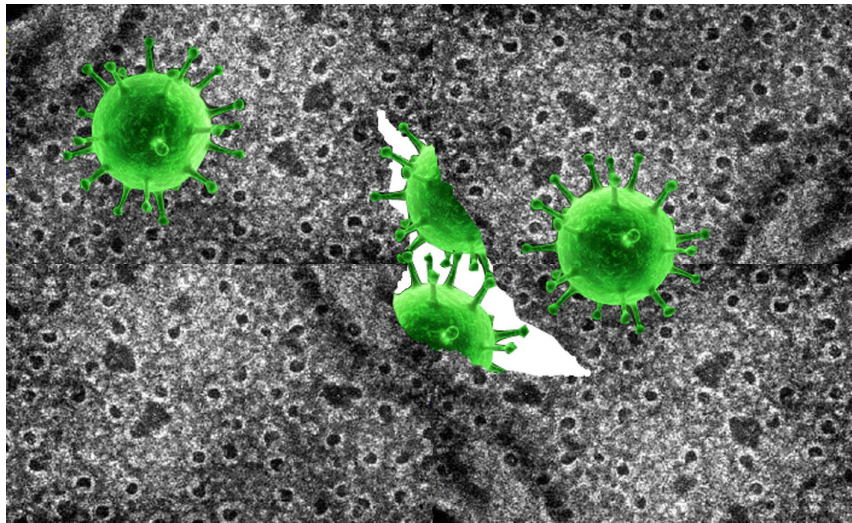# Evasion of the terminal complement pathway by human pathogens

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**Contents**

**About the cover**

The figure on the cover is an artist impression of complement evasion. It depicts the escape of pathogens (green spheres) from the lesions inflicted by membrane attack complexes (electron microscopic photo).

**Abstract**

The complement system is an important part of the human immune system. It can react quickly to entering micro-organisms and eliminate them. Therefore, pathogens have to evade the complement attack in order to have a chance at survival within the host. This can be done by preventing recognition or controlling the complement cascade. Viruses, bacteria, fungi and parasites all developed escape strategies. Many of the proteins involved are targeted against the activation stage of complement, but it is also interesting for pathogens to defend themselves from the terminal pathway. In the final stage the membrane attack complex is formed which causes death through osmotic lysis. The many proteins involved in the terminal complement pathway provide several sites for pathogens to interfere with. Here, an overview is given of all the complement evasion mechanisms related to the terminal pathway known to date.

## Introduction

The human immune system consists of multiple mechanisms to protect the body against pathogenic micro-organisms. The complement pathway is an important part of this system because it is regarded as a first line of defence: upon the entry of a micro-organism complement immediately recognises the invader and is rapidly activated.

The best known function of complement is the lysis of cells, bacteria and viruses. However, many other processes are also induced: promotion of phagocytosis through opsonisation, neutralisation of viruses, clearance of immune complexes and apoptotic cells and the activation of an inflammatory response. Through stimulation of the humoral immune response, complement forms a bridge between innate and adaptive immunity[1, 2].

Pathogens are, as they are resistant to an immune attack, micro-organisms that cause disease. For these pathogens it is necessary to evade the immune system to be able to survive within their hosts. Because the complement cascade is one of the first defence mechanisms the invaders encounter, many pathogens have developed ways to prevent complement recognition and to escape or control complement attack. These evasion strategies can be direct through interaction with the complement proteins or indirect by mimicking or interfering with the complement regulators. For every step in the complement cascade, evasion mechanisms have been described for different pathogens; viruses, bacteria, parasites and fungi all have developed evasion strategies.

The evasion mechanisms have been described mostly for the initiation part of the complement pathway. This is also the most beneficial for the pathogen because when the cascade is stopped early it affects the pathogen the least. But, the final molecules of complement are the most dangerous for pathogens as those form the membrane attack complex (MAC). The MAC is inserted into the membrane forming a pore which causes osmotic lysis leading to the death of the micro-organism or infected cell. Thus, also for the terminal part of the complement system evasion mechanisms exist. Moreover, despite the fact that one blocking step is usually sufficient, evasion is most efficient when multiple steps in the pathway are inhibited[3]. Many pathogens therefore possess more than one complement evasion molecule.

Here, an overview will be given of the evasion mechanisms of human pathogens related to the terminal part of complement. To show that different organisms developed

similar mechanisms, the complement escape strategies are separated by their moment of action instead of by organism.

## Complement

The complement system is a compilation of more than 30 proteins that interact with each other, resulting in a cascade that ends with the lysis of infected cells and micro-organisms. One of the most confusing parts of this system is the nomenclature. Several of the involved proteins are zymogens which become active upon cleavage. The two generated fragments are then designated 'a' for the smaller fragment and 'b' for the larger fragment. There is an exception for C2 of which the large fragment is called C2a and the small fragment C2b. To make the nomenclature even more confusing, the complement proteins were numbered in order of discovery. However, this appeared not to correspond with the order of reaction: C1 is sequentially followed by C4, C2, C3 and C5, but the cascade ends in numerological order with C6 through C9.

Complement is a very ancient system that already existed before the emergence of vertebrates. In the sea urchin, an invertebrate, homologues of C3 and factor B have been found. Similarly, in several protochordates complement regulatory-like molecules and lectin pathway associated molecules are identified. Jawless fish, the most primitive vertebrates, seem to be the first to have the alternative and lectin activation pathways of complement. This is based on the presence of a mammalian C3 homologous protein with opsonic activity and a MASP protein. Teleost fish are the first animals in which all three activation pathways (alternative, lectin and classical) are present[4].

