Complement evasion strategies

of bacteria, viruses, fungi and parasites

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ABSTRACT

The human immune system consists of multiple subsystems and cascades to recognize and eliminate pathogenic intruders. The complement system is a vast and important component of this protective machinery. However, numerous of pathogens have developed ways to evade the complement system through a range of mechanisms, often by exploiting host regulators.
Understanding these processes gives insight in the pathology of infectious and inflammatory diseases, eventually resulting in novel therapeutic treatments.
This thesis gives an overview of the complement system and it's host regulators. In addition the differences and similarities between evasion strategies and mechanisms of pathogens from different domains will be given.

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Introduction

The complement system, which is available in blood and on mucosal surfaces, is a crucial mechanism of the innate immune system and can be considered as one of the first lines of defense against pathogens. This elegant and evolutionary preserved system also modifies the adaptive immune response and is important for cellular integrity and tissue homeostasis[1]. When the complement system is activated it recognizes and eliminates invading pathogens by opsonization or membrane lysis. Thus it is not surprising that pathogens have developed a wide range of complement-modulation strategies during evolution[2-5].

These modulations allow pathogens to slow down or stop the complement cascade, thereby creating an opportunity to replicate and create a microenvironment with better conditions for survival.

The steps in the complement cascade develop in a sequential manner, allowing regulation which modulates the intensity of the response and adjust the type of reaction. The complement system has many host regulators that can modulate the complement cascade at different levels to prevent damage to the host[6-11].

The complement has three different activation routes to recognize pathogens: the classical (CP), the lectin (LP) and the alternative (AP) pathway. After recognition, these different pathways use comparable mechanisms to generate C3 convertases. These convertases cleave C3 in C3a and C3b, which is a crucial step in complement activation.

C3a and C5a, of which C5a is formed later in the cascade, are anaphylatoxins which initiate inflammation. The complement cascade is prolonged by C3b, which is crucial for C5 convertase formation. The second function of C3b is opsonization by covalently binding to the pathogenic surface. C3b can be degraded to iC3b which also is an opsonin.

The C5 convertases cleave C5 in C5a (anaphylatoxin, enhancing inflammation) and C5b, which recruits C6, C7, C8 and multiple C9 molecules to form the membrane attack complex (MAC)[10, 12]. This complex, also called terminal complement complex (TCC), kills the pathogen by pore formation in the membrane, causing osmotic lysis of the pathogen[5, 13, 14].

The AP is constitutively activated, at a low level, resulting in an immune surveillance system[8, 9]. Therefore the AP is strictly regulated. The AP also functions as an amplification loop when the cascade is started by the CP and LP (fig. 1)[8]. In 10 minutes one C3b molecule can be expanded to 10^{10} C3b molecules by amplification of the AP[15].

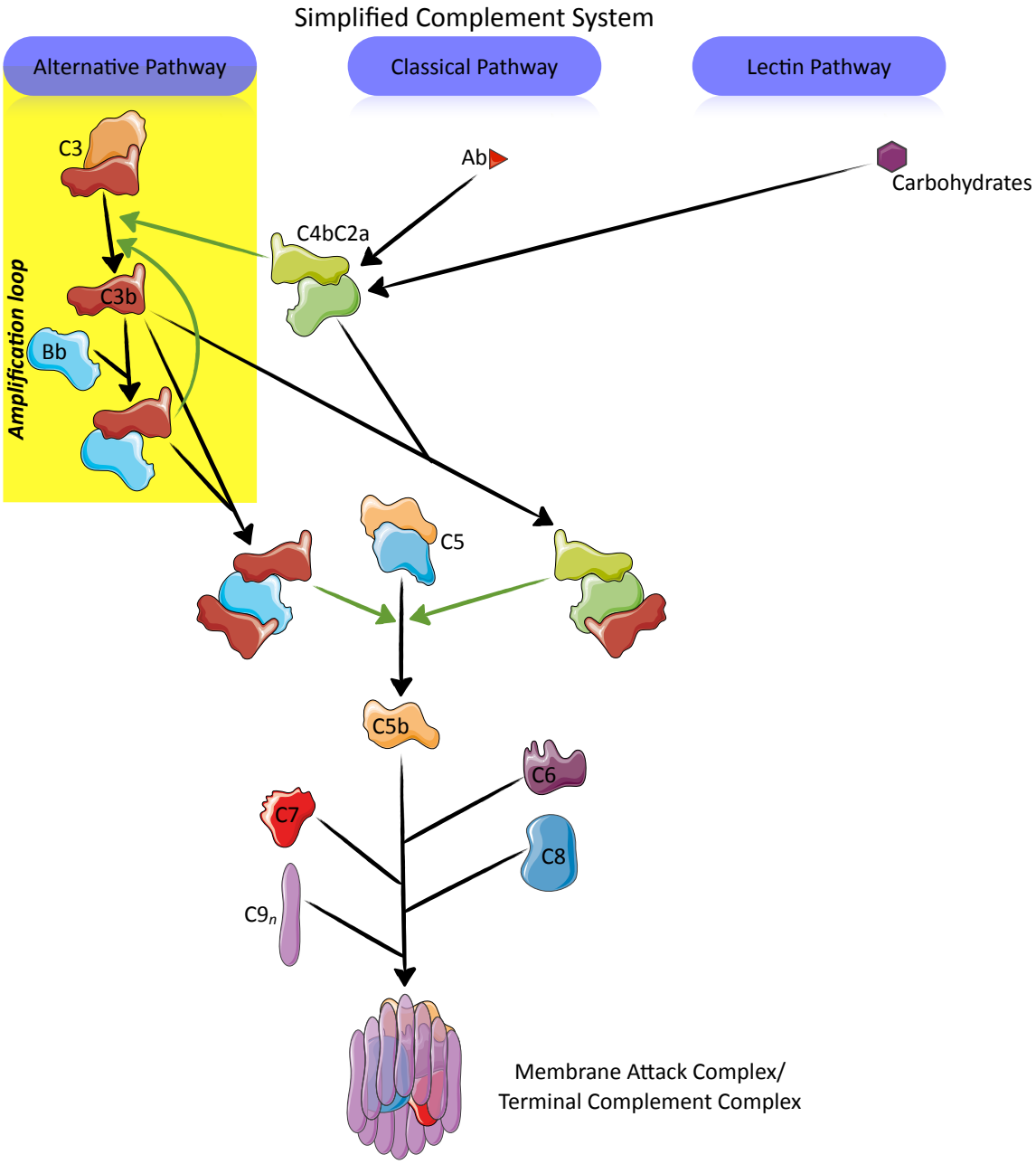

fig. 1 | Simplified representation of the complement cascade. The accent is on amplification loop of the AP which creates more C3b and thus more C3 convertases and C5 convertases.
After activation by the CP or the LP, the AP amplificates, the cascade and the formed C5 convertases mediates C5 conversion into C5a and C5b. C5b recruits C6, C7, C8 and multiple C9 molecules to form the MAC.

