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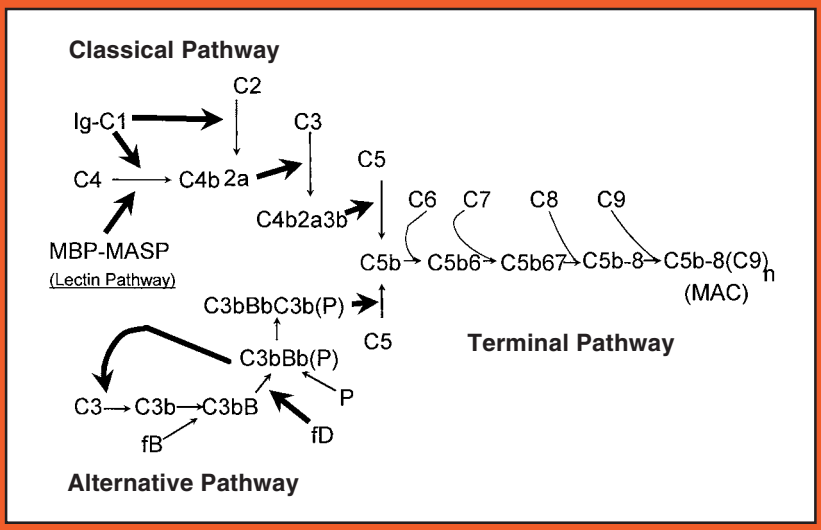
Complement Methods and Protocols

Edited by

B. Paul Morgan



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Complement Methods and Protocols

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Edited by

B. Paul Morgan

Department of Medical Biochemistry
University of Wales College of Medicine
Cardiff, UK

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Totowa, New Jersey 07512

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Preface

The complement system, first described more than a century ago, was for many years the ugly duckling of the immunology world, but no more. Complement in recent years has blossomed into a fascinating and fast moving field of immediate relevance to clinical scientists in fields as diverse as transplantation biology, virology, and inflammation. Despite its emergence from the shadows, complement retains an unwarranted reputation for being "difficult." This impression derives in large part from the superficially complicated nomenclature, a relic of the long and tortuous process of unraveling the system, of naming components in order of discovery rather than in a systematic manner. Once the barrier of nomenclature has been surmounted, then the true simplicity of the system becomes apparent.

Complement comprises an activation system and a cytolytic system. The former has diverged to focus on complement to distinct targets—bacteria, immune complexes, and others—so that texts now describe three activation pathways, closely related to one another, but each with some unique features. The cytolytic pathway is the same regardless of the activation process and kills cells by creating pores in the membrane. Complement plays an important role in killing bacteria and is essential for the proper handling of immune complexes. Problems occur when complement is activated in an inappropriate manner—the potent inflammation-inducing products of the cascade then cause unwanted tissue damage and destruction.

Complement's renaissance has been driven in large part by the discovery of the complement regulatory molecules and the realization that these molecules and other agents can provide effective anticomplement agents for use in therapy. As newer and better anticomplement agents become available, the requirement for laboratories to assess complement activation in clinical samples and to monitor the effects of anticomplement agents will grow.

Complement Methods and Protocols aims to provide a comprehensive source of up-to-date protocols for the study of the complement system, both for the basic scientist interested in understanding the mechanisms of activation and the clinical scientist wishing to quantify complement activation. In the first

chapter, the complement system is briefly reviewed to set the stage for the methods chapters to follow. The next two chapters describe methods for purifying complement components, using classical chromatography and immunoaffinity approaches, respectively. Chapters 4 to 6 describe methods for the functional analysis of complement components, regulators, enzymes, and complexes, including a detailed description of the generation of the depleted sera essential for complement assays. Methods for measurement of complement activation fragments and complexes deposited on cells, in tissues, or in biological fluids are detailed in Chapters 7 to 10. Chapter 11 provides an overview of screening methods for identifying and assessing complement deficiency and Chapter 12 a detailed account of methods needed to assess deficiency of C1 inhibitor. Other clinically relevant protocols for analysis of complement autoantibodies, immune complexes, and complement allotypes are provided in Chapters 13 to 15. Chapter 16 departs from the main theme of the book to describe protocols for generating gene-deleted mice, included here because of the enormous influence such methods are now having on complement research. The final chapter reviews complement deficiencies in experimental animals, listing the different complement deficiencies defined in animals and the experimental models in which these deficient animals have been examined.

