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Bacterial Evasion of Host Immune Responses

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CHAPTER 4

Evasion of complement system pathways by bacteria

Michael A. Kerr and Brian Henderson

4.1 INTRODUCTION

Paul Ehrlich, better known for his work on chemotherapeutics, coined the term "complement" in the 1890s to denote the activity in sera that could "complement" the lysis of bacteria induced by specific antibody. By the early 1900s, complement was recognised as composed of two components, and by the 1920s it was believed that at least four serum factors were involved. However, it was not until the 1960s that analytical biochemistry was sufficiently rigorous to allow the identification of the majority of the known complement pathway components. Individual components were named as they were discovered, which accounts for the still confusing nature of the nomenclature for describing the complement pathways (for comprehensive reviews of complement, see Law and Reid, 1995; Fearon, 1998; Crawford and Alper, 2000; Kirschfink, 2001; Walport, 2001a, 2001b).

Three pathways of complement activation have now been described (Fig. 4.1). The classical pathway, first to be discovered, is generally considered to require immune complexes for activation. A second pathway, termed, naturally enough, the alternative pathway, was first proposed by Pillemer in the late 1950s but was not taken seriously until the late 1960s when sufficient evidence had accrued. This pathway is now generally considered to be activated by cell surfaces that are not protected by host-derived complement inhibitors (see Lindahl et al., 2000). A third pathway was elucidated in the late 1980s–early 1990s. This has been termed the lectin pathway and is activated by the collectin (i.e., collagen-like lectin), mannose-binding lectin (MBL) (Gadjeva et al., 2001) and by ficolins (proteins containing both a collagen-like and a fibrinogen-like domain; Matsushita and Fujita, 2001). These serum proteins can opsonise bacteria and then interact with proteinases homologous to

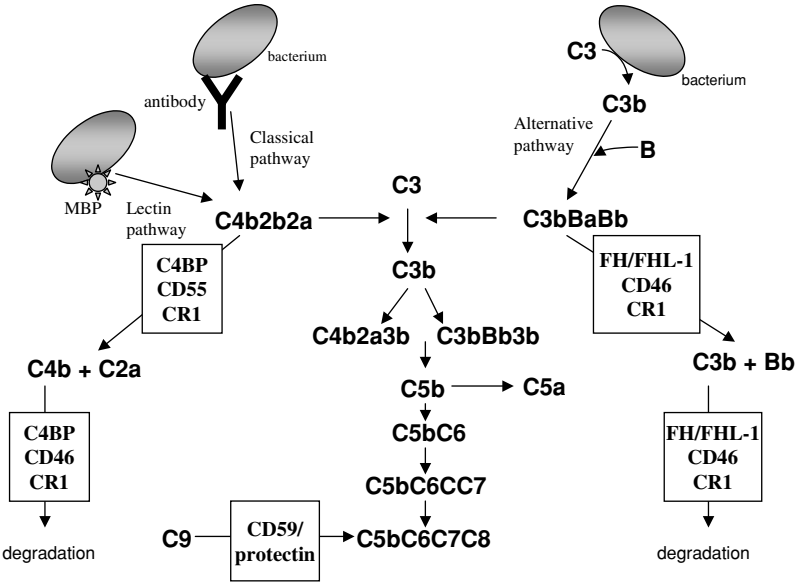

Figure 4.1. The complement pathways and their controlling proteins. The three pathways (classical, lectin, and alternative) produce C3 convertases – C4bC2b2a in the case of the classical and lectin pathways and C3bBaBb in the case of the alternative pathway. These enzyme complexes are very labile; they are formed when the C4bC2 complex is cleaved by C1s or MASP or when the C3bB complex is cleaved by D. The decay of the activity is the result of the loss of the enzymic subunits, C2a and Bb, from the complexes. The smaller subunits C2b and Ba contain the binding sites of C2 and B for C4b and C3b, respectively. The convertases dissociate even more quickly in the presence of appropriate RCA proteins (boxed). The association of C9 with the rest of the lytic complex is inhibited by the GPI-anchored protein, CD59 (protectin).

C1r and C1s, known as mannose-binding lectin associated serine proteinases (MASPs). The activated MASPs, in turn, cause the antibody-independent activation of the classical pathway. These three pathways overlap in terms of their activators and activity and must not be thought of as being totally discrete.