The CP and the LP form the C3 convertase C4bC2a (C4b2a) by conversion of C4 and C2 using different proteins. The CP activation is antibody (Ab) mediated, C1q binds the Ab[16]. When the globular heads of two C1q molecules bind, the associated C1r is auto activated and in turn activates C1s. C1s cleaves C4 to generate C4b and the anaphylatoxic peptide C4a. The surface bound C4b binds C2 which in turn is cleaved by C1s from the nearby C1 complex releasing the small C2b fragment resulting in formation of the C3 convertase C4b2a[17].

The LP also forms the C3 convertase C4b2a, however the pathway leading to this is different. The LP recognizes carbohydrates with mannan-binding lectin (MBL) and ficolin (L-, H- or M-ficolin). MBL recognizes neutral sugars (manose, *N*-acetylglucosamine, fucose) through it's C-type lectin domain in a Ca^{2+} dependent manner. Ficolin binds GlcNAc and peptidoglycan[18, 19]. Both MBL and ficolin are, in circulation, associated with several proteases called MBL-associated serine proteases (MASPs). These proteases fulfill the same function as C1s and C1r, however only MASP-2 is known to cleave C4 and C2 and thereby generating the C3 convertase C4b2a[20]. Recently it has been reported that the SIGN-R1 lectin is able to bind C1q and activates the CP in a different matter. The question is if this can be seen as a completely new pathway. It looks like SIGN-R1 mediates between a substrate and C1q and is another activator of the CP and not a completely new pathway.
The complement component C4b2a functions as a C3 convertase and thereby activates the complement cascade via the AP amplification loop.
The AP can be activated independent of the CP and the LP, this can be achieved by hydrolysis of the internal thioesther bond in C3, forming soluble $C3H_2O$[21]. Factor B can be bound by surface bound C3b or soluble $C3H_2O$ and can be cleaved by Factor D when in complex. Factor D is the only complement serine protease that is found in its active form in serum and not as a zymogen. By cleaving factor B the small peptide Ba is released resulting in the formation of C3bBb, a surface-bound C3 convertase, or $C3H_2OBb$, a highly unstable fluid-phase C3 convertase.
Binding of an additional C3b molecule to the C3 convertases results in the formation of the C5 convertases C3bBbC3b (C3bBb3b) and C4bC2aC3b (C4b2a3b). These C5 convertases preferentially cleave C5 instead of C3, resulting in the formation of C5a and C5b. C5a is, like C3a and C4a, an anaphylatoxic and a chemotactic factor which recruits and activates phagocytes by binding to the C5a receptor[22].
C5b forms a soluble complex with C6, subsequent binding of C7 induces amphiphilic site which results in insertion into the target lipid bilayer. Incorporation of C8 and multiple (10 to 15) C9 molecules results in formation of transmembrane channels (MAC/TCC), which induces osmotic lysis[23] (fig. 2).

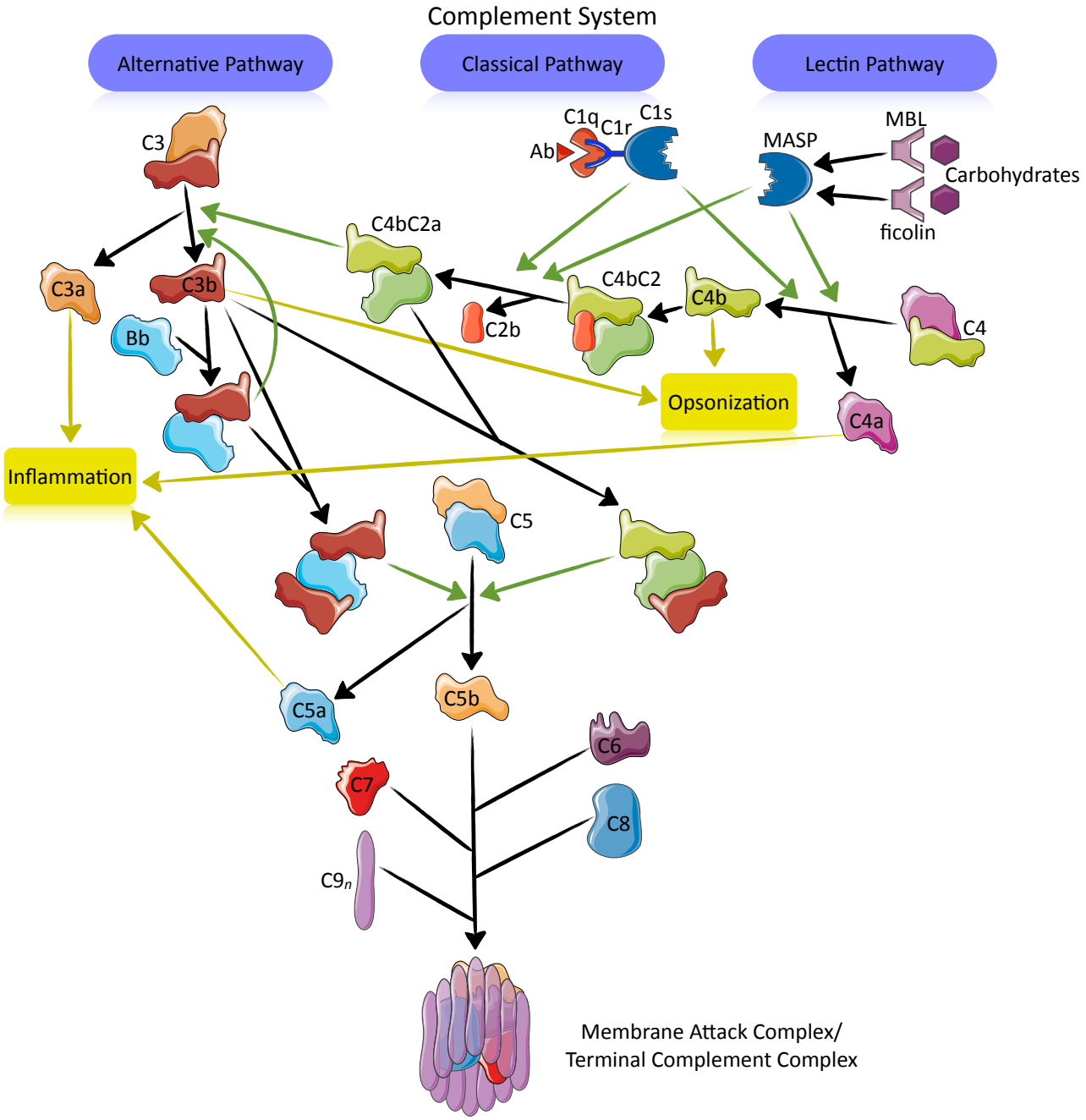

fig. 2 | Overview of the complement cascade without regulators. The LP binds carbohydrates via MBL and ficolin which bind MASP. MASP cleaves C4 and C2, creating the C3 convertase C4bC2a. The CP is activated when an Ab is bound by C1q, which activates C1r. C1r binds and activates C1s which cleaves C4 and C2. This results in the C3 convertase C4bC2a. The AP is activated and creates its own C3 convertase (C3bBb) by cleavage of C3 and factor B. Addition of C3b to on of the C3 convertases creates the C5 convertases (C4bC2aC3b and C3bBbC3b). C5 is cleaved by the C5 convertases and C5b recruits C6 and C7 which induce incorporation in the membrane. In addition C8 and multiple C9 molecules are recruited to create the MAC which causes osmotic lysis via pore formation.
In addition C4b and C3b cause opsonization of the pathogen and enhances phagocytosis. C3a, C4a and C5a are anaphylatoxins enhancing inflammation.