The evolution of the terminal or lytic pathway of complement started later than the activation parts of the cascade. A protein similar to the complement regulatory protein CD59 in hagfish and the isolation of C8 and C9 indicate the presence of a terminal complement pathway in primitive vertebrates. However, the first functional terminal pathway that also resembles the mammalian membrane attack complex was found in teleost fish. Comparing the C6, C7, C8 and C9 molecules showed that these proteins are structurally and biochemically very similar. Further sequence analysis suggests that C6 and C7 evolved first, followed by the emergence of C8 and C9, probably through gene duplication[4, 5].

The mammalian complement system has been extensively studied and described[1, 2, 6, 7] and we have a good idea of how it works (fig.1). The cascade can roughly be divided into two parts: activation and the terminal pathway.

### Complement activation

The complement pathway has a special position within the immune system. It is classified as part of the innate immune system, but also connects with adaptive immune responses. This bridge between innate and adaptive immunity can be explained by the stimulation by complement of the humoral immune response, but also by one of the three different ways by which complement can be activated.

The first discovered pathway is the classical pathway. This is the pathway connecting with the adaptive immune system because it begins with antigen-antibody complex formation. This induces conformational changes which allow the first component of complement, C1, to bind to the Fc region of the antibody. Subsequently, C1 is activated and able to cleave C4 and C2. The larger fragments of these molecules together form a C3 convertase: C4b2a. The C3 convertases can hydrolyse many C3 molecules after which some of the convertases combine with C3b (C4b2a3b) to form a C5 convertase.

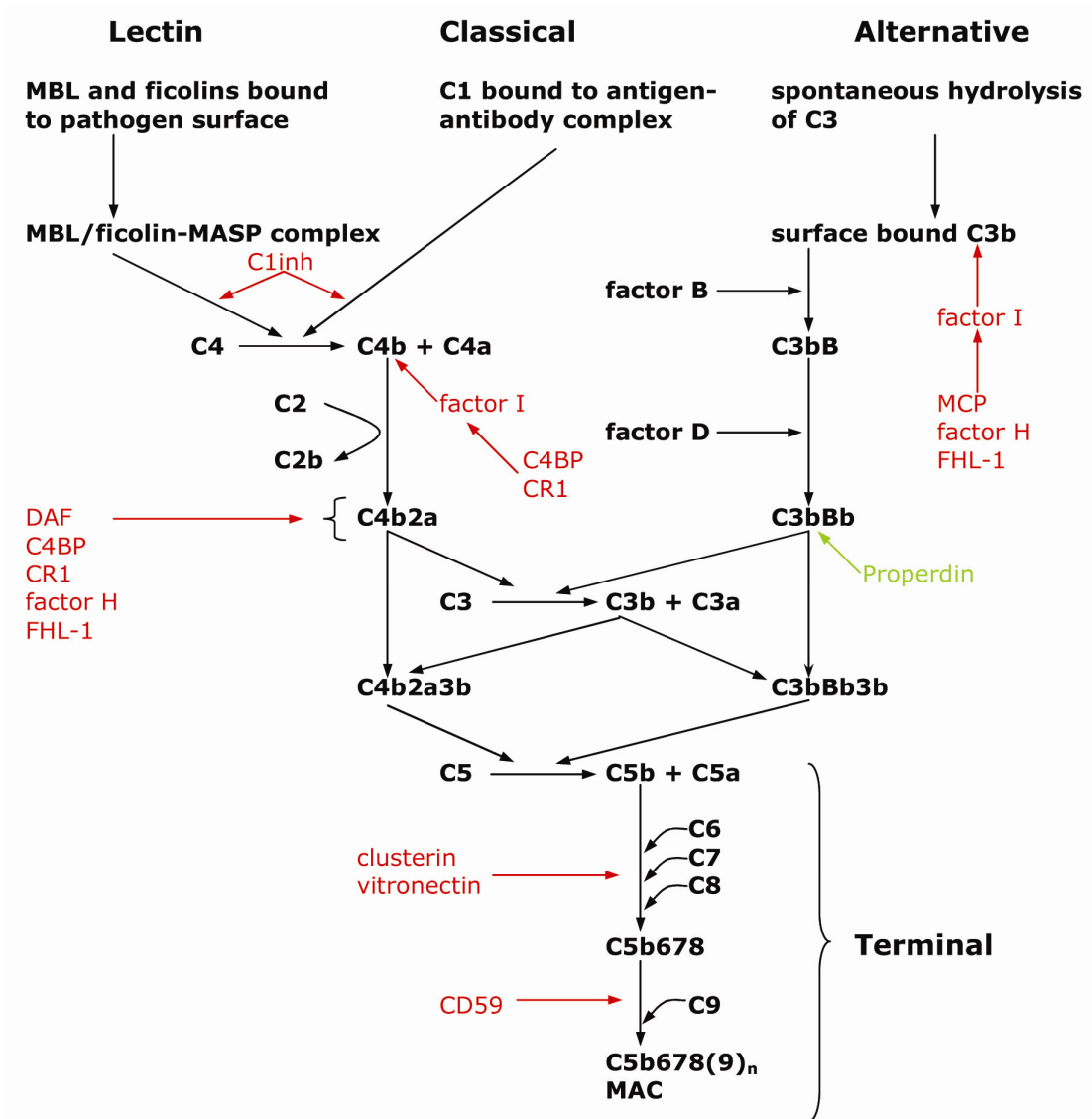The lectin pathway is similar to the classical

**Figure 1. The complement cascade.** Complement can be activated by three different activation pathways: the lectin, classical and alternative pathway. All activation routes lead to the terminal pathway which results in the formation of the membrane attack complex (MAC). The cascade is regulated by several inhibitory proteins (indicated in red) and one stabilising protein (indicated in green) that interfere with the different steps of the pathway.

pathway but is initiated differently. Mannose binding lectin (MBL) and ficolins bind to mannose residues on carbohydrates and glycoproteins on the surface of micro-organisms. MBL-associated serine proteases, MASP1 and MASP2, can now bind to MBL and ficolin upon which the complex becomes active. Because of their structural similarity to C1 they can mimic C1 activities and thereby cleave C2 and C4.