I am grateful to my friends and colleagues who have contributed to this volume for their willingness to make time in their busy schedules. In particular, I wish to thank the members of the Complement Biology Group in Cardiff, many of whom have contributed chapters to this volume and others who have reviewed parts of the manuscript or contributed to the tedious task of assembling the appendices. I promise I won't do it again in a while! Finally, thanks to The Wellcome Trust for their continued and generous support of complement research in Cardiff.

B. Paul Morgan

Contents

Contributors

Marina Botto • *Rheumatology Section, Division of Medicine, Imperial College School of Medicine, Hammersmith Campus, London, UK*

Anne E. Bygrave • *Rheumatology Section, Division of Medicine, Imperial College School of Medicine, Hammersmith Campus, London, UK*

Kevin A. Davies • *Rheumatology Section, Division of Medicine, Imperial College School of Medicine, Hammersmith Campus, London, UK*

C. Erik Hack • *CLB and Department of Internal Medicine, Academic Hospital of the Free University Amsterdam, Amsterdam, The Netherlands*

Juha Hakulinen • *Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki, Helsinki, Finland*

Claire L. Harris • *Department of Medical Biochemistry, University of Wales College of Medicine, Heath Park, Cardiff, UK*

Stuart Linton • *Department of Medical Biochemistry, University of Wales College of Medicine, Cardiff, UK*

Seppo Meri • *Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki, Helsinki, Finland*

B. Paul Morgan • *Department of Medical Biochemistry, University of Wales College of Medicine, Heath Park, Cardiff*

Julian T. Nash • *Rheumatology Section, Division of Medicine, Imperial College School of Medicine, Hammersmith Campus, London, UK*

Peter Norsworthy • *Rheumatology Section, Division of Medicine, Imperial College School of Medicine, Hammersmith Campus, London, UK*

Ann Orren • *Department of Microbiology, National University of Ireland, Galway, Galway, Ireland*

O. Brad Spiller • *Department of Medical Biochemistry, University of Wales College of Medicine, Heath Park, Cardiff, UK*

Antti Väkevä • *Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki, Helsinki, Finland*

Carmen W. van den Berg • *Department of Pharmacology, University of Wales College of Medicine, Heath Park, Cardiff, UK*

Reinhard Würzner • *Institut für Hygiene, Innsbruck University, Innsbruck, Austria*

1

The Complement System: *An Overview*

B. Paul Morgan

1. Introduction

The complement (C) system consists of a group of 12 soluble plasma proteins that interact with one another in two distinct enzymatic activation cascades (the classical and alternative pathways) and in the nonenzymatic assembly of a cytolytic complex (the membrane attack pathway) (**Fig. 1**; **Table 1**). A third activation pathway, termed the lectin pathway, has recently been described ***(1,2)***. Control of these enzymatic cascades, essential to prevent rapid consumption of C in vivo, is provided by 10 or more plasma and membrane-bound inhibitory proteins acting at multiple stages of the system. C plays a central role in innate immune defense, which provides a system for the rapid destruction of a wide range of invading microorganisms.

The purpose of this volume is to provide a balanced account of the methods which have been applied to the study of the C system in clinical and research laboratories. In the early chapters, methods for the isolation of the individual C components, regulators and receptors will be described and assays for the measurement of C activity in the various pathways will be detailed. Later chapters will describe aspects of C methodology of relevance to the clinical laboratory—protocols for screening for C deficiency and deficiency of C1 inhibitor, methods for measurement of C activation products in biological fluids and tissues and methods for allotyping C components. Other chapters will describe advanced technologies which are now having enormous influence on C research—structural analysis of the components and regulators and gene targeting to generate animals deficient in individual components and regulators.