This aggressive defense mechanism needs to be strictly regulated to prevent damage of host cells. The complement system is regulated by the host through numerous proteins. These proteins can be soluble as well as surface bound, including complement receptors (fig. 3).

The host regulation will be discussed in this introduction while the evasion of different pathogens will be discussed in the complement evasion paragraph.

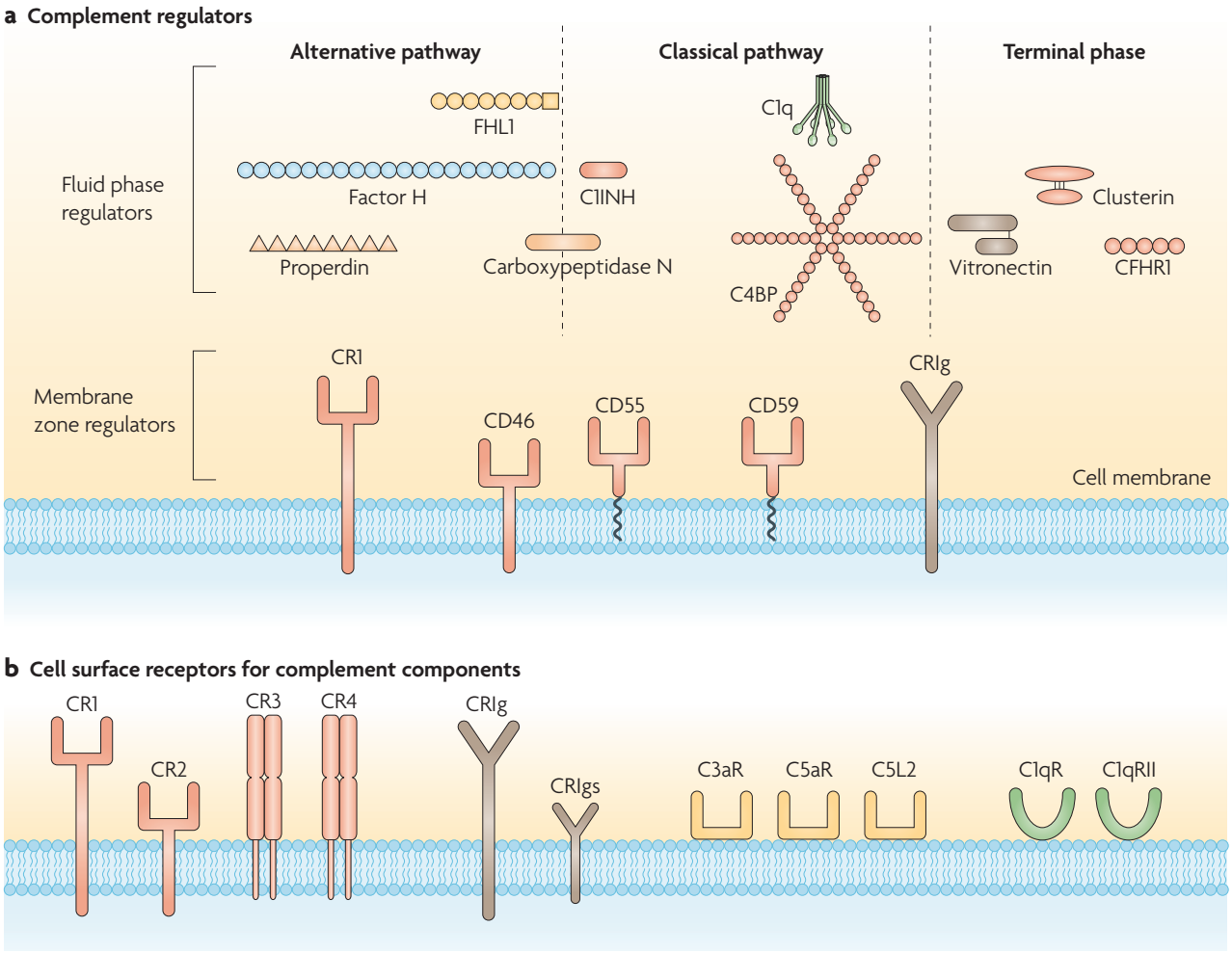

fig.3[13] | Complement regulators and surface receptors. **a. |** Complement regulators exist at the cell membrane and in the fluid phase. There are multipe regulatory proteins, each controlling different steps of the cascade. Fluid phase regulators of the AP are factor H, factor H-like 1 (FHL1) and the activator protein properdin. Carboxypeptidase N works in the CP, the LP and the AP. The CP and LP are further regulated by the soluble proteins C1q, C1 inhibitor (C1-INH) and C4 binding protein (C4BP). The terminal pathway is regulated by the soluble proteins clusterin, vitronectin and complement factor H related protein 1 (CFHR1). Regulators residing on the membrane include CR1, CD46 (MCP), CD55 (DAF), CD59 (protectin) and complement receptor of the Ig superfamily (CRIg). These regulators all have different working mechanisms. **b. |** Host cells are covered with complement receptors, including CR1, which binds C3b, iC3b and C4b, and CR2, which binds C3d and C3dg, and CRIg. Host cells are also equiped with CD46, CD55 and CD59 (not shown). CR1 and CR2 act as a co-receptor for surface bound Igs and regulate B-cell maturation and differentiation. CR3 and CR4 act as integrins and mediate phagocytosis of of C3b-opsonized cells. CRIg has a major role in the clearance of iC3b- and C3d-tagged pathogens and autologous cells.

The complement cascade is regulated by the host on different levels, here divided into three groups: C3 conversion inhibition, C5 conversion inhibition and terminal pathway inhibition regulators.

C3 conversion is inhibited by the C1 inhibitor (C1-INH) in an indirect manner by preventing C4 and C2 conversion through binding to the C1s complex or MASPs and thus the C3 convertase C4b2a is not synthesized[24]. The other regulators work in a later stadium of the cascade, mostly affecting the C3 convertases.