Because the alternative complement pathway is spontaneously and continuously activated, and could thus cause tissue damage, a number of genes encoding proteins termed regulators of complement activation (RCAs) have evolved (Fig. 4.1). These include membrane bound proteins such as CR1 (CD35), CD46 (membrane cofactor protein – MCP), and CD55 (decay accelerator factor – DAF) and soluble proteins such as C4 binding protein

Table 4.1. *Bacterial components (or host responses to bacteria) associated with activation of the three pathways of complement activation*

Complement pathway	Bacterial component or host response
Classical Pathway	Natural antibody (IgM, IgG) via C1q
	Direct binding via C1q
	Lipid A and LPS (*Klebsiella, Escherichia, Shigella, Salmonella*)
	Lipoteichoic acid (group B streptococci)
	Capsular polysaccharide (*H. influenza*)
	OMPs (*Proteus mirabilis, Sal. minnesota, Klebsiella pneumoniae*)
	C1q binding via C-reactive protein (CRP) (*Strep. pneumoniae*)
Lectin Pathway	Mannose-binding lectin
	Other collectins?
	Ficolins
Alternative Pathway	Bacterial cell wall components (LPS, peptidoglycan, teichoic acid)

(C4BP), factor H (FH), and factor H-like protein 1 (FHL-1), also known as reconectin and factor H-related proteins 1–4. These proteins are encoded by closely linked genes on human chromosome 1 and are composed almost entirely of domains of approximately sixty residues known as short consensus repeats (SCRs) or complement control protein repeats (CCPs). The RCAs are major targets for bacterial and viral evasion mechanisms (Lindahl et al., 2000). A further complement inhibitory protein is protectin (CD59), a GPI anchored protein that inhibits the C5b-8 catalysed insertion of C9 into cell membranes (Davies et al., 1989). The total number of proteins involved in complement activation must be approaching forty and for this reason we will refer to complement as the complement system throughout this chapter. Examples of the constituents of bacteria able to trigger the three complement activation pathways are listed in Table 4.1.

4.2 BIOLOGICAL FUNCTIONS OF THE COMPLEMENT SYSTEM

As is highlighted in other chapters, multicellular organisms have evolved protective mechanisms, which can be grouped under the umbrella term

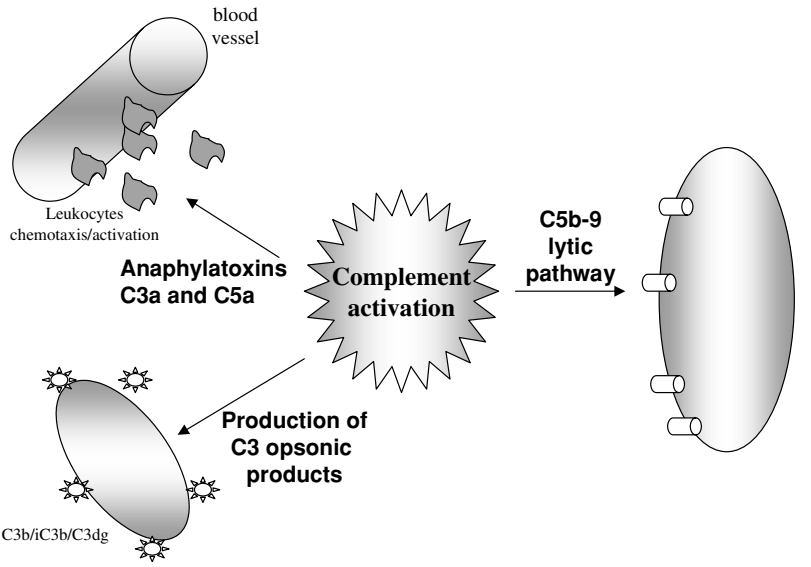

Figure 4.2. Main actions of the complement system. The major antibacterial components are the breakdown products of C3 (C3b, iC3b and C3dg) that covalently bond to the bacterial surface and enhance the process of phagocytosis and bacterial killing. The anaphylatoxins (C3a and C5a) are chemotactic and activate recruited leukocytes. The lytic pathway of complement forms a complex with the ability to insert into the bacterial membrane and form a damaging pore.

inflammation. The complement system is one of the major effector arms of both the innate and adaptive immune responses and is recognised to be involved in: (i) the killing of microorganisms; (ii) the solubilisation and clearing of immune complexes, and (iii) the enhancement of B lymphocyte responses (the latter effect is reviewed by Fearon and Locksley, 1996; Carroll, 1998; Carroll, 2000). There is also growing evidence for the ability of the complement system to act to control the key regulatory cytokine, IL-12 (Karp and Wills-Karp, 2001).