The third pathway is the alternative pathway. Slow spontaneous hydrolysis of the C3 component occurs due to an unstable thioester bond. C3b is then able to bind to foreign surface antigens and then recruit factor B. Upon the cleavage of factor B by factor D, the C3 convertase C3bBb is generated. Like C4b2a it is able to cleave C3 and bind C3b forming the C5 convertase C3bBbC3b. Because the alternative pathway is not actively initiated, it could be considered an amplification route on top of

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the classical and lectin pathways.

In all three pathways a C3 convertase is formed which cleaves C3. This is an important point in the complement cascade because this is the major amplification step. Upon the cleavage of C3, C3b is generated which can combine with factor B forming new C3 convertases resulting in great amounts of C3b.

The smaller fragments generated by the cleavage of C3, C4 and C5 are called anaphylatoxins. After they are clipped off, the fragments diffuse away and bind to specific receptors triggering several inflammatory responses. Specially, degranulation of mast cell and basophils is induced, resulting in the smooth muscle contraction and increased permeability of the blood vessels. Furthermore, more adhesion molecules are expressed on the surface of macrophages and monocytes and the secretion of IL-1 and IL-6 is increased. C3a, C4a and C5a are also chemoattractants, drawing particularly neutrophils to the site of complement activation.

The active components C3b and C4b, besides forming convertases, can also bind to receptors on a variety of cells. In this way, these molecules facilitate opsonisation, through which phagocytosis of the immune complexes and antigens bound to C3b or C4b is promoted.

*Terminal complement pathway*
All three activation pathways lead to the cleavage of the fifth component of complement, C5. With the generation of C5b the terminal part of the complement pathway is induced. Once bound to the surface of the target cell C5b can bind C6 and C7. A structural transition of the complex exposes the hydrophilic domains which enables the insertion of the complex into the plasma membrane. Another conformational change results from the binding of C8. Hereby, the C5b678-complex is stabilised in the membrane forming a

small pore. However, this pore is too small to induce cell lysis. To achieve cell death, the C9 molecule is necessary. The binding and polymerisation of C9 completes the formation of the membrane attack complex (MAC) (fig.2). The MAC forms a larger pore in the phospholipid bilayer causing a loss of osmotic stability which results in the death of the target cell.

*Regulation of complement activation*
Strict regulation of complement is necessary to make sure that only pathogens are attacked and not the host cells. Various components of complement form an initial, passive level of control as they are highly labile components: when no reaction with other components occurs, the molecules become inactive rapidly.

The only factor that has a positive effect on complement is properdin. Properdin binds C3bBb, stabilising the complex and thereby lengthening the limited half-life.

The most regulators are part of a group of proteins called RCA: regulators of complement activation. These proteins all contain repeating amino acid motifs called short consensus repeats (SCR), which cause the proteins to be structurally similar. RCA are particularly involved in the formation and activity of C3 convertases. C4 binding protein (C4BP), complement receptor type 1 (CR1), Factor H and membrane cofactor protein (MCP or CD46) block the assembly of C3 convertases by binding to C3b or C4b and thereby preventing the binding of C2 or factor B. C4b bound to CR1 or C4BP and C3b bound to Factor H, Factor H-like protein (FHL-1) or MCP are susceptible for cleavage by Factor I, leaving them inactive. Factors that accelerate the decay of C3 convertases are decay-accelerating factor (DAF or CD55) and the previously mentioned CR1, Factor H, FHL-1 and C4BP.

Important for the classical and lectin pathway is C1-inhibitor (C1inh). This protein can bind to C1 and MBL-MASP

complexes and blocks their enzymatic activity needed for cleavage of C2 and C4.

## Regulation of terminal complement components

In the terminal complement pathway there are also some regulators present. Vitronectin, also known as S-protein, and clusterin show similar functions in complement regulation. They both bind soluble C5b67 complexes such that it can no longer insert into cell membranes. C8 and C9 are still able to bind, but C9 polymerisation is inhibited. In this way, cells surrounding the complement activation site are protected from bystander lysis.

Vitronectin is found in blood plasma and in the extracellular matrix. In plasma, vitronectin can occur in two forms: the whole single chain with a length of 459 amino acids and a molecular weight of 75kDa and a cleaved form of two chains (65 and 10kDa) which are held together by a disulfide bridge formed by $Cys^{274}$ and $Cys^{453}$. The protein is anchored in the extracellular matrix through its collagen binding domain and glycosaminoglycan binding domain. The amino terminal segment contains domains which can bind to integrins and the urokinase receptor enabling cell adhesion and migration. Hemostasis is influenced through the interaction of vitronectin with the thrombin-antithrombin III complex and regulation of fibrinolysis is achieved by binding plasminogen activator inhibitor-1 and plasminogen[9]. Finally, vitronectin is able to bind C5b67 and inhibit complement. This is mainly important for neighbouring cells of a complement attacked cell, because released C5b67 complexes can still insert into
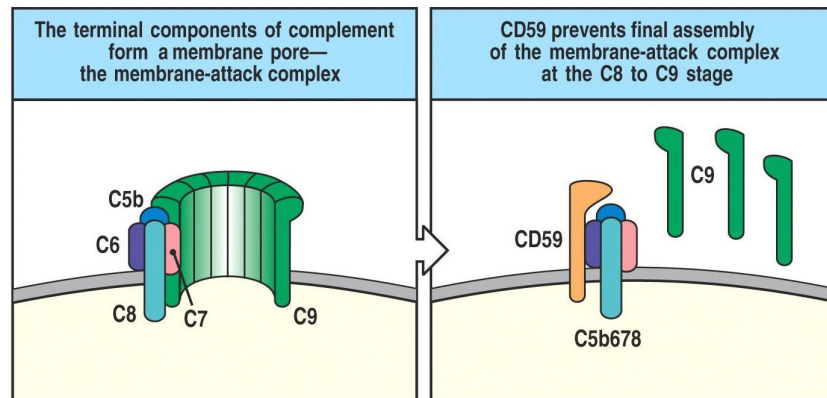


