled water at 0°C for 18 to 36 h. Buchner concluded that the lytic activity of fresh serum was due to enzymatic activity.

Bordet (1896, 1898) proved that at least two factors in immune serum were required for bacteriolysis. A heat-stable factor, increased by immunisation and reacting specifically with the immunising organism, is now known to be antibody. The second factor Bordet termed alexin was shown to be heat-labile and was present in both immune and non-immune animals. The heat-stable factor was incapable of killing bacteria in the absence of alexin. Alexin was also capable of lysing antibody-coated erythrocytes (Bordet, 1898). During the early part of the present century a number of workers showed that alexin, or complement, was not a single serum constituent, and four factors were identified on the basis of euglobulin precipitation, heat-sensitivity treatment with zymosan or cobra venom, and ammonia sensitivity. Euglobulin contains $C\bar{1}$ or midpiece, while the pseudoglobulin contains endpiece. Heat-inactivated serum was devoid of $C\bar{1}$ and C2, zymosan or cobra venom-treated serum was deficient in C3, and ammonia destroyed

Table 1.1 Human complement proteins

	Component	Molecular weight	Electrophoretic mobility	Serum concentration (μg/ml)	Polypeptide chain structure	Genetic polymorphism	Cleavage products
Classical pathway	C1q	400 000	α	250	18 (6 × 3)	—	—
	C1r	90 000	β	100	1	?	H & L chains
	C1s	90 000	β	80	1	?	H & L chains
	C4	204 000	β	430	3	+	C4a, C4b
	C2	100 000	β	20	1	+	C2a, C2b
Alternative pathway	B	93 000	β	150	1	+	Ba, Bb
	$\bar{D}$	25 000	α	2	1	?	—
	P	22 000	γ	30	4	?	—
Terminal sequence	C3	190 000	β	1300	2	+	C3a, C3b, C3c C3d, C3e
	C5	185 000	β	75	2	—	C5a, C5b
	C6	128 000	β	60	1	+	—
	C7	121 000	β	60	1	+	—
	C8	153 000	γ	80	3	+	—
	C9	79 000	β	50	1	—	—
Control proteins	$C\bar{1}$-inhibitor	105 000	α	180	1	+	—
	C4 binding protein	540 000 590 000	β	?	8	?	—
	C3b inactivator (I)	90 000	β	50	2	—	—
	β1H (H)	150 000	β	300	1	?	—
	S protein	88 000	α	?	1	?	—
	Anaphylotoxin inactivator	300 000	α	?	8	?	—

Table 1.2 Biological activites of complement

Activity	Product
Pro-inflammatory	
1. Increased vascular permeability	C4a, C3a, C5a, C2-kinin
2. Chemotaxis	C5a, $C5a_{desarg}$, $\overline{C5b67}$, $\overline{C3bBb}$
3. Lysosomal enzyme secretion	C3b
Cytolytic	$\overline{C5b\text{-}9}$
Bystander lysis	$\overline{C5b\text{–}7}$
Opsonic	
1. Adherence to receptors	C4b, C3b, C3bi, C3d
2. Enhancement of phagocytosis	C3b
3. Increased intracellular killing of bacteria	C3b
4. Increased intracellular degradation of immune complexes	C3b
Solubilisation of complexes	C3b ± C4b
Antibody production	
1. T-dependent responses	C3
2. Generation of B memory cells	C3

C4 (reviewed by Rapp & Borsos, 1970). These studies showed the sequential nature of complement activation and the existence of cellular intermediate products in immune haemolysis. C3 activity was considered to be due to a single component until 1958 when Rapp (Rapp, 1958) showed that a mathematical analysis of the haemolytic reaction of C3 was consistent with it being more than one factor. It is now known that C3 activity, determined by these old techniques, is a complex of six proteins (Nelson et al, 1966).

With the advent of improved methods of plasma protein fractionation, the complement system has been shown to consist of 20 proteins (Table 1.1). As a consequence of its activation, biologically-active cleavage products, which influence many cell types, are generated (Table 1.2).

The system is a cascade mechanism, similar to the coagulation and fibrinolytic systems.

Molecules circulate in an inactive (zymogen) form, and as a result of their limited proteolysis, themselves acquire proteolytic activity, the substrate being the next protein in the activation sequence.