In order to appreciate the methodologies to be described, it is essential first to understand the basics of the system.

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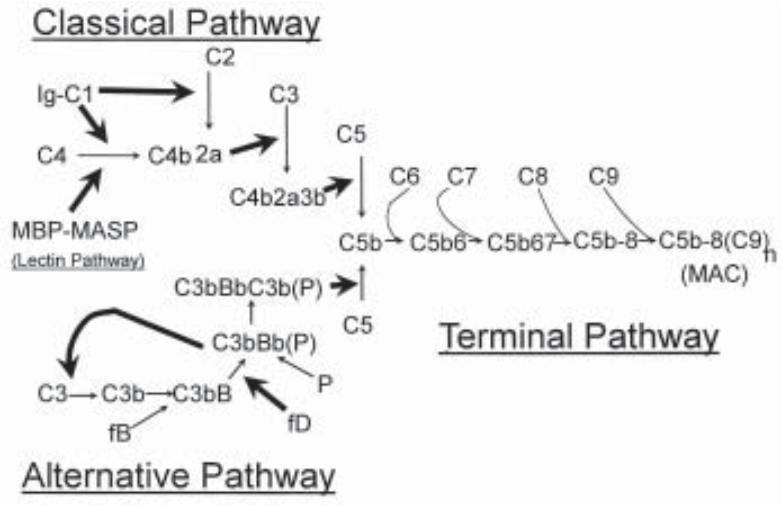

Fig. 1. The complement system and its control. The constituent pathways of the C system and the component proteins are shown. Enzymatic cleavages are represented by thick arrows. The lectin pathway differs from the CP only in that the MBP-MASP complex replaces the C1 complex. Regulators act to inhibit either the enzymes of the activation pathways (activated C1, C3 convertases, C5 convertases) or assembly of the MAC.

2. Activation of C

2.1. The Classical Activation Pathway

The classical activation pathway (CP), so called because it was the first pathway to be described, is triggered by antibody bound to particulate antigen. Many other substances, including components of damaged cells, bacterial lipopolysaccharide, and nucleic acids, can also initiate the CP in an antibody-independent manner.

The CP is initiated by the binding of C1q ***(3)***. Activation in vivo involves binding of C1q to aggregated or immune complex bound IgG or IgM antibody. C1 is a large heterooligomeric complex (molecular weight approx 800 kDa) consisting of a single molecule of C1q and two molecules each of C1r and C1s associated noncovalently in a Ca^{2+}-dependent complex. C1q contains no enzymatic activity, but conformational changes occur upon binding of multiple heads of the C1q molecule by aggregates of IgG, which trigger activation of the other components of the C1 complex. IgM is a multivalent molecule and can thus activate C1q efficiently without the need for aggregate formation. The enzymatic activity of the C1 complex is provided by C1r and C1s. These are

Table 1
The Component Proteins of the Complement System

Component	Structure	Plasma conc (mg/L)
Classical Pathway		
C1	Complicated molecule, composed of 3 subunits, C1q (460 kDa), C1r (80 kDa), C1s (80 kDa) in a complex ($C1qr_2s_2$)	180
C4	3 chains (α, 97 kDa; β, 75 kDa, γ, 33 kDa); from a single precursor	600
C2	single chain, 102 kDa	20
Alternative Pathway		
fB	single chain, 93 kDa	210
fD	single chain, 24 kDa	2
Properdin	oligomers of identical 53 kDa chains	5
Common:		
C3	2 chains: α, 110 kDa, β, 75 kDa	1300
Terminal Pathway		
C5	2 chains: 115 kDa, 75 kDa	70
C6	single chain, 120 kDa	65
C7	single chain, 110 kDa	55
C8	3 chains: α, 65 kDa, β, 65 kDa γ, 22 kDa	55
C9	single chain, 69kDa	60