**Figure 2. Assembly of the membrane attack complex (MAC) and inhibition of assembly by CD59.** After cleavage of C5, C6 and C7 bind to C5b. The C5b67 complex binds to the membrane through C7. With the binding of C8 the complex is stabilised in the membrane. Finally, multiple C9 molecules bind and are inserted into the membrane forming a pore through the lipid bilayer called the MAC (left panel).
CD59 binds to C8 and C9, preventing the polymerisation of C9 and thus the final assembly of the MAC (right panel). Figure adjusted from Janeway *et al*[8].

membranes and cause bystander lysis.

If soluble C5b67 manages to insert itself into a neighbouring cell, the CD59 protein (or protectin, homologous restriction factor (HRF), membrane inhibitor of reactive lysis (MIRL)) can prevent completion of the MAC. CD59 is a through glycosyl phosphatidyl-inosytol (GPI) membrane bound protein, has a weight of 18-20kDa and is a member of the leukocyte antigen 6 protein family. CD59 has been associated with T-, B- and NK-cell regulation and might be a co-stimulator for the T cell receptor CD2. Intermedilysin, a bacterial toxin produced by *Streptococcus intermedius*, has been shown to use CD59 as a receptor. But the best known role of CD59 is inhibition of the complement system and it is expressed in almost all tissues in the body to protect cells from bystander lysis. CD59 is highly expressed on reproductive cells, for instance, so that a complement attack can be blocked during fertilisation and pregnancy[10]. The membrane bound CD59 protein contains three β-strands, a small helix followed by a helix and a small β-ribbon (fig 3). Several cysteine bonds (3-26, 6-13, 19-39, 45-63 and 64-69) stabilise the structure of the protein. Between the helix and the second β-strand (D in fig 3) there is a hydrophobic gap which provides the binding sites for C8 and

C9[11]. A consensus sequence of VSLAFS has been identified for the primary binding site for CD59 in C9. This sequence is located at the residues 365-371. There is substantial sequence homology between C8 and C9 and the CD59 binding peptide derived from C9 is able to inhibit CD59-C8 interaction. Therefore, it is suggested that the binding region of C8 overlaps the CD59 binding site of C9[12]. Upon binding of CD59 to C5b678 or C5b6789, binding and polymerisation of C9 to the complex is blocked. By inhibiting the completion of the MAC, the cells expressing CD59 are protected from complement attack (fig. 2) [13].

## Mechanisms of evading complement activation

The complement system is the most important host defence system for pathogens to watch out for. The first attack on microorganisms is performed by complement and it can activate the innate as
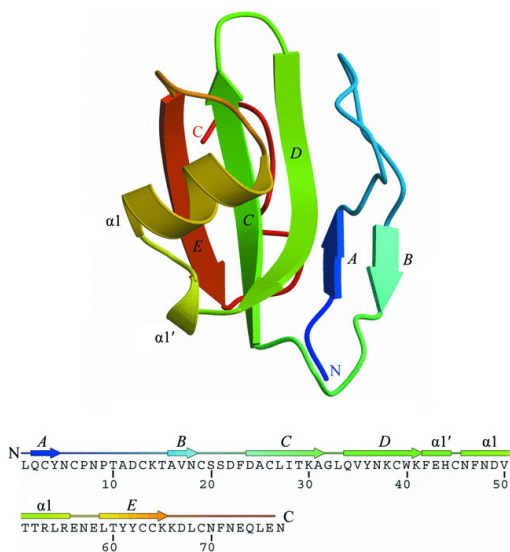


**Figure 3. Structure of human CD59.** At the top the structure of CD59 is displayed in ribbon format and is coloured from blue to red in the N-terminal to C-terminal direction. The secondary structure contains a β-ribbon (A and B), β-strands (C-E), a small helix (α1') and a helix (α1). At the bottom the amino acid sequence is shown in relation to the secondary structure elements. Figure obtained from Huang *et al*[11].

well as the active immune response. Thus, naturally, many pathogens have evolved at least one mechanism to evade complement in order to have a chance at survival within the host. The many steps and molecules involved in the complement cascade ensure an effective antimicrobial response, but also offer multiple sites for pathogens to control the cascade and thereby prevent recognition or escape an attack.

Some pathogens even use complement for their own benefit. Predominantly, viruses use complement receptors or surface bound regulators to enter host cells. The most intriguing mechanism is carried out by HIV-1 and *Mycobacterium tuberculosis* that both activate complement on purpose so they can use the deposited C3b to bind to receptors on host cells[14]. Also, *Candida* recruits C4BP that mediates adhesion of the fungus to endothelial cells, suggesting facilitation of tissue invasion[15].

Most complement evasion mechanisms have been described for bacteria, but other pathogenic organisms, like viruses, parasites and fungi, also have developed strategies. Some mechanisms are shared between different organisms.

The evasion strategies can be divided into three main mechanisms: degradation of complement components, recruiting or mimicking of complement regulators and direct inhibition.

