Understanding the complement resistance mechanisms of Klebsiella pneumoniae

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Abstract

Klebsiella pneumoniae is a gram-negative bacteria that has become a serious public health concern due to the emergence of multidrug-resistant and hypervirulent variants. In order to thrive within the human host, K. pneumoniae has acquired a number of virulence factors and evasion mechanisms. The complement system is an important component of the innate immune system that acts as a first line of defence against infections such as K. pneumoniae. It recognizes and kills gram-negative bacteria by inserting a membrane attack complex into the bacterial membrane's outer membrane or by marking the bacteria for phagocytosis. K. pneumoniae has several mechanisms which contribute to complement resistance. In this review, we aim to give a comprehensive overview of the diverse complement evasion strategies of K. pneumoniae. Having a thick capsule, long O-antigens and outer membrane proteins are the most important complement evasion mechanism of K. pneumoniae. Understanding these mechanisms of complement evasion is crucial for developing alternative K. pneumoniae treatment options.

Introduction

Klebsiella pneumoniae is gram-negative bacteria that is considered a major public health treat. K. pneumoniae possesses a high number of accessory genomes on plasmids and chromosomal gene loci. (Martin & Bachman, 2018). K. pneumoniae strains are classified into three categories based on their accessory genomes: opportunistic, hypervirulent, and multidrug-resistant (MDR). Infections caused by opportunistic strains are particularly common in hospitalized or immunocompromised people, and they can cause urinary tract infections, bacteraemia, and pneumonia. (Wang et al., 2020). Hypervirulent strain infections cause liver abscess, endophthalmitis, and meningitis in healthy individuals. MDR strains have antibiotic resistance genes like as carbapenemases and Extended Spectrum Beta-Lactamases, making these infections extremely difficult to treat (Martin & Bachman, 2018). The emergence of multi-drug resistance strains requires the development of new antibiotic classes or other therapeutic approaches.

K. pneumoniae has developed a variety of virulence traits and immune evasion mechanisms in order to survive within the human host. Examples of this are the capsular polysaccharides (CPS), LPS, siderophores, fimbriae, a type VI secretion system, outer-membrane proteins, porins, efflux pumps, an iron transport system and biofilms (Patro & Rathinavelan, 2019). Among these virulence factors, siderophores, CPS, LPS and fimbriae are well-characterized. These virulence factors help K. pneumoniae evade the host's innate immune response and survive in many regions within the host (Paczosa & Mecsas, 2016). They form a physical barrier that makes it harder for the immune system to recognize them. It is unclear if K. pneumoniae actively suppresses host immune system components, like Staphylococcus aureus does with its secreted proteins (Pietrocola et al., 2017).

Increased synthesis of the siderophore aerobactin is seen more often in hypervirulent strains as the uptake of iron via siderophores is critical for the growth and virulence within the host (Russo et al., 2018). The capsule protects K. pneumoniae against antimicrobial peptides, phagocytosis and complement-mediated lysis (Patro & Rathinavelan, 2019). K. pneumoniae strains can also be classified based on their capsular serotype (K-typing) and there are currently more than 130 different serotypes identified. K1 and K2 serotypes are usually found in the hypervirulent strains (Wyres et al., 2016). Despite the fact that the majority of infections are negative for K1 and K2, little is known about the other K-types (Choi et al., 2020). Increased synthesis of the capsule results in a hypermucoviscous phenotype. Hypermucoviscosity is seen more in hypervirulent stains, however classical strains can also show this phenotype (Russo et al., 2018). Furthermore, not all hypervirulent strains are hypermucoviscous (Russo et al., 2018; Walker et al., 2019, 2020). Hypervirulent and complement resistant strains/isolates are frequently used in K. pneumoniae research. NTUH-K2044 is a hypervirulent strain with a K1 capsule and was the first K. pneumoniae isolate to cause a liver abscess (Wu et al., 2009). ATCC 43816 is a K2 strain that is often utilized in mice virulence research (Broberg et al., 2014).