The proteins that constitute the classical, alternative, and membrane attack pathways are listed together with their approximate concentration in plasma. Modified from: Morgan, B.P. and Harris, C. L. (1999) *Complement Regulatory Proteins.* Academic, London.

both single-chain molecules of molecular weight 80 kDa, encoded by closely linked genes on chromosome 12 and sharing a high degree of homology. In the presence of Ca^{2+}, C1r and C1s associate with each other to form an elongated $C1r_2$–$C1s_2$ complex, which binds between the globular heads of C1q ***(4,5)***. Binding of C1q through the globular heads to Fc regions of aggregated IgG triggers activation through conformational changes that trigger the autoactivation of the proenzyme C1r, a process which involves cleavage at a single site within the molecule.

C1r then activates C1s in the complex, again by cleaving at a single site in the molecule ***(4)***. C1s in the activated C1 complex will enzymatically cleave and activate the next component of the CP, C4.

C4 is a large, plasma protein (200 kDa) containing three disulphide-bonded chains (α, β, and γ) ***(6,7)***. C4 is encoded by two closely linked genes in the class III region of the major histocompatibility complex (MHC) on the short

arm of chromosome 6, which give rise to the two isotypic variants, C4A and C4B ***(8,9)***. These variants differ by only six amino acids, but these small changes cause significant differences in function, C4A binding preferentially to amino groups after cleavage and C4B to hydroxyl groups. C1s cleaves plasma C4 at a single site near the amino-terminus of the α-chain, releasing a small fragment, C4a (M_r approx 9 kDa) and, in the process, exposing a reactive thioester group in the α-chain of the large fragment, C4b. The thioester group is crucial to the function of both C4 and C3. In native C4, the thioester is buried deep within a hydrophobic pocket in the molecule, rendering it nonreactive. Once exposed in C4b, the thioester can form covalent amide or ester bonds with exposed amino or hydroxyl groups, respectively, on the activating surface, locking the molecule to the activating surface. The thioester group is extremely labile because of its propensity to inactivation by hydrolysis, restricting C4b binding to the immediate vicinity of the activating C1 complex. Membrane-bound C4b provides a receptor for the next component of the CP, C2.

C2 is a single-chain plasma protein of molecular weight 102 kDa. Membrane-bound C4b expresses a binding site which, in the presence of Mg^{2+} ions, binds C2 and presents it for cleavage by C1s in an adjacent C1 complex. The 70 kDa carboxy-terminal fragment, C2a remains attached to C4b to form the C4b2a complex, the next enzyme in the CP.

C3 is the most abundant (1–2 mg/mL in serum) of the C components, essential for activity of both the CP and AP. It is a large (185 kDa) molecule composed of two chains (α, 110 kDa and β, 75 kDa) held together by disulphide bonds ***(10)***. C3 binds noncovalently to C2a in the C4b2a complex and is then cleaved by the C2a enzyme at single site in the α-chain (between *Arg*77 and Ser78), releasing the small fragment, C3a (9 kDa), from the amino-terminus and exposing in the large fragment, C3b, a labile thioester. C3b binds either to the activating C4b2a complex ***(11,12)*** or to the adjacent membrane. Only C3b bound to the activating C4b2a complex takes any further part in activation, although C3b bound further afield has other important roles in mediating interactions with phagocytic cells. The enzyme so formed, C4b2a3b is the C5 cleaving enzyme (convertase) of the CP.

The next component, C5, is a two-chain plasma protein of 190 kDa molecular weight, structurally related to C3 and C4, but lacking a thioester group. C5 binds noncovalently to a site on C3b in the C4b2a3b convertase and is presented for cleavage by C2a in the complex. Cleavage occurs at a single site (after residue 74) in the α-chain of C5, releasing a small amino-terminal fragment, C5a (approx 10 kDa), and exposing in the larger fragment, C5b, a labile hydrophobic surface-binding site and a site for binding C6.