### *Degradation of complement components*

Evasion is effectively done by cleaving the attacking molecules into smaller, inactive parts. This degradation is performed by proteases which are produced and secreted by the pathogen, but they can also be acquired from the host itself. The most well known example of using the host's own regulators are the plasminogen activators of bacteria. Upon activation of plasminogen (a zymogen within the coagulation system), the active molecule plasmin is generated which

in turn is able to cleave C3[14].

Plasmin is not the only protease known to cleave dangerous components off bacterial surfaces. There are several bacteria that produce proteases that cleave off surface bound IgG, C1 or C3, like group A streptococci, *Pseudomonas aeruginosa* and *Porphyromonas gingivalis*[3, 16]. But also soluble complement molecules are inactivated; group B streptococci, for instance, degrade the anaphylatoxin C5a with C5a peptidases[16].

It is striking that the cleaving evasion mechanisms are almost only used by bacteria; viruses and parasites are not known to have proteases against complement and in fungi only two species (*Candida albicans* and *Aspergillus fumigatus*) have been found to secrete a C3 protease[15].

*Recruiting and mimicking regulators*

The most widely used evasion mechanism by pathogens is the recruitment and mimicking of host complement regulators. The required regulators are bound to the surface and thereby protect the pathogen against complement attack. This is an energetic advantageous and smart way of fooling the host immune system. The proteins are produced by the host itself and are thus the natural regulators, perfectly fitted to inhibit the attack. Furthermore, many regulators contain SCR domains which allows the pathogen to bind different host regulators with the same recruiting protein directed against SCR domains [14].

Viruses mainly recruit regulators expressed on the host cell surface, like DAF and MCP. This strategy is followed by enveloped viruses (*vaccinia*, *HIV*, *HTLV*, *PRV*) that bud off from the host cell membrane which, naturally, contains host regulators[17, 18].

Bacteria recruit host regulators not by taking the host cell membrane but by capturing them with specialised proteins on their surface. Well known examples are the complement regulator acquiring surface proteins (CRASPs) of *Borrelia burgdorferi* and the M protein family in *Streptococus pyogenes*. The regulators than captured are soluble proteins like factor H or C4BP[19]. The same mechanism has also been found in several fungi[15]. The bacterium *E. coli*O157:H7 secretes StcE, a C1inh binding protein. By binding and cleaving this inhibitor, StcE enhances the function of C1inh and thus the inhibion of the classical pathway[20].

The mimicry of complement regulators is almost only seen in viruses. Particularly the proteins with not just a functional but also a genetic similarity with host regulators has only been found in viral pathogens. The vaccinia virus control protein (VCP), small pox inhibitor of complement enzymes (SPICE) and open reading frame (ORF) 4 of HVS show high similarity with host regulators like DAF and C4BP. These viral proteins contain several SCRs and bind C3b and C4b, function as decay accelerators of C3 convertases and even show co-factor activity for factor I[17, 18].

The ticks *Ixodes scapularis* and *I. Ricinus* express the *Ixodes scapularis/ricinus* anticomplement protein (ISAC/IRAC). These proteins inhibit C3b deposition and accelerate the decay of the C3 convetase C3bBb3b. ISAC and IRAC show functional resemblance to the host regulator factor H. However, unlike the viral mimicked regulators, these proteins are not genetically similar to the host proteins[21, 22].

*Direct inhibition of complement proteins*

In contrast with the regulatory proteins, C1 through C9 are little interfered with by pathogens. This is surprising, because those are the proteins that form the core of the complement system and if one of these proteins would be taken out, the cascade fails. There are only a few species that directly inhibit the key components, but still for several components of C1-C9 a blocking protein is known. Direct inhibition occurs

through the binding of the inhibitory molecule to a complement component in such a way that activation of or binding to another component is blocked.

For example, the hookworm *Necator americanus* secretes a calreticulin-like molecule that can bind to C1 and thereby inhibit lysis via the classical complement pathway[23]. All three activation pathways are blocked by the gC1 protein of *herpes simplex virus* which binds C3b[17]. And so does the extracellular fibronectin binding molecule (Efb) of *S. aureus*: it binds to C3 and alters its conformation in a way that activation can no longer take place and C3b cannot be deposited on the pathogenic surface[3].

*S. aureus* also has a C3 convertase inhibitor. Staphylococcal complement inhibitor (SCIN) is able to bind both C3 convertases. Through this stabilisation of C3bBb and C4b2a their enzymatic activity is impaired and no additional C3a and C3b are generated[3].

Moreover, several binding proteins for the terminal components of complement have been discovered and these will be discussed later on.

## Evasion of the terminal complement pathway

C3 is considered the centrepiece of the complement pathway because that is where all activation routes meet and where amplification of the cascade is established. It would therefore not be surprising if pathogens try to evade complement at this point. Interfering at another, later point, at the level of the MAC for instance, only seems partially beneficial. Then, the pathogen is already opsonised through the deposition of C3b and anaphylatoxins have been released to activate other immune responses. However, the defences of several pathogens against complement lie within the final steps of the pathway and are sufficient to survive within the host. For some pathogens these late escape mechanisms are an addition to evasion at other points in the complement cascade, increasing the chances of survival, but other organisms rely on that one protein that protects them from the killer molecule MAC.

Here, the evasion mechanisms are discussed for every step in the terminal complement pathway. Some mechanisms are unique for a single bacterium, parasite or virus, some are shared between organisms. Of the three active evasion mechanisms discussed earlier, degradation of complement components does not occur in the terminal pathway. The two other strategies, however, are used extensively and particularly the complement regulators are a popular target among pathogens. For the MAC, an extra evasion mechanism exists which is probably the simplest as it is passive.