The activation of the complement system provides three sets of antimicrobial proteins: (i) opsonins (principally C3b and its products and also C4b) to bind to bacteria and enhance bacterial phagocytosis and antibody formation, (ii) anaphylotoxins (C3a, C5a) to enhance inflammatory events and cause leukocyte activation/chemoattraction, and (iii) a lytic complex to kill bacteria (Fig. 4.2). C3 is present at a concentration of 1.3 mg ml^{-1} in serum and is the key participant in the antimicrobial actions of the complement system. Cleavage of C3 by one of the C3 convertases (C4b2a of the classical pathway or

this complex, in turn, binds C7 (forming C5b67). The interaction of C7 results in the complex having the ability to insert itself into lipid bilayers. C8 then binds to this membrane-associated complex (C5b678), which can then associate with as many as fourteen C9 monomers to form a membrane pore. It is thought that this pore has antibacterial actions (Joiner et al., 1985) and, as will be discussed, there is increasing evidence for this. However, opsonophagocytosis still seems to be the major antibacterial defence mechanism of the complement system.

4.3 THE INVOLVEMENT OF THE COMPLEMENT SYSTEM IN ANTI-BACTERIAL DEFENCES

Now that we have briefly described the salient features of the complement system, what is the evidence for its role in protection against bacterial infections? The main evidence supporting the role of the complement system in antibacterial protection comes from individuals with deficiencies in individual complement genes (Table 4.2). Such genetic deficiencies can be broadly divided into seven categories: (i) classical pathway genes, (ii) mannose-binding lectin, (iii) alternative pathway genes, (iv) C3, (v) genes encoding the MAC, (vi) regulatory protein genes, and (vii) complement receptors. Further evidence is now emerging from the generation of complement gene transgenics (Mold, 1999). Deficiencies in the components of the classical pathway result largely in individuals with the symptoms of SLE or immune complex disease. These individuals can also suffer recurrent infections. Low levels of serum mannose-binding lectin are associated with recurrent infections in young children, but not in adults. Deficiency in the gene encoding the alternative pathway protein, D, results in recurrent upper respiratory tract infection while deficiency in alternative pathway protein, P (properdin), results in an enhanced susceptibility to fatal fulminant meningococcal infections. The importance of C3 is demonstrated by the finding that individuals deficient in this gene have recurrent infections. In contrast, deficiencies in the genes involved in construction of the MAC are manifest by an enhanced susceptibility to recurrent infections with *Neisseria* spp. Individuals deficient in C9 are generally healthy suggesting that the C5-8 complex is sufficient to cause damage to bacterial cell walls. However, C9 deficiency can be associated with recurrent Neisserial infection. Even in Japan where C9 deficiency is rather common (1 in 1,000), usually without symptoms, patients with recurrent meningococcal meningitis are most likely to be C9 deficient (Ngata et al., 1989). Deficiency in complement control proteins, such as factor I, can result in recurrent infections. Complement receptor protein deficiencies are

Table 4.2. *Human infections associated with genetic deficiencies of complement components*

Complement component deficient	Infection or condition
C1q	Chronic bacterial infections with encapsulated organisms (22%)
C2, C4	Primarily immune complex disease. Some infections (20%)
H, I	Recurrent infections with pyogenic organisms (40–100%)
Properdin, D	Meningococcal meningitis (70%)
C3	Recurrent infection with pyogenic organisms (*Strep. pneumoniae, Strep. pyogenes, H. influenzae, Staph. aureus*) (80%)
CR3	Recurrent infection with pyogenic organisms (*Strep. pneumoniae, Strep. pyogenes, Pseudomonas* spp, *Staph. aureus*) (100%)
C5	Recurrent meningococcal and gonococcal infections and recurrent infections with staphylococci, streptococci, proteus, pseudomonas, and enterobacter (60%)
C6, C7 or C8	Recurrent meningococcal and gonococcal infections (75%)
C9	Recurrent meningococcal and gonococcal infections (8%)

Note: Table indicates the most frequently observed infections associated with complement deficiencies together with an indication of the frequency of those infections that have been observed in individual cases. For example, individuals with C9 deficiency almost always suffer from Neisserial infection but only 8% of such people seem to suffer from recurrent infection.

also associated with pathology (see reviews by Colten and Rosen, 1992; Mold, 1999; Walport 2001a, 2001b).