2.2. The Alternative Activation Pathway

The alternative pathway (AP) provides a rapid, antibody independent route for C activation and amplification on foreign surfaces. C3 is the key component of the AP, but three other proteins, factor B (fB), factor D (fD), and properdin, are also required. FB, a single-chain 93 kDa plasma protein that is closely related to C2, binds C3b in a Mg^{2+}-dependent manner. Binding renders fB susceptible to cleavage by fD, a 26-kDa serine protease present in plasma in its active form, which cleaves fB at a single site, exposing a serine protease domain on the large (60 kDa) fragment, Bb ***(13)***. The C3bBb complex thus formed is the C3-cleaving enzyme (C3 convertase) of the AP. Properdin binds and stabilizes the C3bBb complex, extending the lifetime of the active convertase three- or fourfold ***(14,15)***. Properdin is a basic glycoprotein made up of oligomers—mainly dimers, trimers, and tetramers—of a 53-kDa monomer ***(16)***. The AP requires Mg^{2+} ions for assembly of the C3bBb complex, whereas the CP requires both Mg^{2+} ions (for assembly of the C4b2a complex) and Ca^{2+} ions (for assembly of the C1 complex). This provides a most useful means of distinguishing the two pathways in serum samples. Ethylenediaminetetraacetic acid (EDTA), by chelating both ions, will block both pathways, while ethylene glycol-bis[β-aminoethylether]N,N'-tetraacetic acid (EGTA) (with supplemental Mg^{2+}) chelates only Ca^{2+} and blocks specifically the CP.

Initiation of the AP on a surface occurs spontaneously, the phenomenon of "tickover." C3 in plasma is hydrolyzed to form a metastable $C3(H_2O)$ molecule, which has many of the characteristics of C3b, binds fB in solution, and renders it susceptible to cleavage by fD to form a fluid phase C3 convertase thus formed ($C3(H_2O)Bb$) ***(17,18)***. The surface features of many microorganisms and foreign cells favor amplification of the AP (activator surfaces) and rapidly become coated with C3b molecules.

As in the classical activation pathway, bound C3b acts as an essential receptor for C5, permitting the cleavage of C5 by Bb in an adjacent C3bBb complex. The AP C5 convertase comprises two molecules of C3b, one binding Bb in the C3bBb complex and an adjacent (or attached) C3b acting as receptor for C5. The site of cleavage in C5 is identical to that utilized by C2a in the CP convertase.

2.3. The Lectin Pathway

The lectin pathway represents a recently described activation pathway, which provides a second antibody-independent route for activation of C on bacteria and other microorganisms. The key component of this novel pathway is mannan-binding lectin (MBL; also termed mannan-binding protein, MBP), a high-molecular-weight serum lectin made up of multiple copies of a single

32 kDa chain ***(2,19)***. MBL binds mannose and N-acetyl glucosamine residues in bacterial cell walls. MBL has a structure similar to that of C1q—a multimeric molecule with globular binding regions and a collagenous stalk, associated with a novel serine protease termed MBL-associated serine protease (MASP), a 100-kDa protein that is homologous with C1r and C1s ***(20)***. The MBL-MASP complex activates C4 in a manner analogous to that described for C1, providing a rapid, antibody-independent means of activating the CP on bacteria.

2.4. The Membrane Attack Pathway

The membrane attack pathway involves the noncovalent association of *C5b* with the four terminal C components to form an amphipathic membrane-inserted complex.

While still attached to C3b in the convertase, C5b binds C6, a large (120 kDa) single-chain plasma protein. Binding of C6 stabilizes the membrane binding site in C5b and exposes a binding site for C7, a 110-kDa single-chain plasma protein that is homologous to C6 (*see* below). Attachment of C7 causes release of the complex from the convertase to the fluid phase. The hydrophobic membrane binding site in C5b67 allows the complex to bind tightly with the membrane.