Because of the complexity of the system, it is useful to divide it into four groups of proteins: the classical pathway, the alternative pathway, the terminal sequence and a group of control proteins.

Nomenclature

Each of the components of the classical pathway and the terminal sequence is denoted by the letter C followed by a number, e.g. C1, C4, C2, C3, C5, C6, C7, C8 and C9. The alternative pathway components are termed factors

and each is represented by a letter, e.g. factor B, factor $\bar{D}$, properdin (P). The control proteins are referred to by their trivial names, $C\bar{1}$-inhibitor ($C\bar{1}$-INH), C4 binding protein (C4BP), C3b inactivator (C3bINA) and β1H globulin (β1H). The abbreviated forms of their names are shown in brackets. Recently the symbols I and H have been assigned to C3bINA and β1H respectively.

The enzymatically active forms of components have a bar over the symbols, e.g. $C\bar{1}$, $\overline{C42}$, $\bar{D}$. Cleavage fragments are indicated by suffixed lower-case letters, e.g. C4a, C4b, C4c, C4d. The polypeptide chains of components are suffixed with Greek letters, e.g. α for the largest then β and γ e.g. C4α, C4β and C4γ.

CLASSICAL PATHWAY ACTIVATION

The classical pathway (Fig. 1.1) consists of five proteins C1q, C1r, C1s, C4 and C2, which are responsible for the assembly of the C3-cleaving enzyme $\overline{C42}$, and three control proteins $C\bar{1}$-inhibitor ($C\bar{1}$-INH), C4 binding protein (C4BP) and C3b/C4b inactivator (C3bINA; I).

Antigen-antibody complexes containing antibody molecules of the IgM class, or IgG1, IgG2 or IgG3 subclasses, interact with the first complement component (C1), to convert it to its active form, $C\bar{1}$. C1 is a macro-molecule consisting of 1 molecule of C1q, 2 molecules of C1r and 2 molecules of C1s, held together by calcium ions (Gigli et al, 1976). Activation of C1 proceeds by three distinct steps: (1) binding of C1 to antibody; (2) activation of C1r to $C\bar{1}r$; (3) activation of C1s to $C\bar{1}s$.

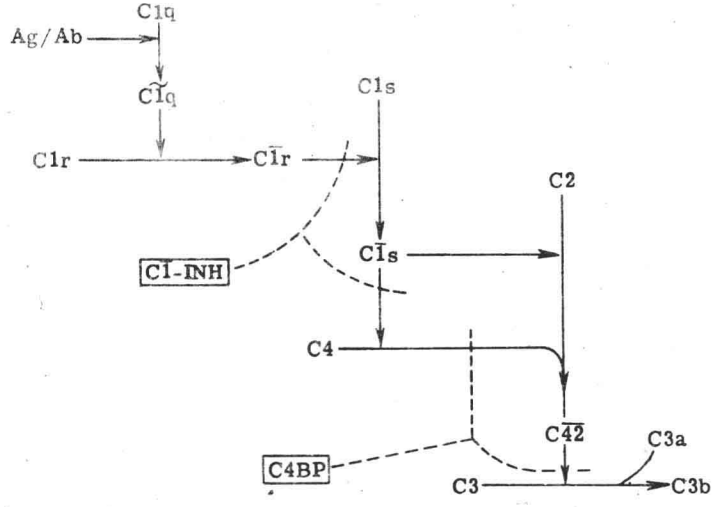

Fig. 1.1 Classical pathway activation. A ~ above a symbol indicates that the component has undergone a conformational change. A bar above a symbol indicates that the component is in its activated state.