In addition to K-typing K. pneumoniae strains are also typed based on their O-antigen (O-typing). The O-antigen is the outermost domain of lipopolysaccharide (LPS), which is a key component of the outer membrane of gram-negative bacteria. In K. pneumoniae, 11 O-

serotypes of LPS have been found and described (Follador et al., 2016; Trautmann et al., 2004). O1, O2, O3, and O5 are the most prevalent serotypes as they account for approximately 90 percent of the K. pneumoniae isolates, with serotype O1 being the most dominant (45%). The O1 serotype is also associated with multidrug resistance. (Choi et al., 2020).

Once K. pneumoniae has entered the human host it will encounter the hosts immune system. The complement system is part of the innate immune system and it serves as a first line of defence against pathogens. The complement system consists of a network of soluble and cell membrane proteins that work in a coordinated manner. Complement activation is initiated upon recognition of microbial patterns or antigen-antibody complexes, which trigger a cascade of proteases (Ling & Murali, 2019). Activation leads the binding of complement proteins to the bacterial surface, marking them for phagocytotic uptake or initiating direct killing of the bacteria by inserting a membrane attack complex into the outer membrane. Three different pathways initiate complement activation; the classical pathway, the lectin pathway and the alternative pathway .

The classical pathway is initiated by C1. C1 undergoes a conformational change when it detects particular target molecules like antigen-antibody complexes. This leads to activation of the C1r and C1s domain of C1 to generate active C1qr2s2. Following that, active C1qr2s2 cleaves C4 and subsequently C2 to produce C4b2a, the classical pathway C3 convertase. (Ling & Murali, 2019). The lectin pathway is initiated by pattern recognition receptors that recognize carbohydrate structures on bacteria. These include mannose binding lectin (MBL) and ficolins. MBL-associated serine proteases (MASP-1 and MASP-2) are evolutionary related to C1r and C1s and have a similar function. MBLs form a complex with MASPs, which cleave C4 and C2 to generate C4b2a, the lectin pathway C3 convertase (Garred et al., 2016). The alternative pathway is initiated by hydrolysis of C3, which constitutively happens at a low level, forming C3b. Complement activation only proceeds when factor B binds to C3b, after which factor D cleaves factor B into Bb. C3b together with Bb form the alternative pathway C3 convertase C3bBb (Nilsson & Nilsson Ekdahl, 2012). Properdin stabilizes C3bBb activity and is only found on surfaces with low sialic acid content like bacterial cell membranes, but not on sialic acid-rich host cell membranes. As a result, complement activation occurs exclusively on foreign cell membranes and not on the host cell membrane. C3b formed by the classical or lectin pathways can both activate and increase the alternative pathway.

Complement protein C3 is cleaved into C3b and C3a by the C3 convertases generated in the each of the three pathways. C3b binds to the bacterial surface and is recognized by complement receptors on immune cells. The opsonised bacteria are phagocytised by these cells and taken up in the phagosome, where they are attacked by a variety of antimicrobial proteins as well as reactive oxygen species. Generated C3b also form C5 convertases together with the C4b2a and C3bBb (C4b2a3b and C3bBbC3b). The C5 convertase then cleaves C5, releasing an inflammatory mediator C5a and C5b (Ling & Murali, 2019).

C3a and C5a activate mast cells that release inflammatory mediators and thereby contribute to the vascular phase of inflammation. C5a is also a chemoattractant which attracts neutrophils and monocytes, contributing to inflammation's cellular phase. C5b initiates the formation of the membrane attack complex (MAC), which consists of C5b and C6, C7, C8, and multiple copies of C9 (C5b-C9) (AF & P, 2014). MACs can only be formed on Gram-negative bacteria and not on Gram-positive bacteria. The cell envelope of Gram-negative bacteria is made up of an outer membrane and a periplasmic region with a thin peptidoglycan layer, which are both located outside the inner membrane. The MAC kills Gram-negative bacteria by generating a pore in the outer membrane. Gram-positive bacteria are not affected by the MAC because they have a thick peptidoglycan layer, which protects the inner membrane (Doorduijn et al., 2016).