C8 is a complex molecule made up of three chains, α, β, and γ (molecular weights 65, 65, and 22 kDa, respectively). The β-chain in C8 binds C7 in the C5b67 complex and the resulting complex, C5b-8, becomes more deeply buried in the membrane and forms small pores, causing the cell to become slightly leaky ***(21)***. C9, a single chain plasma protein (molecular weight 69 kDa), binds the α-chain of C8 in the C5b-8 complex and undergoes a major conformational change from a globular, hydrophilic form to an elongated, amphipathic form which traverses the membrane and exacerbates membrane leakiness. Additional C9 molecules are recruited into the complex to form a pore (the membrane attack complex, MAC), which can cause lysis of the target cell.

3. Regulation of C

The C system is tightly controlled by regulatory proteins present in the plasma and on cell membranes (**Table 2**). The first step of the CP is regulated by C1-inhibitor (C1inh), a serine protease inhibitor that binds activated C1 and removes C1r and C1s from the complex ***(22,23)***. C1inh is the only plasma inhibitor of activated C1 and even partial deficiency can result in uncontrolled activation of C in peripheral sites with resultant inflammation (*see* Chapter 12). Control later in the activation pathways is provided by fI, a serine protease which, in the presence of essential cofactors, cleaves C3b and C4b to inactivate the convertases. In plasma, two proteins act as cofactors for fI. fH, a large, single-chain glycoprotein that catalyses fI cleavage of C3b in the AP convertases ***(24)***. C4bp is a large, multimeric plasma protein that calalyses cleav-

Table 2
C Regulatory Proteins

Molecule	Structure	Se. conc. (mg/L)	Target
Plasma			
C1inh	single chain, 90 kDa(?)	200	C1
FH	single chain, 90 kDa	450	C3/C5 conv.
FI	2 chains: α, 50 kDa; β, 38 kDa	35	C3/C5 conv.
C4bp	6 or 7 α chains (70 kDa), 1 or 0 β chain (25 kDa)	250	CP C3 conv.
S protein	single chain, 83 kDa	500	C5b-7
Clusterin	2 chains: α, 35 kDa; β, 38 kDa	50	C5b-7
CPN (Anaphylatoxin Inactivator)	dimeric heterodimer, 290 kDa chains, 85 kDa, 50 kDa	30	C3a, C4a, C5a
Membrane			
MCP	single chain, 60 kDa; TM	—	C3/C5 conv.
DAF	single chain, 65 kDa; GPI	—	C3/C5 conv.
CR1	single chain, 200 kDa; TM	—	C3/C5 conv.
CD59	single chain, 20 kDa; GPI	—	C5b-8/C5b-9

RCA, Regulators of Complement Activation gene cluster; TM, transmembrane; GPI, glycosyl phosphatidylinositol; CPN, carboxypeptidase N. Modified from: Morgan, B. P. and Harris, C. L. (1999) *Complement Regulatory Proteins.* Academic, London.

age of C4b in the CP convertase. Both fH and C4bp also inhibit in a second way by acting to break up (decay) the multicomponent convertases, a property termed *decay acceleration* ***(4,25)***. On the membrane, decay accelerating factor (DAF) is a 65-kDa single-chain protein tethered to the membrane by a glycosyl phosphatidylinositol (GPI) anchor. DAF binds to and breaks up the convertase. Membrane cofactor protein (MCP) is a 60-kDa transmembrane protein which, like DAF, binds to the activation pathway convertases but, instead of causing dissociation, acts as cofactor for the cleavage of C4b and C3b by fI, thus irreversibly inactivating the enzyme. **C receptor 1** (CR1) is a large (approx 200 kDa) transmembrane protein that inactivates the activation pathway convertases both by causing dissociation/decay and by acting as a cofactor for cleavage by fI. A unique feature of the AP is the existence of a protein that stabilizes the C3 and C5 cleaving enzymes. Properdin (P) was the first of the components specific to the AP to be discovered ***(26)***. It is a large, oligomeric (3, 4, or more identical 56-kDa subunits) plasma protein which binds C3b in the convertase and inhibits the spontaneous and accelerated (fH, DAF, CR1) decay ***(27,28)***.