C1q is the recognition unit of the C1 macromolecule. It possesses a hexameric structure, each of six globular heads being joined by collagen-like fibrillarystalks (Porter & Reid, 1978). Each of the heads is able to bind to the CH2 of the IgG heavy chain or the CH3 domain of the IgM heavy chain. The affinity of C1q for monomeric immunoglobulin is weak, but is increased when the immunoglobulin has been aggregated, physically or chemically, or is part of an antigen-antibody complex. In the presence of Ca^{2+}, C1r binds to the central region of the stalks of the C1q molecule and acts as a bridge between C1q and C1s. C1r and C1s have structural and functional similarities. Both are single polypeptide chain zymogens which, when activated by proteolytic cleavage, are converted to active enzymes consisting of disulphide-linked heavy and light chains. The enzymatic site of both is located on the light chains. The mechanism of activation of C1 is only partially understood. The heads of C1q upon complexing with immunoglobulin, undergo a conformational change, which is transmitted along rigid collagen-like stalks to C1r. The shearing force presumably results in a realignment of the C1r molecule so that a susceptible peptide bond is exposed to an enzymatic site in another part of the molecule. Auto-catalysis results in the generation of $\overline{C1r}$ which has only one known natural substrate, C1s. It is of interest that activation of C1r can only occur in the presence of C1s (Dodds et al, 1978). The close proximity of substrate to enzyme probably permits activation of C1s before $\overline{C1}$-INH can inhibit $\overline{C1r}$. $\overline{C1r}$ produces a single proteolytic cleavage of C1s to convert it to $\overline{C1s}$, which carries the enzymatic site of C1, and which cleaves C4 and C2. $\overline{C1}$-INH binds to the active sites of $\overline{C1r}$ and $\overline{C1s}$ and limits the proteolysis of C4 and C2. As a result of this action the C1 macromolecule is dissociated leaving a fluid-phase $\overline{C1r}$, $\overline{C1s}$, $\overline{C1}$-INH complex, the molar ratio of which is 1:1:2 (Sim et al, 1979; Ziccardi & Cooper, 1979).

C4 consists of three polypeptide chains, α, β and γ, held together by disulphide bridges. $\overline{C1}$ or $\overline{C1s}$ produces a limited proteolytic cleavage in the α-chain, separating a 6000 mol. wt peptide, C4a, from the N-terminus (Schreiber & Müller-Eberhard, 1974). The remainder of the C4 molecule, C4b, possesses an evanescent ability to bind to antigen-antibody complexes. Failure to bind within 50 ms results in loss of the labile binding site, and the molecule becomes fluid-phase C4b. The ability of C4b to bind to surfaces is due to the presence of an internal thiolester group in C4. Cleavage of the α chain by $\overline{C1s}$ results in the generation of a reactive acyl group which is transferred from the thiol to a hydroxyl group on the acceptor molecule (Campbell et al, 1981). Thus C4b binds by covalent bond formation, possibly proceeding by means of a transesterification reaction as proposed for C3b (Law et al, 1979).

C4b possesses a Mg^{++}-dependent binding site for C2. C2 is cleaved by $\overline{C1}$ or $\overline{C1s}$ to yield two fragments, C2a (73 000) and C2b (34 000). Although $\overline{C1}$ and $\overline{C1s}$ can activate C2 in the absence of C4b, the reaction occurs more efficiently in the presence of the latter molecule (Gigli & Austen, 1969). C2

binds to C4b by the C2b fragment, which remains bound to C2a by non-covalent bonds. C2a contains the enzymatic site of this molecule (Nagasawa & Stroud, 1977a).

$C\overline{42}$ is an unstable enzyme, having a very short half-life at 37°C. C2a decays from the complex, which can be regenerated by fresh C2 in the presence of $C\overline{1}$ or $C\overline{1}s$.

Control of C4b activity and $C\overline{42}$

C4b is degraded by the action of the enzyme C3bINA and its cofactor C4BP. C4BP binds to C4b and renders it susceptible to cleavage by C3bINA. Two cleavages occur in the α chain, the β and γ subunits remaining intact. The N-terminal fragment of the α chain (47 000) is C4d and the remainder of the chain and the intact β and γ chains comprise C4c (Fujita et al, 1979). C4c appears in the fluid-phase, with the C4d fragment remaining bound to the immune complexes which can no longer bind to C4b/C3b receptors (CR_1) on erythrocytes or leukocytes.

C4BP will displace C2a from C4b as it binds to the latter protein. The rate of decay of this already labile enzyme is therefore accelerated (Gigli et al, 1980).

THE ALTERNATIVE PATHWAY

When polysaccharides such as zymosan or endotoxin are added to normal serum, C3 consumption occurs without significant utilisation of C1, C4 or C2. Furthermore, C3 consumption occurs when these polysaccharides are added to C4- or C2-deficient sera. Obviously an alternative pathway for C3 cleavage and activation of the terminal sequence exists (Fig. 1.2).