### Passive evasion strategies

Some pathogens escape from complement attack simply due to their structure: gram positive bacteria and fungi have a cell wall that is too thick to be perforated by the MAC[14, 15]. Furthermore, some parasites and Gram-negative bacteria express glycoproteins on their surface which makes it impossible for complement components to attach to them. In the case of the bacterium *Salmonella minnesota*, it causes the MAC to be unable to insert into the membrane and be released from the surface[24].

### C5 – C9: direct component inhibition

The terminal complement pathway starts with the cleavage of C5. *S. aureus* is known to express several staphylococcal superantigen like (SSL) proteins of which SSL7 is able to bind to C5. It thereby probably inhibits cleavage of the protein and thus prevents formation of the MAC. It is feasible that the production of the anaphylatoxin C5a is then prohibited as well which is likely to be of more value because *S. aureus* is resistant to lysis by MAC

through a peptidoglycan-rich cell wall[25].

Soft ticks do not enter the human body, but take blood meals that can take a few hours. Therefore, they still have to interfere with several processes in the host, like complement. In the saliva of the soft tick *Ornithodorus moubata* a protein was found with a function similar to SSL7 of *S. aureus*; OMCI (*O. moubata* complement inhibitor) binds directly to C5 and thereby inhibits C5 cleavage[5, 26]. Like OMCI, TSGP2 and TSGP3 of *Ornithodorus savigny* belong to the lipocalin protein family of soft ticks. Between these three proteins high levels of sequence identity exists and the most important similarity is a conserved loop. This loop appeared to be involved in the interaction with C5. OMCI, TSGP2 and TSGP3 bind C5 with similar affinity while lipocalin family members that do not contain this loop also do not bind C5[27].

At the level of C6 the K12 strain of *Escheria coli* counteracts complement with the TraT protein. TraT is an outer membrane protein that inhibits the final complement steps after C5 and before C7. Probably, it either prevents C5b6 complex formation or inactivates the complex by structural changes[28].

A little further downstream the complement pathway the insertion of C5b67 into the membrane can be blocked by the streptococcal inhibitor of complement (SIC) of the *Streptococcus pyogenes* strains M1, M12, and M55. The extremely polymorphic SIC protein is encoded by a gene in the mga-regulon which also contains other virulence proteins like M proteins and the C5a peptidase. In function SIC is similar to the hosts natural regulator clusterin: SIC binds the C5b67 complex and prevents lysis through MAC formation with equal efficiency as clusterin[29, 30].

In the M57 serotype of *S. pyogenes* a SIC-like protein is located outside the mga-regulon. This protein closely related to SIC (CRS) has similar structural and biochemical characteristics to SIC: it also is an excretory product and is able to bind to C6 and C7, inhibiting the terminal complement pathway[31].

A second variant of the SIC protein has been found in the *S. pyogenes* strains that contain the emm12 and emm55 genes. DRS (distantly related to SIC) shows less sequence similarity to SIC than CRS, but is also able to bind to C6 and C7. However, this has little effect on the lytic capacity of complement. And although SIC and DRS are expressed in the same serotypes and compete with each other for binding to C6 and C7, DRS does not affect the inhibitory function of SIC. The function of DRS thus remains unclear[32].

The final step of MAC assembly is prevented by the Rck protein of *Salmonella enterica* serova Typhimurium, a bacterium that can cause gastroenteritis. The 17kDa outer membrane protein prevents polymerisation of C9[33] for which the third outer membrane loop region seems to be essential[34]. Rck belongs to a protein family which also includes Ail of *Yersinia enterocolitica*. Like Rck, Ail is involved in the serum resistance[35], but it is unclear whether the protein also inhibits C9 polymerisation. The same accounts for *Yersina pestis*, the causative agent of plague, which also expresses Ail[36].

## Mimicking CD59

Over 200 million people, mainly in Africa and South America, are affected by schistosomiasis or bilharzia caused by *Schistosoma mansoni*[37]. This helminth is a successful complement evader, knowing to block the cascade at multiple steps by binding several complement components. One of these proteins was first described as SCIP-1 (*Schistosoma* complement inhibitory protein)[38] but later appeared to be paramyosin (Pmy)[37]. Paramyosin is an invertebrate muscle protein expressed on the surface of *S. mansoni*. This protein shares some characteristics with the human

regulatory protein CD59: a through GPI membrane bound protein and able to bind C8 and C9[38]. Next to binding the final components polymerisation of C9 is also inhibited, leading to incomplete MAC assembly. The binding site for C8 and C9, or the complement regulatory domain, has been mapped to the C-terminal region of Pmy[37].

Other parasites that produce a CD59-like molecule are the amoebas *Entamoeba histolytica* and *Naegleria fowleri*. The latter is rare (200 cases between 1960 and 2000[39]) but fatal as it causes the nervous system destroying disease meningoencephalitis. *E. histolytica* affects more people (50 million/year) but an infection is not necessarily fatal when drugs are available[40]. Their CD59-like proteins function similarly to the human CD59 as blockers of MAC formation through binding of C8 and C9. The protein expressed on the surface of *N. fowleri* is cross-reactive with antibodies against human CD59 and also has a comparable molecular mass of 18kDa (18-20kDa for human CD59[13])[41]. Although the protein first found in *E. histolitica* shared epitopes with human CD59, it was very different in molecular weight (260kDa)[42]. However, another protein with antigenic similarity to CD59 was recently discovered of which the molecular weight is more in proportion to the natural complement regulator (21kDa)[43].

The complement resistant strains of the Lyme disease causing bacterium *Borrelia burgdorferi* have almost no MAC bound to their membrane. Further analysis showed that this micro-organism expresses a protein on its surface with antigenic and functional similarity to human CD59[44].

The last pathogen mimicking CD59 is a member of the herpesviruses. In *Herpesvirus saimiri* (HVS) the open ORF15 encodes a protein that shares amino acid sequence similarity to CD59[45]. Later, HVSCD59 was also shown to inhibit the terminal complement pathway[46].