Factor H degrades C3b to C3bH which can further and irreversible be degraded to iC3b under influence of factor I, in turn iC3b can be degraded to the inactive C3c. C4b is degraded to C4c and C4d in an irreversible manner by factor I[25].
C4 binding protein (C4BP) is a cofactor of factor I mediated C4b degradation and enhances the decay of the C3 convertase C4b2a[26, 27]. In addition C4BP contributes as factor I cofactor in the cleavage of C3b and thereby may down regulate the AP[28]. C4BP appears as a spider-like structure by electron microscopy with tentacles protruding from the central core[29]. Just like factor I, factor H and factor H like protein (FHL1) act as cofactors in the degradation of C3b and C4b and thereby prevent formation of C3 convertases[30, 31], factor H also accelerates the decay of the AP C3 convertase and fulfills an important role in the discrimination between self (non-activating) and non-self (activating) surfaces[32]. The high affinity of factor H for C3b on self surfaces is regulated by sialic acids and glycosaminoglycans which are abundantly present on all self cell surfaces. By this mechanism the AP activation can be regulated on self surfaces. FHL1, which is a splice variant of factor H, regulates the AP activation via similar mechanisms[33]. The surface bound CD46 binds to C3b and C4b and stimulates factor H and factor I, accelerating C3b and C4b degradation[34]. The cell bound CR1 works in a similar way as CD46, stimulating degradation by factor I and factor H[35]. The surface bound protein CRIg mediates phagocytosis by binding iC3b and inhibits AP activation[36]. iC3b is a degradation product of C3b and is a surface bound protein. CD55 (DAF), another surface bound protein, degrades the C3 convertases C3bBb and C4b2a and thereby inhibits prolongation of the complement cascade[37].
At this level of the complement cascade properdin, the only known positive regulator of complement activation, is found. Properdin binds and stabilizes the unstable AP C3 convertase C3bBb[38, 39].
The anaphylatoxic peptides C3a, C4a and C5a can be inactivated by carboxypeptidase N to prevent inflammation. The complement factor H related protein 1 (CFHR1) inhibits the C5 convertases C3bBbC3b and C4bC2aC3b and thereby prevents prolongation of the terminal pathway[40, 41]. The surface bound protein CD59 binds complement factor C8 and thereby inhibits formation of the MAC[42]. The soluble proteins clusterin and vitronectin inhibit insertion into the membrane by binding to the membrane binding site of the C5b-7 complex. Interestingly, in the case of vitronectin, the C5b-7 complex is still able to bind C8 and C9 to form soluble C5b-8 and C5b-9 complexes, but these are unable to insert into the membrane and are thereby not lytic anymore. It has also been shown that vitronectin in solution is able to prevent C9 polymerization, hence it indirectly and directly prevents pore formation, which both prevent osmotic lysis[43-45].
These regulatory proteins are often referred to as RCA, regulators of complement activation.
The complement system is strictly regulated by host factors (fig. 4) which can be grouped in a CP and LP regulation group, a C3 convertase regulation group, an inflammation regulation group, a C5 convertase regulation group and a terminal pathway regulation group.
Further simplified this can be divided into two main streams, C3 convertase regulation and terminal pathway regulation.

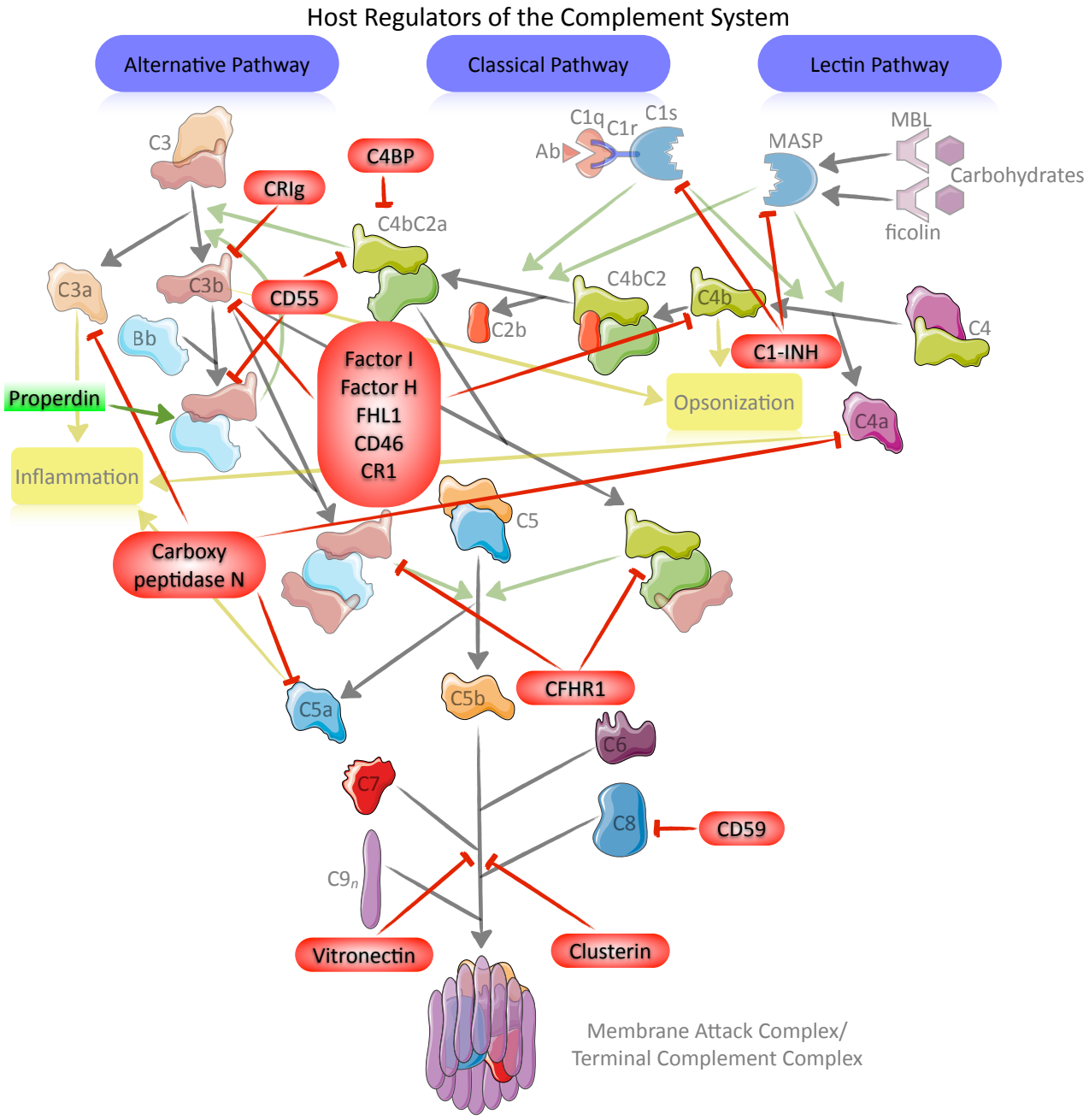

fig. 4 | Overview of the complement cascade including regulators. The CP and the LP are regulated by the C1-INH which binds and inactivates C1s and MASPs. Factor I, factor H, FHL1, CD46 and CR1 bind and degrade/ inactivate C3 convertase components. CD55 degrades AP and CP/LP convertases. C4BP accererates CP/LP C3 convertase decay and degrades C4b. CRIg binds C3b, inhibiting binding of other complement components. The anaphylatoxic proteins are inactivated by Carboxypeptidase N. If the cascade has prolonged and has formed C5 convertases this can be regulated by CFHR1 which inhibits C5 convertase function. The terminal pathway prolongation can be prevented by CD59, which binds C8; clusterin and virtonectin, which bind to C5b-7 and C5b-8. This were all negative regulators of complement, however there is also one known possitive regulator of complement. Properdin binds and stabilizes the AP C3 convertase, preventing decay and enhancing activity. Resulting in more C3b formation.