K. pneumoniae has emerged as a multi-drug resistant pathogen, so it is critical to better understand the mechanisms by which it can survive in the human host. Aside from virulence factors, complement evasion is one the important strategies to evade the immune system. Whereas our collective understanding of K. pneumoniae complement evasion strategies has grown, the mechanisms of complement resistance in K. pneumoniae are still poorly understood. The aim of this study is to provide a comprehensive overview of K. pneumoniae's diverse complement evasion strategies and to hypothesize the complement resistance mechanisms. Understanding these complement evasion mechanisms is critical for developing novel K. pneumoniae treatment options.

Complement activation by K. pneumoniae

K. pneumoniae activates the classical pathway via outer membrane proteins and LPS. C1q can for instance bind to outer membrane protein K36 (OmpK36) of K. pneumoniae to initiate the classical pathway, which leads to deposition of C3b and C5b-9 (Albertí et al., 1993). The process of C1q binding to Ompk36 is antibody-independent, in contrast to the conventional classical pathway complement activation via antigen-antibody complexes (Ling & Murali, 2019). In this case, the antibody binds to a specific domain of the bacteria, allowing C1q to attach to it. LPS and outer membrane proteins (OMPs) activate the classical pathway because they are main targets for C3b deposition (Albertí et al., 1996). Purified LPS from K. pneumoniae can also activate the alternative complement pathway (Albertí et al., 1993). Some K. pneumoniae strains, containing mannobiose or rhamnobiose capsular polysaccharide structures can activate the lectin complement pathway by interacting with MBL (Sahly et al., 2009).

Complement susceptible strains of K. pneumoniae show rapid surface-deposition of C3 (Nypaver et al., 2010). A study showed that in ten isolates of K. pneumoniae isolates, alternative pathway activation was observed, despite serum resistance of seven isolates (Jensen et al., 2020). One of the serum-sensitive isolate activated two or all three pathways, had several MACs formed in the outer membrane and was lysed. When both the classical and alternative pathways are activated, effective clearance of K. pneumoniea is observed. Decreased or absent activation of the classical pathway is seen in complement-resistant strains (Jensen et al., 2020). Complement-mediated killing can occur when only the alternative pathway is activated. However, it is more effective when combined with the classical pathway. In some K. pneumoniae strains/isolates, C5b-C9 can be deposited, while it does not generate a pore in the outer membrane (Jensen et al., 2020; Merino et al., 1992). Pores can form on a number of surfaces, including LPS and capsules. This is because there is no specific receptor or lipid dependency during the initial C5b membrane-binding phase (Bayly-Jones et al., 2017). As a result, we only refer to it as a MAC if the pore is formed in the bacteria's outer membrane.

A thick capsule protects K. pneumoniae against complement activation and killing

K. pneumoniae is able to evade complement activation and killing, and having a thick layer capsular polysaccharide is one the key evasion mechanisms (Dorman et al., 2018). Aside from being a virulence factor, the capsule of K. pneumoniae is also linked to complement resistance (Álvarez et al., 2000; Astorza et al., 2004; Short et al., 2020). Poorly encapsulated and nonencapsulated mutant bacteria have more C3b deposition than K. pneumoniae clinical isolates with more extensive capsules (Astorza et al., 2004). C3b deposition is also influenced by the thickness of the K. pneumoniae capsule, with reduced C3b deposition observed in K. pneumoniae strains with thicker capsules. This is most likely because the capsule shields antibody epitopes on the bacterial surface, preventing complement activation (Astorza et al., 2004). In some complement resistant isolates complement accumulation is observed in the capsules of these isolates, resulting in morphological capsule alterations and capsule shedding, but no bacterial lysis (Jensen et al., 2020). Immunoelectron microscopy indicated no pore-like structures in the outer membrane of a complement resistant strains after incubation in serum. However, there was a considerable amount of C5b-9 deposition found in the capsule (Jensen et al., 2020). This marks the importance of the capsule as a defensive mechanism against complement-mediated killing. The capsule acts as a physical barrier, preventing C5b-9 from reaching the outer membrane and so preventing MAC formation. K. pneumoniae strains have a very diverse capsular polysaccharide composition and some K. pneumoniae strains evade the lectin pathway by altering their capsular composition (Sahly et al., 2009). These strains lack the carbohydrates mannobiose and rhamnobiose which are recognized by MBL's.