Table 3
Receptors for C components, Fragments, and Complexes

Receptor	Ligand	Characteristics	Distribution
cC1qR	C1q, collagenous region	70 kDa; calmodulin-like	Broad
gC1qR	C1q, globular heads	33 kDa; 80 kDa	Leukocytes; platelets.
CR1 (CD35)	C3b/C4b	180–200 kDa, sc, tm, app. 30 SCRs.	E, B cells, neutrophils, monocytes etc. (broad).
CR2 (CD21)	C3d (and EBV)	145 kDa, sc, tm, 15 or 16 SCRs.	B cells, FDCs, epithelia, glia, ? others?
CR3 (CD11b/18)	iC3b (and matrix)	heterodimer	Myeloid and NK cells
C5aR (CD88)	C5a	40 kDa; 7-tm-spanning	Neutrophils, macrophages, mast cells, muscle, ?others?
C3aR	C3a	app. 60 kDa; 7-tm-sp.	as above
C4aR	C4a	nd	nd
C5b-7R	C5b67	nd	nd

The cellular receptors for C are listed together with their CD asignments (where known) natural ligands, molecular characteristics and cell distribution. Abbreviations: sc, single chain; tm, transmembrane; sp, spanning; SCR, short consensus repeat; E, erythrocyte; FDC, follicular dendritic cell; NK, natural killer cell; nd, not determined. Modified from: Morgan, B. P. and Harris, C. L. (1999) *Complement Regulatory Proteins.* Academic, London.

The membrane attack pathway is tightly regulated by inhibitors present in the fluid phase and on membranes. The hydrophobic membrane binding site in the fluid-phase C5b-7 complex is the target of S-protein (vitronectin) and clusterin, abundant serum proteins which, among their many roles, help regulate C activation. On the membrane, CD59 antigen (CD59), a 20-kDa GPI-anchored molecule, binds to C8 in the C5b-8 complex and blocks incorporation of C9, thereby preventing formation of the lytic MAC ***(29,30)***.

4. Receptors for C Components and Fragments

Cells express surface receptors specific for *C1q*, for the large fragments of C3 and C4 generated during C activation and for the small fragments of C3, C4, and C5 (**Table 3**). Receptors for C1q (C1qR) were first described in the early 1980s ***(31)***. Several different receptors for C1q, binding either the collagenous stalk or the globular head, have now been described, although their precise roles remain to be ascertained ***(32–34)***. Multiple receptors also exist for the large products of cleavage of C4 (C4b) and C3 (C3b) generated during C activation. **C receptor 1** (CR1; CD35) has dual roles as C regulator and C receptor. The ligands for CR1

are C3b and C4b, which bind to separate sites on the large CR1 molecule ***(35)***. CR1 on erythrocytes plays a key role in the transport of immune complexes. **C receptor 2** (CR2; CD21) is the receptor for C3d, the surface-bound fragment, which is the end-product of the cleavage of C3b by fI and serum proteases ***(36)***. CR2 does not function as a C regulator. **C receptor 3** (CR3; CD11b/CD18) is the receptor for iC3b and also binds numerous extracellular matrix proteins. It is a member of the β-2 integrin family of cell adhesion molecules and, like other members of this family, is a heterodimer composed of one molecule of the common integrin β-chain, CD18, and one molecule of a specific α-chain, which in CR3 is CD11b ***(37,38)***. **C receptor 4** (CR4; CD11c/CD18), like CR3, is a β-2 integrin with wider roles in cell adhesion.