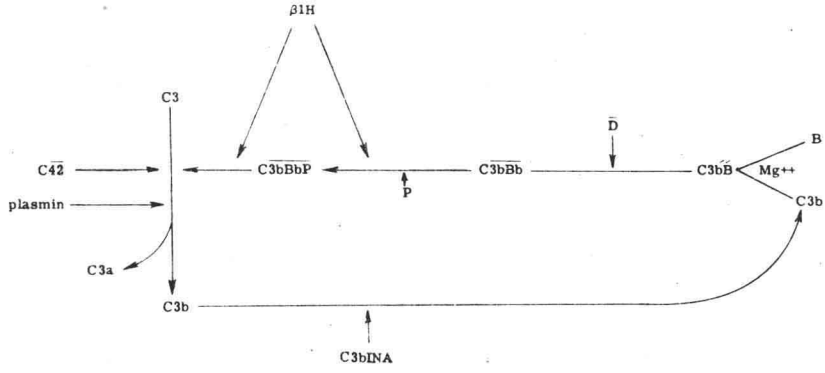

Fig. 1.2 Alternative pathway activation. A ~ above a symbol indicates that the component has undergone a conformational change. A bar above a symbol indicates that the component is in its activated form. (From Whaley & Ferguson, 1981.)

Four proteins are involved in the assembly of the alternative pathway C3 convertase, C3, factors B and $\bar{D}$ and properdin (P). C3bINA (I) and β1H (H) regulate the extent of alternative pathway turnover.

The alternative pathway C3 convertase is formed when C3b complexes with B, in the presence of Mg^{2+}, to form C3bB. $\bar{D}$. a serine protease, then cleaves B to form $\overline{C3bBb}$ the unstable alternative pathway C3 convertase; Ba appears in the fluid-phase. This convertase is said to be unstable as Bb decays rapidly from the complex. The enzyme can be regenerated by the addition of fresh B in the presence of $\bar{D}$ (Fearon et al, 1973). The enzyme is stabilised as a result of P binding to C3b; in some way the decay of Bb from the complex is retarded (Fearon & Austen, 1975). The properdin-stabilised C3 convertase is designated $\overline{C3bBbP}$.

$\overline{C42}$ and $\overline{C3bBbP}$ cleave the α chain of C3 to release a small peptide C3a (9000 mol. wt) from the N-terminus. The residual part of the molecule is termed C3b, and comprises the remainder of the α chain and the intact β chain. Cleavage of the α chain of C3 by C3 convertases exposes a labile binding site by which C3b binds to antigen-antibody complexes or polysaccharides. This bond is covalent, probably an ester linkage to the carbohydrate residues on the polysaccharide or immunoglobulin (Law & Levine, 1977). Failure to bind promptly to a suitable acceptor results in loss of the binding activity and C3b remains in the fluid-phase. The mechanism of binding of C3b to surface structures is the same as that for C4b. Native C3 contains an internal thiolester bond (Tack et al, 1980). Cleavage of the α chain of C3 by C3 convertase action results in the appearance of a single thiol group (Janatova et al, 1980). Covalent bond formation results from the transfer of an acyl group from the thiol to a hydroxyl group contained on the acceptor molecule (Law et al, 1980; Pangburn & Müller-Eberhard, 1980; Tack et al, 1980). Thus it has been proposed that covalent bond formation occurs by a transesterification reaction (Law et al, 1979). C5 lacks this thiolester bond (Law et al, 1980).

C3b, a product of the enzymatic action of C3 convertases on C3, is itself a constituent of the alternative pathway C3 convertases. Thus a positive feedback loop exists which in the absence of control mechanisms, when set in motion, should continue to cleave C3 until the supply of C3 or B becomes exhausted.

Control of the alternative pathway

C3bINA, in the presence of its cofactor β1H, cleaves the α chain of C3b to form C3bi which can no longer bind B to form a C3 convertase. β1H is an absolute requirement for the cleavage of fluid-phase C3b (Pangburn et al, 1977), whereas C3bINA can digest membrane-bound C3b in the absence of β1H (Whaley & Ruddy, 1976a). Further degradation of C3bi gives rise to C3c, C3d and C3e if tryptic-like proteases are used (Nagasawa & Stroud, 1977b). However, in serum C3bi is degraded to C3c and a fragment termed