Over the past decade, transgenic mice have been produced in which a small number of complement genes have been inactivated (Table 4.3). The response of these mice to bacterial infection or endotoxin challenge is generally deficient. Surprisingly, the lack of C3 appeared to have no influence

Table 4.3. *Response to infection by mice with complement gene knockouts*

Gene inactivated	Response	Reference
C1q	increased mortality/SLE	Botto 1998
C3/C4	reducedLD_{50} to GBS[a]	Wessels et al. 1995
C3/C4	enhanced response to endotoxin	Fischer et al. 1997
C3/C4	increased lethality to CLP[b]	Prodeus et al. 1997
C3/C4	enhanced *E. coli* colonisation	Springall et al. 2001
CR3	no influence on *Mycobacterium tuberculosis* infection	Hu et al. 2000
C3a receptor	enhanced lethality to endotoxin	Kildsgaard et al. 2000
C5	decreased clearance of *Pseudomonas aeruginosa*	Cerquetti et al. 1986
C5	hypersusceptibility to *M. tuberculosis* infection	Jagannath et al. 2000
C5	deficit in granulomatous response to *M. tuberculosis*	Actor et al. 2001
C5a receptor	decreased mucosal clearance of *Ps. aeruginosa*	Hopken et al. 1996
Urokinase	decreased clearance of *Ps. aeruginosa*	Gyetko et al. 2000

[a] GBS: Group B streptococci.

[b] CLP: Caecal ligation and puncture-induced peritonitis.

on infection by *Mycobacterium tuberculosis* (Hu et al., 2000) although the lack of C5 decreased the host protective response to this organism. These findings generally support the clinical information available from the investigation of natural complement deficiencies in *Homo sapiens*.

4.4 BACTERIAL EVASION OF THE COMPLEMENT SYSTEM

With such a plethora of mechanisms for the activation of complement, it is clear that in any infection the susceptibility of the microorganism will depend on which mechanisms are activated and to what extent. This will also change during the course of an infection as the balance of innate and adaptive immunity changes. Many aspects of the interaction of an organism with the immune system might indirectly or directly affect the amount of complement activation, e.g., the type and amount of antibody produced, or the intensity of the acute phase response. That bacteria can directly evade the complex defences of the complement system has also been recognised for some time

Table 4.4. *Bacterial strategies to evade the complement system*

Evasion strategy	Bacterium	Molecules involved
Bacterial capsule	GAS	hyaluronic acid-containing capsule
	Group B streptococci	type III capsular polysaccharide and sialic acid
	Neisseria spp	capsule and capsule containing sialic acid/LPS
	Staph. aureus	
	Haemophilus spp	
	E. coli	lipopolysaccharide
	Salmonella spp	lipopolysaccharide
	Meningococci	lipopolysaccharide
Proteinases	*P. gingivalis*	gingipain
	GAS	C5a peptidase
	Ps. aeruginosa	elastase
Chemical inactivation	*H. pylori*	Urea/ammonia
	Ps. aeruginosa	
Binding to RCA proteins	GAS	M protein family
		Protein H
	Strep. pneumoniae	Hic
	Bord. pertussis	filamentous haemagglutinin
	N. gonorrhoeae	Por1A/Por1B
	B. burgdorferi	many (CRASP/OspE etc.)
	Y. enterocolitica	YadA
Ibhibition of lytic pathway	GAS	Streptococcal inhibitor of complement (SIC)
	Y. enterocolitica	Ail
	S. typhimurium	Rck and Trat
	E. coli	Trat and binding protectin
	H. pylori	binding protectin
	Moraxella catarrhalis	?

but only in the last decade or so has the range of mechanisms that bacteria utilise to achieve this begun to be defined (Mold, 1999; Rautemaa and Meri, 1999; Lindahl et al., 2000; Table 4.4). Viruses have also been shown to be able to evade complement-mediated attack by, for example, encoding proteins with homology to host complement control proteins such as protectin (Albrecht et al., 1992).