The small anaphylactic peptides C5a and C3a generated during C activation express important biological activities. Each peptide is 74–77 aminoacids in length and is highly cationic. The presence of specific and distinct membrane receptors for C5a and C3a on neutrophils and macrophages was first demonstrated by classical methods using labeled ligands ***(39,40)***. The C5a receptor (C5aR; CD88) was cloned and shown to be a member of the 7-transmembrane-spanning receptor family ***(41)***. The precise distribution of the receptor is still unclear. The receptor for C3a (C3aR) was finally cloned in 1996 ***(42)***. C3aR is also a member of the 7-transmembrane-spanning receptor family and is highly homologous with C5aR, the major difference being a large extracellular loop, absent in C5aR.

5. Deficiencies of C

Deficiencies of almost every C protein and regulator have been described and more detailed accounts of the various C deficiencies can be found in several recent reviews ***(43–45)***. A description of the considerations and methods applied to the identification of C deficiencies is provided by A. Orren in Chapter 11 and deficiency of C1 inhibitor is described by E. Hack in Chapter 12.

Deficiencies of components of the CP (C1, C4, or C2) are associated particularly with an increased susceptibility to immune complex disease, a consequence of the failure of immune complex solubilization. The frequency and severity of disease is greatest with deficiencies of one of the subunits of C1 (C1q, C1r, C1s), closely followed by total C4 deficiency, each giving rise to a severe immune complex disease, which closely resembles systemic lupus erythematosus (SLE). Subtotal deficiency of C4 is common, because of the extremely high frequency of null alleles at both the C4A and C4B loci, but total C4 deficiency is very rare. Deficiency of C2 is the most common homozygous C deficiency in Caucasoids, but causes much less severe disease; it appears that deposition of the early components, C1 and C4, provides some solubilization of immune complexes.

C3 is an essential component of both activation pathways and is vital for efficient opsonisation of bacteria. Deficiency thus causes a marked susceptibility to bacterial infections. Immune complex disease is not a common finding in C3 deficiency, although solubilization of preformed immune complexes is severely compromised in the absence of C3. Individuals with C3 deficiency run a stormy course through childhood, but with prompt therapy of infections can survive. In adulthood, the number and severity of infections is much reduced as other arms of the immune system take up the challenge. Deficiencies of the regulators fI and fH cause a secondary deficiency of C3 and present with similar symptoms.

Deficiencies of components of the AP are rare and do not predispose to immune complex disease or pyogenic infections. A few individuals deficient in fD have been described, all of whom have presented with recurrent *Neisseria* infections, usually meningococcal meningitis. Very recently, two cases of fH deficiency have been described, again presenting with meningococcal disease. Deficiency of the positive regulator, properdin, is the commonest disorder of the AP.

Deficiencies of terminal pathway components (C5, C6, C7, C8, or C9) also cause susceptibility to infection with organisms of the genus *Neisseria*. Deficiency of C6 is the second most common C deficiency among Caucasians and is frequently associated with meningococcal meningitis and systemic infection with the meningococcus. C9 deficiency, rare in Caucasians, is by far the most frequent C deficiency in Japan with an incidence approaching 1 in 1000 ***(46)***.

6. C in Inflammatory Disease

The C system contributes to tissue damage in a large number of autoimmune diseases. Autoantibodies and immune complexes deposit in the affected organs where they trigger activation of C and exacerbate inflammation. In many autoimmune diseases, from the organ specific (e.g., autoimmune thyroid disease) to the disseminated (e.g., SLE), C deposition can be detected in the affected tissues and products of C activation are found in the plasma (methods for detection detailed in Chapters 8 and 7, respectively). In all these autoimmune diseases, C is just one of several factors that contribute to pathogenesis. In SLE, autoantibodies are present that recognize DNA and other components of normal cells. Following any stimulus to cell death, these components will be released and immune complexes will form. Immune complexes deposit in capillary beds, particularly in skin and kidney, where they activate C to cause inflammation and further tissue destruction (*see* Chapter 15). A vicious cycle is triggered in which more cell killing drives the production of more immune complexes, which in turn exacerbates C activation and tissue destruction.