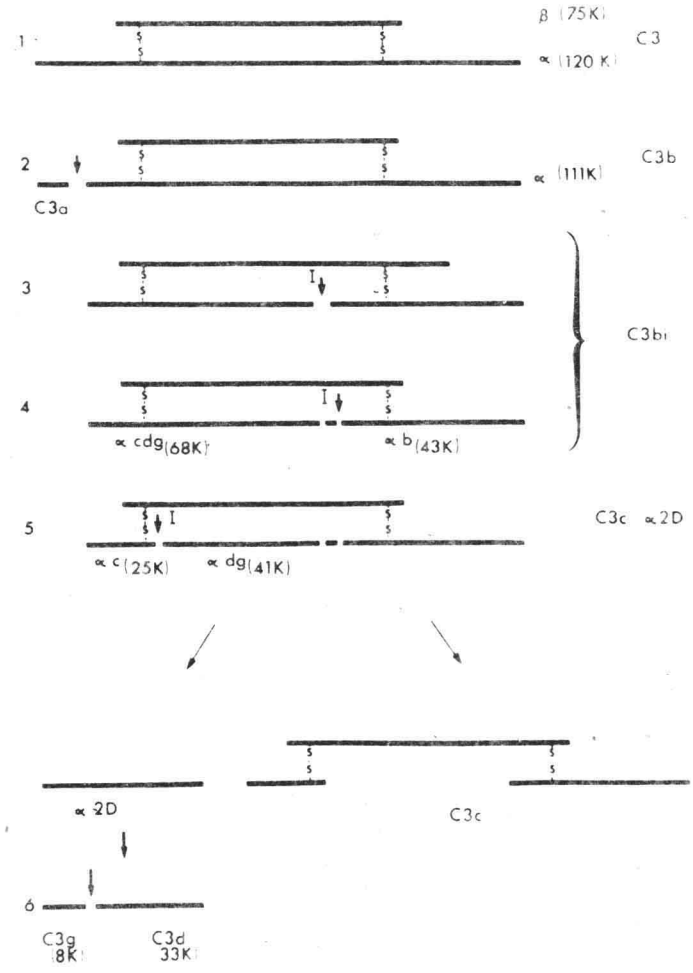

Fig. 1.3 Patterns of cleavage of C3. (Adapted from Lachmann et al, 1982.).

α_2D (Fig. 1.3) (West et al, 1966). α_2D consists of C3d and a second fragment C3g (Lachmann et al, 1982) which can be released by trypsinisation of α_2D (Chaplin et al, 1982). The serum protease converting C3bi to C3c and α_2D has not yet been identified, but plasmin remains a possibility. Bound C3b can be degraded to α_2D and C3c by C3bINA if the C3b receptor (CR1) acts as a cofactor, rather than β1H (Medof et al, 1982).

In addition to its role as cofactor for the catalysis of C3b by C3bINA, β1H has another and perhaps more important role in the regulation of the alternative pathway. β1H binds to C3b (Whaley & Ruddy, 1976b) enabling

it to compete with B (Conrad et al, 1978) and accelerate the rate of decay of $\overline{C3bBbP}$ (Weiler et al, 1976; Whaley & Ruddy, 1976a). C3b is therefore re-exposed to the proteolytic activity of C3bINA.