## Using host regulators

While HVS mimics the CD59 protein, many other viruses simply (mis)use the hosts own regulators. They upregulate them while in the target cell or incorporate them into their virions.

The *human herpesvirus 7* (HHV-7) is a betaherpesvirus that specifically infects T cells. Higher levels of the CD46 and CD59 proteins were found in infected cells with western blot and flow cytometry. This suggests more production and subsequent expression on the surface of the proteins induced by HHV-7. The virus could hereby protect the host cell and thus itself from complement lysis[47].

Other viruses incorporate host complement regulators into their envelope. This mechanism has been described for *human immunodeficiency virus* (HIV), *human T cell leukaemia virus type 1* (HTLV-1), *human cytomegalovirus* (HCMV) and *vaccinia virus* [48-51]. These are all enveloped viruses which acquire their envelope by taking part of the host cell membrane during budding off the cell. It is therefore not surprising that only the extracellular enveloped virion of the *vaccinia virus* is more resistant to complement than the intracellular immature virion: the latter does not express, for example, CD59 because it has no envelope[51]. It has been proposed that because different kinds of enveloped viruses use this evasion mechanism, incorporation of host regulators might be a common mechanism for enveloped viruses[50]. For HIV it is indicated that budding of the virions occurs at lipid rafts in the host cell membrane. Since lipid rafts contain GPI-linked proteins such as CD59 and the disruption of lipid rafts causes a decrease of CD59 on HIV envelopes[52], it has been suggested that the presence of CD59 is one of the reasons for enveloped viruses to bud from lipid rafts and be protected from complement[17].

The first bacterium found to be able to bind CD59 was *Escherichia coli*. The lipid-A rich bacterial membrane can associate with the phospholipid tail of CD59. As a result, the incorporation of C5b-9 is inhibited and protection from complement killing is accomplished similarly for *E. coli* as for the host cells[53].

A second bacterial pathogen that also makes use of the host regulator CD59 is *Heliobacter pylori*. The *H. pylori* bacteria infect the human stomach and must escape complement attack to colonise. The bacteria in the gastric pits usually have CD59 bound which is correlated with an absence of C5b-9 on their surface. Misusing the host regulator thus contributes to the survival of the pathogen[54].

Vitronectin, another regulator of the terminal complement pathway, is bound by the Gram-negative pathogen *Haemophilus influenzae* serotype b (Hib). From the bacterial surface short, thin fibrils extrude which function as adhesins and are encoded by the highly conserved *hsf* gene. These proteins called *Haemophilus* surface fibrils (Hsf) are the major vitronectin binding proteins in encapsulated Hib. When challenged with normal human serum (NHS), survival of an Hsf mutant bacterium was strongly decreased compared to the wild type Hib. Binding of vitronectin inhibits the insertion of the C5b-7 complex, through which Hsf contributes to serum resistance of *H. influenzae*[55].

Likewise, *Moraxella catarrhalis* interferes with complement. *Moraxella catarrhalis* is a Gram-negative bacterium and a very common cause of otitis media in young children. Binding of vitronectin was shown to lead to serum resistance of the bacterium as incubation with vitronectin-depleted NHS caused the bacteria to be more susceptible to killing. The protein responsible is UspA2, which is expressed on the bacterial surface

| Pathogen | Evasion molecule | Evasion mechanism |
|---|---|---|
| Bacteria | | |
| *S. aureus* | SSL-7 | Binding C5 |
| *E. coli* | TraT | Preventing C5b6 complex formation |
| | | Incorporation of CD59 |
| *S. pyogenes* | SIC | Binding and inhibiting insertion of C5b67 complex |
| | CRS | Binding C6 and C7 |
| | DRS | ? |
| *S. enterica* | Rck | Inhibiting C9 polymerisation |
| *Y. enterocolitica* | Ail | ? |
| *Y. pestis* | Ail | ? |
| *B. burgdorferi* | CD59-like | Mimicking CD59 |
| *H. pylori* | | Incorporation of CD59 |
| *H. influenzae* | Hsf | Binding vitronectin |
| *M. catarrhalis* | UspA2 | Binding vitronectin |
| | | |
| Viruses | | |
| *HHV-7* | ? | Upregulating CD46 and CD59 |
| *HIV* | | Incorporation of complement regulators |
| *HTLV-1* | | Incorporation of complement regulators |
| *HCMV* | | Incorporation of complement regulators |
| *Vaccinia virus* | | Incorporation of complement regulators |
| *HVS* | HVSCD59 | Mimicking CD59 |
| | | |
| Parasites | | |
| *O. moubata* | OMCI | Binding C5 |
| *O. savigny* | TSGP2 and TSGP3 | Binding C5 |
| *S. mansoni* | Paramyosin | Mimicking CD59 |
| *N. fowleri* | CD59-like | Mimicking CD59 |
| *E. histolytica* | CD59-like | Mimicking CD59 |

**Table 1. Overview of pathogens evading the terminal complement pathway, the molecules responsible and their mechanisms of evasion.**

as short projections and can also bind C4BP and fibronectin[56]. The AGAT nucleotide repeat in the 5'-UTR of the *uspA2* gene is essential for normal expression of UspA2. The number of repeats affects the levels of expression of the UspA2 protein. More repeats result in higher expression levels of the protein UspA2 which in turn lead to increased binding of vitronectin and less polymerised C9 bound to the bacteria[57]. UspA2 is thus a major virulence factor for *M. catarrhalis*.