In addition to possessing a capsule, the capsular type also influences complement susceptibility. There are already over 130 distinct K-types recognized, with K1 and K2 serotypes being the most common in hypervirulent strains (Wyres et al., 2016). In a recent K. pneumoniae clinical isolate study, complement resistance was observed in all K1 and K10 isolates (both 10/10), while complement susceptibility was seen in K2 (6/16), K51 (6/11), and K24 (3/7) isolates (Loraine et al., 2018). Interestingly, all three K74 isolates were susceptible to complement. Strains expressing the K1, K10, and K16 antigens can disguise their LPS, while strains expressing the K2 antigens cannot (Merino et al., 1992). As LPS is a target for C3b deposition (Albertí et al., 1996), this might explain why the K1 and K10 serotypes are more complement resistant than the K2 serotype.

Currently there are several capsule related genes identified in K. pneumoniae which are involved in complement resistance. Mutant K. pneumoniae strains that produce less CPS than wild type, are more sensitive to serum killing than strains that produce CPS levels higher than or similar to wild type (Mike et al., 2021). Transposon-directed insertion site sequencing (TraDIS) is a high-throughput approach for testing huge libraries of bacterial mutants, revealing gene essentiality, gene function, and genetic interaction (Barquist et al., 2016). Mutant libraries are generated by using transposon delivery plasmids, which are delivered and randomly conjugated into the recipient strain. Genomic DNA is sheared and transposon-containing fragments are amplified by PCR. Following that, Illumina sequencing is performed to identify the mutant gene. Transposon-directed insertion site sequencing

(TraDIS) studies revealed the genetic basis of serum survival of K. pneumoniae (Short et al., 2020). In a particular study, four different serum-resistant K. pneumoniae strains (NTUH-K2044, B5055, ATCC 43816, and RH201207) were examined by their recognition by key complement factors. NTUH-K2044, B5055 and ATCC 43816 showed minimal to no C3b binding, whereas RH201207 serum treatment resulted in a significant increase in C3b and C5b-9 levels over time. The capsule production of these strains varies, with B5055 and NTUH-K2044 having a high capsule production and ATCC 43816 and RH201207 a lower production. B5055 (K2, O1v2, ST66), NTUH-K2044 (K1, O1v2, ST23), ATCC 43816 (K2, O1v1, ST493), RH201207 (K2, O2v2, ST258) also have different K-types, O-types and sequence types. In the four strains, capsule biosynthetic genes were among the putative factors for serum survival. However, the number of cps locus genes involved in complement resistance, as well as the extent of the serum survival, differed between the strains.

In all four strains (NTUH-K2044, ATCC 43816, B5055 and RH201207), an RfaH mutation resulted in a significant reduction in serum survival, and complementation with plasmid-encoded RfaH restored wild-type survival, demonstrating the significance of RfaH in complement resistance (Short et al., 2020). In E. coli and other Gram-negative bacteria, RfaH regulates the transcription of operons that direct the synthesis, assembly, and export of the LPS and CPS (Bailey et al., 1997). RfaH mutants lose their capsule and, most likely, also their O-antigen. As a result, RfaH mutants have significantly higher levels of C3b and C5b-9 binding than wild types and are killed by MAC formation in the outer membrane.