Most of the complement cascade takes place at the cellular membrane because many interactions are dependent of accumulation. Proteins are more likely to find each other on a two dimensional surface than in a three dimensional space and it is more efficient to concentrate complement component formation on the pathogenic surface. Bound complement factors also function as opsonins for host adaptive immune cells (fig. 5a). The anaphylatoxins C3a, C4a and C5a induce pro-inflammatory and chemotactic responses by binding to respectively the C3aR, C4aR and C5aR.

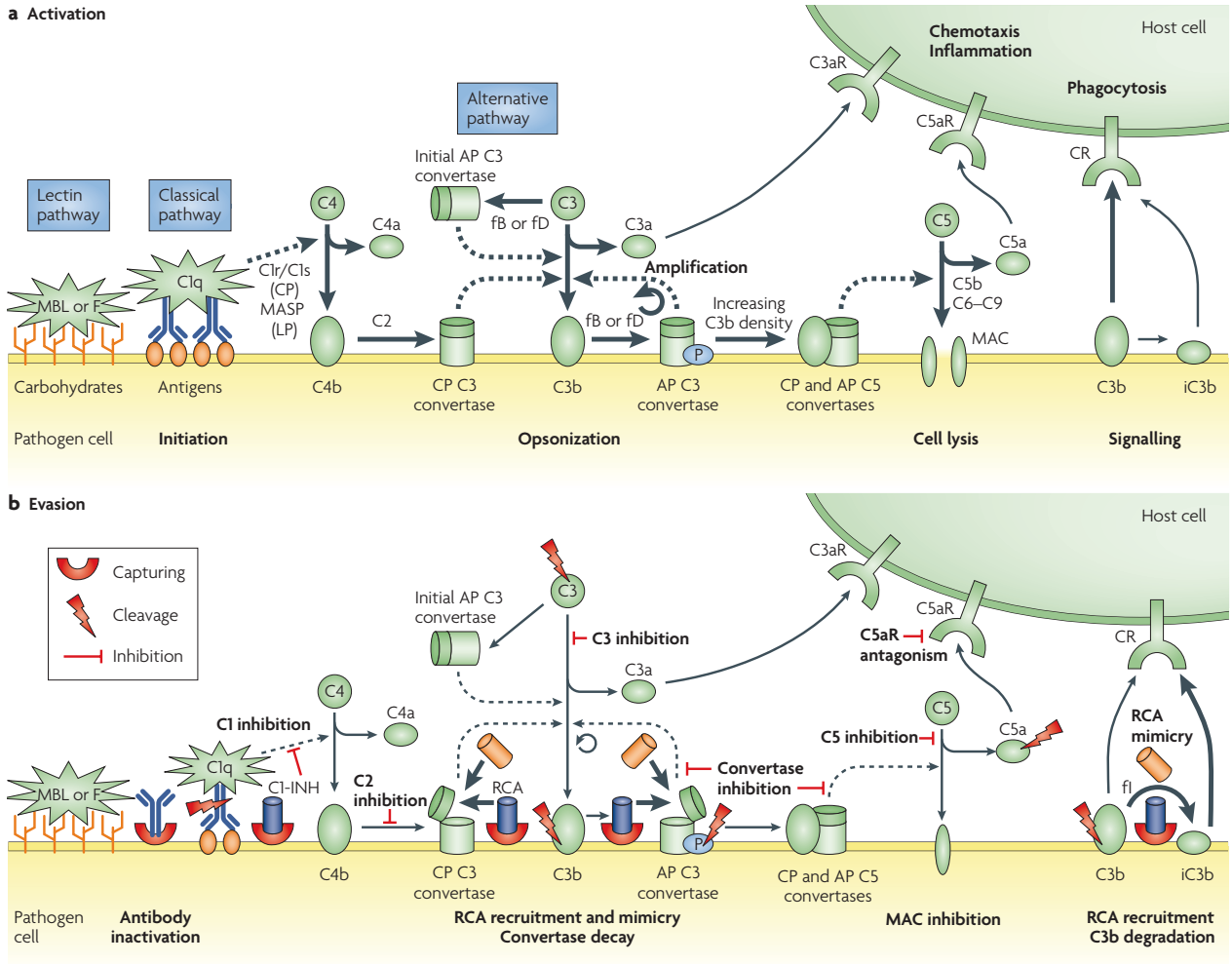

fig 5[131] **| Activation and evasion of complement at the pathogenic surface. a. |** If the complement system is activated by antibody complexes (CP), carbohydrates (LP) or by spontanious and induced C3 hydroysis (AP), the C3 convertases cleave C3 to C3a and C3b. The surface is covalently bound by C3b, opsonizing the the surface and amplifying the complement cascade. C3b mediates phagocytosis and adaptive immune response by binding to complement receptors (CRs). The accumulation of C3b on the surface leads to C5 convertase assembly. C5 convertases can activate C5 to C5a and C5b. The MAC formation is iniated by C5b which recruits C6, C7, C8 and multiple C9 molecules forming a pore. The anaphylatoxins C3a, C4a and C5a induce pro-inflammatory and chemotactic responses by binding to respectively the C3aR, C4aR and C5aR. Properdin stabilizes the AP C3 convertase on pathogenic surfaces, enhancing complement activity.
b. | Pathogens have developed many ways to circumvent the complement system. The CP can be suppressed by capturing the Ab Fc-tail or degrading the Ab. The LP and the CP can be suppressed by capturing the C1-INH. Further evasion can take place by degrading or inactivation of C3, C5 or there convertases. Capturing or mimicking of host regulators also suppresses complement activation at this level. MAC formation can be prevented by recruitment of host regulators like CD59, vitronectin or clusterin. Increased and decreased activity is represented by respectively thick and thin arrows.

Evasion strategies

Pathogens can evade the complement system in different manners, they exploit multiple strategies to overcome this type of innate immunity. Multiple surface and soluble proteins are expressed to either cleave complement components using proteases, inhibit or modify complement proteins, recruit or mimic host inhibitory proteins or disguise themselves from surveillance. Pathogens also interfere with the complement system through direct interactions and in addition of these active evasion strategies, many microorganisms use passive mechanisms to evade complement. A prominent example of passive complement evasion is the cell wall of Gram-positive bacteria which inhibits lysis by the MAC.

Active evasion strategies include capturing of host regulators, conversion inhibition and cleavage of complement components (fig. 5b). This can be achieved at the level of detection, C3 conversion or terminal complement complex formation.

In the following sections different active evasion mechanisms of pathogens will be discussed. We hope to find an answer to the question whether pathogens from different domains or kingdoms use comparable mechanisms to evade the complement system.