MECHANISM OF ACTIVATION OF THE ALTERNATIVE PATHWAY

It is probably true to say that the alternative pathway is continuously undergoing low-grade turnover. C3b which is formed can bind to surfaces, but both the fluid-phase and membrane-bound C3b are susceptible to C3bINA-mediated proteolysis. However, if this C3b binds to an 'activating particle' such as zymosan, it binds in such a way as to be protected from the regulatory effects of β1H and C3bINA, as a consequence of which uncontrolled assembly of $\overline{C3bBbP}$ occurs on the surface (Fearon & Austen, 1977). This allows micro-organisms to become coated with large quantities of C3b, a mechanism which prepares the micro-organism for phagocytosis by C3b receptors (CR1), or for lysis by the generation of C5 convertase activity and initiation of the terminal membrane attack sequence. From these observations it is obvious that the alternative pathway plays a major role inh host-defence; particularly in the protection of the non-immune individual against bacterial and possibly other infections. Alternative pathway activators therefore shift the low-grade fluid-phase turnover to rapid solid-phase turnover, and as such they really act to *amplify* pre-existing turnover. One of the surface properties which determines whether a particle is an 'activator' is the carbohydrate composition. Surfaces low in sialic acid such as zymosan, rabbit erythrocytes, and neuraminidase-treated sheep erythrocytes activate the alternative pathway in human serum (Fearon, 1978; Pangburn & Müller-Eberhard, 1978). On these surfaces the affinity of β1H for C3b is reduced, thus favouring the binding of B to C3b. Analysis of alternative pathway activation in other situations in which control proteins are present in normal concentrations has shown that the effect of the control proteins is somehow circumvented. For instance, cobra venom factor (CoVF) is cobra C3b which is resistant to human C3bINA (Alper & Balavitch, 1976), and decay of $\overline{CoVFBb}$ is unaffected by β1H (Nagaki et al, 1978). Nephritic factor is an antibody to $\overline{C3bBb}$, which binds to the enzyme, and like properdin stabilises it by retarding the decay of Bb (Daha et al, 1978). The nephritic factor-stabilised C3 convertase is resistant to the decay dissociation effect of β1H (Weiler et al, 1976). The question of how the first molecule of C3b is generated remains controversial. Native C3 possesses an internal thiolester bond which is sensitive to nucleophilic attack (Pangburn & Müller-Eberhard, 1980; Tack et al, 1980). C3b-like C3 is thought to occur as a result of spontaneous non-enzymatic hydrolysis of the thiolester bone (Pangburn & Müller-Eberhard, 1980). C3b-like C3 acquires the ability to bind B and form $\overline{C3bBb}$ before it becomes susceptible to attack by C3bINA and β1H (Isenman et al, 1981). Thus the spontaneous formation of C3b-like

C3 perhaps explains how the low-grade fluid-phase turnover of the alternative pathway is initiated.

SIMILARITIES BETWEEN THE CLASSICAL AND THE ALTERNATIVE PATHWAYS (Table 1.3).

$\bar{D}$, a serine protease of 23 500 mol. wt, is equivalent to the light chain of $C\bar{1}s$. C2 and B are structurally and functionally similar. C2 has a mol. wt of 117 000, and B a mol. wt of 110 000. Both are composed of single polypeptide chains which are cleaved at a single site. The active sites of the C3 convertases are located on the larger of the 2 cleavage fragments (C2a and Bb), both of which decay rapidly. The structural genes coding for C2 and B are located on the short arm of the 6th chromosome in man. In fact it is probable that they represent the products of a gene reduplication (Raum et al, 1976). C4b and C3b are analogues binding to C2 and B respectively, reactions which are Mg^{2+}-dependent. C4b and C3b are susceptible to proteolysis by C3b inactivator in the presence of C4BP and β1H respectively. C4BP and β1H also accelerate the decay of the C3 convertases by displacing C2 and Bb respectively from the enzymes.

THE TERMINAL SEQUENCE

The terminal sequence is activated when C3 is cleaved by $\overline{C42}$ or $\overline{C3bBbP}$. C3b binds to the antigen-antibody complexes by its labile binding site. Some

Table 1.3 Similarities between classical and alternative pathways

Classical pathway		Alternative pathway	
C4b	1. Binds C2 + Mg^{2+}-dependent 2. Degraded by C3bINA + C4BP	C3b	1. Binds B, Mg^{2+}-dependent 2. Degraded by C3bINA and β1H
C2	1. Single polypeptide chain 2. Cleaved by $C\bar{1}s$ into C2a + C2b 3. Enzymatic site for C3 on larger fragment (C2a) 4. Decays from $\overline{C42}$ 5. Structural genes on chromosome 6	B	1. Single polypeptide chain 2. Cleaved by $\bar{D}$ into Ba and Bb 3. Enzymatic site for C3 on larger fragment (Bb) 4. Decays from $\overline{C3bBb}$ 5. Structural genes on chromosome 6
$C\bar{1}s$	Serine protease cleaves C4 and C2	$\bar{D}$	Serine protease cleaves B
C3bINA	Enzymatically inactivates C4b in presence of C4BP	C3bINA	Enzymatically inactivates C3b in presence of β1H
C4BP	1. Binds to C4b 2. Cofactor for C3bINA in C4b cleavage 3. Accelerates decay of $\overline{C42}$	β1H	1. Binds to C3b 2. Cofactor fr C3bINA in C3b cleavage 3. Accelerates decay of $\overline{C3bBb}$