## Concluding remarks

Many micro-organisms enter the human body every day and most of them are rapidly cleared by the complement system. Some micro-organisms, however, are able to survive within the host and can cause disease. To do so, the pathogens have to get around the complement attack. It is clear that there exist many different complement evasion mechanisms among pathogens which can be roughly divided into three main strategies: direct inhibition of complement components, degradation of complement molecules and recruiting or mimicking complement regulators. Although inactivation of complement through degradation is not used for the terminal complement pathway (table 1).

Bacteria are the masters of evasion: of all the organisms that evade complement the most are bacteria and they show the greatest variety of evasion mechanisms. While viruses for example, restrict their escape strategies to the regulators of complement. Most viruses recruit host regulators and use them for their own benefit. To a lesser extend host regulator-like proteins are produced by the virus itself or the host regulators are upregulated in virus-infected cells.

Using the hosts own components is a popular strategy among pathogens.

Designed to protect neighbouring host cells from bystander lysis, the proteins in this way also protect the pathogen. The pathogen disguises itself to prevent recognition by complement. This is an advantageous mechanism for pathogens because the regulators are perfectly adapted to the host. This can especially be beneficial when a pathogen infects multiple species.

In the terminal complement pathway, there are several sites available for pathogens to interfere with (fig. 4). However, especially CD59 is a popular target for mimicking and misuse by pathogens. Apparently, it is relatively easy to capture or mimic this regulator. Obviously, it is essential for a pathogen to prevent the direct damage caused by the MAC and CD59 can inhibit MAC assembly, but it is unclear why pathogens prefer to use CD59 and not, for instance, clusterin.

It seems only partial beneficial to inhibit the final steps of the complement system. Direct damage through lysis of the pathogen is blocked, but part of the damage is already induced. Opsonisation by deposition of C3b
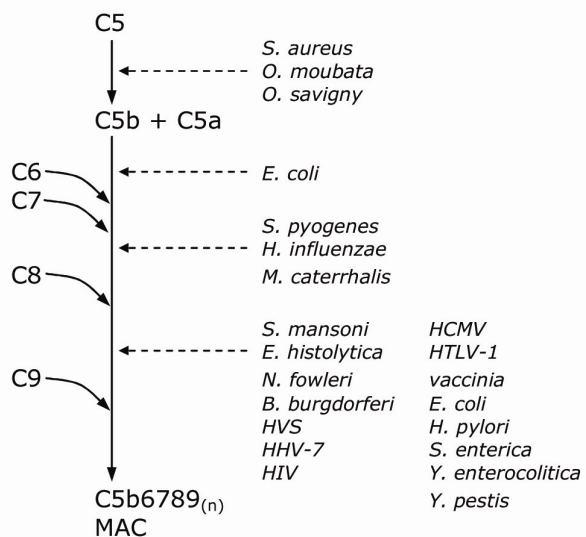


**Figure 4. Terminal complement pathway evasion by human pathogens**
Several pathogens evade complement by interfering with the final steps of the pathway. The dotted arrows indicate at which point in the cascade the different pathogens inhibit complement.

and C4b and the attraction of other immune cells by anaphylatoxins has occurred. Naturally, the most dangerous effect of complement is inhibited when CD59 is used and partial protection is still better than none, but then the pathogen has to protect itself from the other effects of complement too.

Inhibiting a pathway is most efficient when multiple steps are blocked. It is often seen that a pathogen has more than one mechanism to escape from complement attack. The pathogens discussed in this study have only one mechanism to evade complement at the terminal pathway, but many of them have multiple complement inhibitors. The other blockers function more upstream of the complement cascade, in the activation pathways. The most examined and best known example of this is *S. aureus*. This bacterium expresses proteins to block activation via C1, cleave C3b, recruit factor H, block C5a binding to its receptor, inactivate C3 convertases and bind the C5 component[58].

Certain Gram-positive bacteria evade the terminal complement pathway while that is not directly necessary; because of the thick cell wall the MAC cannot perforate the membrane and Gram-positive bacteria are thereby already resistant to lysis. Thus, it is plausible that inhibiting the final steps of complement is not the primary goal of these blocking mechanisms. With inhibiting the cleavage of C5, preventing C5a generation is probably more advantageous for *S. aureus* than preventing MAC assembly. And the C5b67 complex binding protein SIC from *S. pyogenes* is also shown to bind and block two other completely different proteins: secretory leukocyte proteinase inhibitor and lysozyme[59]. These proteins are also involved in the innate immune system but have no relation with complement.

For several pathogens it is shown that they inhibit the terminal complement pathway. However, the mechanism they use or the molecule responsible is often not exactly known. This leaves some research to be done, because it is not only interesting to fully understand the mechanisms of infection, but it could also help in the development of antimicrobial drugs. Newly discovered virulence factors could provide new drug targets. Novel therapeutics are very welcome at the moment because pathogens are getting more and more resistant to the drugs now available. Furthermore, the microbial complement evasion molecules might contain a solution for diseases involving a deregulated complement system.

There will always be interaction between humans and micro-organisms including attack and defence from both sides. As a first line of defence, complement is a key factor in this interplay and the balance between activation and evasion of complement by pathogens will therefore remain an interesting research subject.

**Abbreviations**

| | |
|---|---|
| C1inh | C1 inhibitor |
| C4BP | C4 binding protein |
| CR1 | complement receptor type 1 |
| DAF | decay-accelerating factor (CD55) |
| FHL-1 | Factor H like-protein |
| GPI | glycosyl phosphatidylinosytol |
| HIV | human immunodeficiency virus |
| MAC | membrane attack complex |
| MASP | MBL-associated serine proteases |
| MBL | mannose binding lectin |
| MCP | membrane cofactor protein (CD46) |
| NHS | normal human serum |
| ORF | open reading frame |
| RCA | regulators of complement activation |
| SCR | short consensus repeat |

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