Pathogen detection

Some pathogens evade the complement system through prevention of detection. The following **bacteria** use different methods to evade detection by complement. *Pseudomonas* elastase (PaE) and *Pseudomonas* alkaline protease (PaAP) degrade immunoglobulins (Ig's) and C1q thereby preventing activation by the CP[46]. The *Porphyromonas* degrades immunoglobulin G (IgG) through it's prtH protease. *Staphylococcus* spp[47] uses SAK (Staphylokinase) to bind plasminogen and activate it into plasmin, which can cleave IgG[48]. Sbi (*S. Aureus* IgG-binding protein)[49] and SpA (*S. Aureus* protein A)[50] are used to inhibit the interaction between Ig and C1q. SpA also binds the Fc part of IgG molecules and thereby covers the surface in IgG molecules in a manner that prevents recognition by phagocytes. *Staphylococcus* super-antigen-like 7 (SSL7) can prevent detection by binding IgA. This multiple protein strategy is also used by the *Streptococcus* spp. which uses it's IdeS (IgG-degrading Enzyme of *S. pyrogenes*) to cleave IgG, however IdeS has no interaction with C1q[51]. The PLY (Pneumolysin) protein is used to deplete C1q and IgG[52, 53], while SPE B (Streptococcal pyrogenic exotoxin B)[51, 54] degrades Ig's by cleaving in the hinge region and thereby removing the entire Fc region. SpG (*Streptococcus* protein G)[55] inhibits interaction of IgG's with C1q by binding the Fc-part. *Escherichia* spp. cleaves the host regulator C1-INH with it's StcE protein (secreted protease of C1 esterase inhibitor), this enhances C1-INH activity. On top of that StcE traps C1-INH on the surface, increasing the local concentration and further strengthens its inhibitory activity[56]. So StcE recruits a host regulator and modifies it to function even more effective. C1 itself also can be targeted, for example by the 40kDa porin of the fish pathogen *Aeromonas salmonicida*[57].

These bacteria all prevent CP activation, but there are also numerous of **viruses** that prevent CP activation by averting detection. However some viruses hijack this system by actively trigger it to increase detection and thereby increase uptake into the host cell. Most viruses suppress detection by shedding of viral protein-antibody complexes from there surface. Some viruses use different mechanisms, multiple *Herpes viridae* strains use proteins to decrease the Fc-receptor activation by binding the Fc-tail of IgG, the *Herpes Simplex Virus* (HSV) uses glycoprotein E (gE) together with glycoprotein I (gI) for this[58, 59], while the *Human Cytomegalovirus* (HCMV) uses glycoproteins 34 and 68 (gp34 and gp68)[60, 61]. The *Varicella*

Zoster Virus (VZV) uses gpI and gpIV to decrease Fc-receptor activation according the same mechanism[62].
From the *retroviridea* the *Human Immunodeficiency Virus* (HIV) is a good example of how viruses hijack the complement system to increase uptake, in this case specific by B-cells. The HIV gp41 directly activates the CP through C1q [63-65] and gp120 directly activates the LP through MBL[65, 66], both to enhance uptake. After infection complement is inhibited by induction of expression of the C1-INH protein by the tat (transactivator of transcription) protein[67].
The hitchhiking principle is also used by binding to complement associated proteins. For example the Epstein-Barr virus glycoprotein gp350/220 interacts with the CR2 receptor on B-cells and immature T-cells. Some surface-bound complement regulators can serve as targets for viruses. The membrane cofactor protein (MCP) is targeted by the *measles virus* hemagglutinin and by the *Herpesvirus* glycoprotein H. Another complement regulator which is exploited is the DAF protein, which serves as anchor place for the *coxsachievirus* and the *echovirus*.
Fungi also evade the complement system, however until now no mechanism has been found to actively evade detection. Detection has only been reported to be prevented by shielding of pathogen-associated molecular patterns (PAMPs)[68].
Some **parasites** are able to actively prevent detection. The *Schistosoma spp.* protein paramyosin (Pmy) is able to bind C1q and the Fc-region of IgG, thereby depleting the components for detection[69-72].

Prevention of detection is an effective evasion mechanism that is spread throughout different groups of pathogens. Most evasion tactics are based on binding or degradation of C1q or Ig and this mechanism can be found in bacteria and parasites. Fungi and viruses however, do not solely use this tactic to prevent detection. Fungi use a different mechanism (shielding) and viruses use shedding to remove recognizable proteins from it's surface, however some viruses activate detection and thereby opsonization by complement to enhance virulence.
Binding of the Fc region of Igs is a mechanism shared by bacteria, viruses and parasites. Bacteria and viruses also share the ability to recruit C1-INH. For evasion of detection no other shared mechanisms were found.

C3 conversion

C3 conversion is a crucial step in the prolongation of the complement cascade and therefore this step is tightly regulated by the host, consequently this can be easily exploited by pathogens in numerous ways. For C3 conversion the formation of the convertases is also taken into account.
Different **bacteria** recruit host regulators, such as factor H (fH), FHL-1 and C4BP, to evade complement.
Factor H, FHL-1 and C4BP are recruited by different proteins from different bacteria (Table-1). These factors are thereby present on the bacterial surface, thus osmotic lysis and opsonization by the complement system are prevented. This strategy is very popular among different types of bacteria.
However, bacteria also have other strategies to suppress C3 conversion. The prtH protease of *Porphyromonas* spp. simply degrades C3[47], just like the PaE and the PaAP proteins of *Pseudomonas* spp.[46]. The *Staphylococcal* protein SAK binds plasminogen and activates it into plasmin which cleaves C3b and thereby prevents opsonization and lowers indirectly the convertase concentration at the membrane[48]. C3 and C3b containing convertases are inhibited

by the *Staphylococcus* proteins Efb (extracellular fibrinogen-binding protein)[73-75] and Ehp (Efb-homologous protein)[75, 76]. The *Staphylococcal* complement inhibitor (SCIN) protein inhibits C3 activation to C3a and C3b by binding and stabilizing C3 convertases on the bacterial surface. While stabilizing the C3bBb complex, enzymatic activity is abolished, this suggests binding to the active pocket of Bb. However, conformation of Bb induced by C3b is also important, so another binding place cannot be excluded[75, 77]. *Streptococcus* spp. uses multiple proteins to recruit host regulators (Table-1) and in addition the SPE B protein degrades the C3 convertase stabilizator properdin[51, 54].

Many **viruses** regulate complement at the C3 conversion level, this is done by recruitment of regulators, binding to complement compounds or by mimicking regulators. Herpes viruses can bind to C3b, inhibit properdin and bind C5 by the HSV transmembrane glycoproteins C1,C2 (gC1/2) and as a result of that suppress opsonization and C3 conversion[78]. gC1/2 also accelerates the decay of the AP C3 convertase C3bBb[79]. The envelope glycoproteins gp41 and gp120 of HIV (retrovirus) both recruit factor H to inhibit complement[63-65]. This is interesting because these proteins have a double function, enhancing uptake, but inhibit further pathway activation, which is beneficial for the virus.

Also the nonstructural protein 1 (NS-1) of the West Nile virus (filovirus) recruits factor H as a regulator of complement[80].

The poxviruses mimic regulators and accelerate C3b decay to evade complement. The Cowpox virus binds to C3b and C3 convertases as cofactor and accelerates C3b decay through the inflammation modulatory protein (IMP)[15], which is a homolog of the Vaccinia virus complement control protein (VCP) with the same functionality[81].

The Smallpox inhibitor of complement enzymes (SPICE) also show homology with VCP and control complement in similar matter[82]. The Monkeypox inhibitor of complement enzymes (MOPICE) show homology with VCP, however are not able to bind convertases and are not able to accelerate C3b decay. Thus MOPICE only has cofactor activity by mimicking regulators of C3b[83].

Fungi recruit regulators at the C3 conversion level to evade complement. An unknown factor of *Aspergillus fumigatus* recruits, just like the complement regulator-acquiring surface protein 1 (CRASP-1) of *Candida albicans*; factor H, FHL-1 and C4BP to regulate complement. Another protein of *Candida albicans*, the phosphoglycerate mutase (Gmp1p), recruits factor H and FHL-1. The secreted aspartic protease Sap2 of *Candida albicans* has been shown to cleave complement factor C3, resulting in decreased opsonization of the yeast[84-86].

This are the only active complement evasion strategies of fungi that are known at the moment. These evasion mechanisms are largely aimed at evasion of inflammation and opsonization by C3b and C3a, because the thick fungal cell wall the pathogen is largely resistant to the TCC[87].

Parasites regulate the C3 conversion by recruitment of regulators, cleavage of components, acceleration of decay, prevention of convertase formation by binding or through destabilization of convertases. This wide range of mechanisms shows the diversity in evasion mechanisms.

The Hydatid cyst wall of *Echinococcus* spp. recruits factor H to it's surface [88], just like the microfilariae (mf) of the *Onchocerca* spp[89]. The m28 (a membrane serine protease) protein of *Schistosome* spp. cleaves iC3b to restrict binding to CR3 and thereby prevent opsonization[90, 91].

The IRAC and ISAC, respectively the *Ixodus ricinus*- and the *Ixodus scapularis* anti-complement protein, both accelerate the decay of the AP C3 convertase C3bBb[92, 93].

The CRIT protein, which trispans the complement C2 receptor, of both *Trypanosoms* spp. and *Schistosoma* spp. inhibits the formation of the CP C3 convertase C4b2a[94]. *Trypansoma* spp.

also destabilizes convertases with the T-DAF protein (*Trypansoma* decay-acceleration factor) [95].T-DAF is a CD55 (DAF) homologue.

Suppression of C3 conversion is an effective and commonly used mode of action. Bacteria, viruses, fungi and parasites all recruit host factors at this stage to evade the complement system. This is probably because during evolution this solution was the easiest to acquire, while still very effective. However, this are not the only mechanisms shared. Bacteria and fungi are able to degrade C3, while bacteria and viruses are able to degrade C3b. Acceleration of C3 convertase decay and mimicking of C3 regulators are abilities shared by viruses and parasites.
In bacteria it is striking that *Staphylococcus* spp. are not reported to recruit host factors while *Streptococcus* spp. recruits numerous of host factors. This looks like specialization by the organism. Unfortunately this interesting observation is beyond the scope of this report.

C5 conversion

The conversion of C5 to C5a and C5b is a crucial step in the formation of the MAC because C5b is the central molecule which recruits all other components; in addition the anaphylatoxin C5a induces a pro-inflammatory and chemotactic response by binding to the C5aR.
Bacteria can degrade products, inhibit the convertases or prevent cleavage of C5.
Serratia spp. degrades C5a with a 56kDa protease[96, 97], *Streptococcus* spp. degrades C5a with two *Streptococcal* C5a peptidases (scpA and scpB)[98-100] and *Staphylococcus* spp. antagonizes C5a on the C5a receptor (C5aR) with the CHIPS protein (Chemotaxis inhibitory protein of *S. aureus*)[101].
The *Staphylococcal* super-antigen-like protein 7 (SSL-7) prevents cleavage of C5 and hereby prevents C5a formation, which seems more important for clearance than MAC formation because it's thick cell wall is resistant to MAC mediated lysis[102].
Efb and Ehp, two *Staphylococcal* proteins which also inhibit C3 convertases, inhibit C5 convertases by binding to C3b[73-76].
Pox and *Herpes* **viruses** are also capable of interference with C5 conversion. The *herpes* gC1/2 proteins are able to inhibit binding of C5 to the surface, which is required for cleavage[79]. The *poxvirus* IMP, SPICE and VCP proteins accelerate decay of C5 convertases by binding to C3b[15, 81, 82].
Fungi are not reported to interfere at the C5 conversion level of the complement cascade.
The **parasite** *Ornithodoros* spp. binds to C5 with the *Ornithodoros moubata* complement inhibitor protein (OmCI) and potentially blocks binding to the C5 convertases[103]. This is the only known C5 conversion evasion of parasites.

Evasion at the C5 level seems to be far less popular than evasion at the C3 level. Interruption of the process early on is probably more beneficial because this also prevents opsonization and inflammation at an earlier stage.
Bacteria and parasites share the capability to prevent C5 cleavage as an evasion mechanism. Bacteria and viruses share the ability to inactivate C5 convertases by binding to C3b.
At this stage little similarities are observed, however the total number of methods is also limited.

MAC formation

Evading the MAC is the last resort of the pathogen to prevent lysis (if the pathogen is susceptible to lysis by the MAC). Osmotic lysis by the MAC can be evaded by preventing insertion in the membrane or by preventing formation of the complex.

In **bacteria** the *borrelia* spp. mimics the host CD59 complement regulator with a CD59-like protein and prevents MAC formation by binding C8 and C9[104]. This CD59-like protein is larger than CD59 itself and thus it is not an acquired regulator. In contrast to human CD59, CD59-like can interact with C8 and C9 in absence of the assembling MAC. The *Neisseria meningitides* outer membrane protein A (OpaA), the *Haemophilus influenza* surface fibril and the *Moraxella catarrhalis* UspA2 protein bind vitronectin to prevent lysis by the MAC[14] by inhibiting the C5b-7 complex and directly binding of C9[45].

The *escherichia* spp. TraT protein binds to C5bC6 preventing binding of C7, C8 and C9 to the complex and thereby prevents insertion in the membrane[105]. The *Streptococcal* Inhibitor of Complement (SIC) protein prevents MAC formation by binding C5b-6 and C5b-7, ending the cascade[106, 107]. The protein closely related to SIC (CRS) shares the ability to bind C6 and C7 with SIC. However, because of *Streptococci* resistants to MAC lysis, this are not the sole functions of SIC and CRS.

In **virus** infections the only known protein to interact with MAC formation is the HIV gp41 protein. Gp41 reduces CD59 levels, the advantage for HIV is not clear, however this does explain bystander lysis of neuronal cells in HIV infected patients[63].

In **fungi** there are few evasion proteins known to interfere with MAC formation, this is probably due to the solid cell wall of fungi which makes them impenetrable for the MAC. However, the *Candida albicans* integrin-like protein does recruit vitronectin[14].

The **Parasite** *Schistosoma* prevents MAC formation by paramyosin, which is a CD59 homologue, binding C8 and C9 and thereby prevents pore formation[**69, 72**].

Mimicking of host regulators is shared by bacteria and parasites. Recruitment of host regulators is shared by bacteria and fungi. There is a limit number of MAC regulators and still homology is found between the different domains.

Conclusion

A comparison of different pathogens always should be made with the greatest caution because results are easily biased. This bias can be caused by a variety in research interests and by the popularity of the pathogen studied, giving an incomplete picture.

Different pathogens evade the complement system in a comparable manner, of which recruitment of host factors is the most common strategy (fig. 6).

The recruitment of host factors can be found in bacteria, viruses, fungi and parasites. Mimicking of regulators is most commonly found in viruses and parasites. These mechanisms are shared because it are probably the best mechanisms, otherwise the host also would not use them.

Thus pathogens from different domains share some mechanisms, however it is not clear if comparable mechanisms are acquired from one another or are developed independently through evolution.

Probably comparable mechanisms have developed independently during evolution, because no homologue proteins were found between the different domains. However, further homology studies are required to solve the puzzle.

The complement system is an elegant system which is evaded by pathogens in even more elegant ways. By understanding these evasion mechanisms novel therapeutic approaches might be developed to manipulate the complement system using the knowledge gained from pathogens.

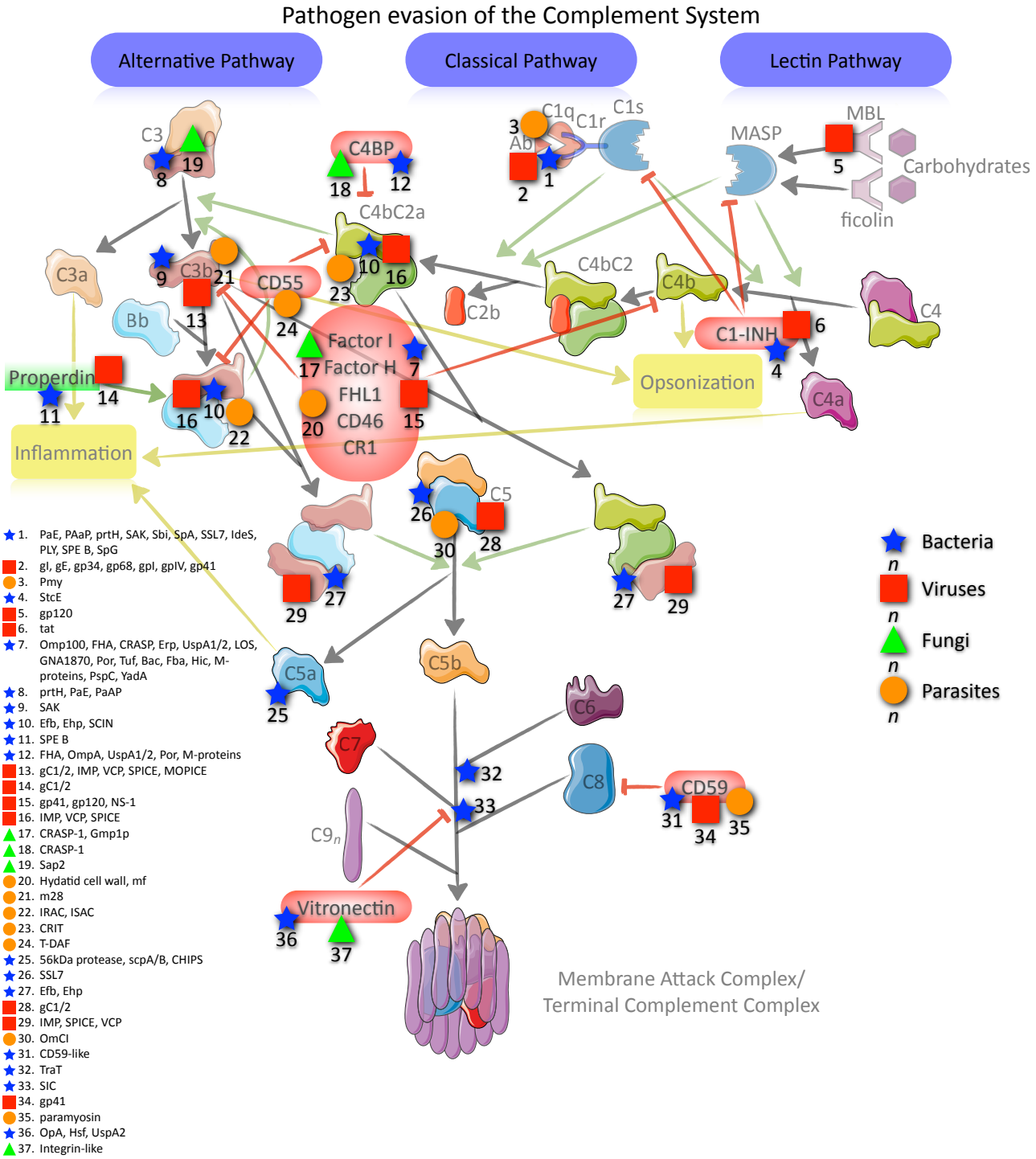

fig. 6 | Complement evasion by pathogens. The different pathogens are compared in one picture. The only mechanism shared by all four pathogens is recruitment of host C3 conversion regulators. Most evasion mechanisms are seen at the C3 level. Recruitment of host factors and blockage of convertases are the most used mechanisms shared by all pathogens.

Tables and Figures

Bacteria	Protein	Target	Ref.
Actinobacillus spp.	Omp100 (outer membrane protein 100)	Factor H	130
Bordetella spp.	FHA (filamentous hemagglutinin)	C4BP, Factor H, FHL-1	129
Borrelia spp.	CRASP (complement regulator-aquired surface proteins) Erp (OspE/F-related proteins)	factor H, FHL-1 factor H	108 127, 128
Escherichia spp.	OmpA (outer membrane protein A)	C4BP	126
Fusobacterium spp.	Unknown factor	factor H	125
Haemophilus spp.	Unknown factor	C4BP, Factor H	123, 124
Moraxella spp.	UspA1/2 (Ubiquitinous surface protein A1/A2)	C4BP	122
Neisseria spp.	LOS (Lipooligosaccharide) GNA1870 (genome-derived neisserial antigen 1870) Por (outer membrane porins) PilC (Type IV pili)	factor H, FHL-1 factor H, FHL-1 C4BP, factor H, FHL-1 C4BP	109 119, 120 14
Pseudomonas spp.	Tuf (elongation factor)	factor H, FHL-1	121
Streptococcus spp.	Bac (Cβ-protein) Fba (Fibronectin-binding protein) Hic (factor H-binding inhibitor of complement) M (M-family surface proteins: Arp, Sir, etc.) PspC (Pneumococcal surface protein C)	factor H factor H, FHL-1 factor H C4BP, factor H, FHL-1 factor H	110 111 112 113 115-118
Yersinia spp.	YadA (Yersinia adhesin A)	factor H	114

Table-1 | Bacteria which recruit the host factors factor H, FHL-1 or C4BP to evade C3 conversion.

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