MagA encodes an outer membrane protein that functions as a capsular polymerase (CT et al., 2010). MagA is associated with a hypermucoviscosity phenotype and is specifically involved in the K1 capsule production in K. pneumoniae. A magA-containing NTUH-K2044 strain possesses a large mucoviscous capsule, actively proliferates in serum, resists phagocytosis, and induces liver microabscess and meningitis in mice. MagA mutants, on the other hand, have a loss of capsule, an increase in serum and phagocytosis susceptibility, and are avirulent in mice. (Fang et al., 2004). This demonstrates that magA is a key complement resistance gene, but exclusively in K. pneumoniae with a K1 capsule.

Deletion of hrtA showed increased C3b deposition and serum killing. HrtA is a gene that codes for a serine protease involved in CPS synthesis (Cortés et al., 2002). In K. pneumoniae htrA mutant strains, CPS synthesis was reduced. This could explain the enhanced sensitivity to complement-mediated killing.

In conclusion, the capsule of K. pneumoniae can protect against complement-mediated killing, but the degree of this protection varies depending on capsule type, capsule thickness, and strain background.

LPS composition influences complement susceptibility.

Besides the capsule, LPS of K. pneumoniae also acts as a physical barrier that prevents complement-mediated lysis. LPS is a crucial component of the outer membrane of gram-negative bacteria, like K. pneumoniae, and it contributes to the protection from the environment and membrane stability. LPS is comprised of three different parts, the conserved lipid A anchor, the core oligosaccharide, and the O antigen (Simpson & Trent, 2019). The lipid A part of LPS can be recognized by TLR4 and initiates a signalling cascades leading to proinflammatory cytokine production. Lipid A modifications, for example the removal of phosphate groups or a change in acylation, can reduce recognition by TLR4 or change which signalling pathway is activated (N et al., 2010). Temperature, metal ion concentrations, pH, anti-microbial peptides, and other factors all influence how LPS is modified. While these adaptation are most known for their function in pathogenesis, they also play a role in controlling outer membrane permeability in a various environments (Simpson & Trent, 2019). Because membrane permeability influences complement activation, lipid A modifications can result in an increase in complement resistance. In a recent TraDis study they found that mutations in genes responsible for lipid A modification showed complement resistance defects in some K. pneumoniae strains (Short et al., 2020). However, a genome-wide density-based screen had shown that mutation of these genes (arnDEF) also decrease K. pneumoniae mucoviscosity (Dorman et al., 2018). Therefore, it is difficult to conclude that the decrease in complement resistance is a direct result of lipid A modifications.

Although changes in the lipid A part of LPS can lead to immune evasion, it does not have a significant impact on complement activation. The O-antigen type can however have a major impact on complement susceptibility (Pennini et al., 2017). LPS with an O-antigen is called 'smooth LPS' and LPS without an O-antigen is called 'rough LPS'. Generally, smooth LPS strains with an O-antigen are more resistant to serum killing than rough LPS strains (McCallum et al., 1989). The O-antigen in smooth LPS likely protects against antibody or C1q binding to the K. pneumoniae surface components (Merino et al., 1992). These serum-resistant strains with smooth LPS bind less C3b than serum-sensitive strains with rough LPS. Furthermore, unlike rough LPS strains, C3b deposition on smooth LPS strains occurs on the O-antigen rather than on the outer membrane. Despite C5b-9 deposition, strains with an O-antigen also resist MAC insertion in the outer membrane. Because the C5b-9 deposition is most likely on the O-antigen rather than the outer membrane, the MAC cannot reach the outer membrane (Merino et al., 1992). This demonstrates that LPS with an O-antigen of K. pneumoniae can suppress MAC formation either by preventing the MAC from reaching the outer membrane or by decreasing complement deposition of C1q and C3b.

Because the O-antigen is a prominent surface-exposed component, host selection induces structural variability. However in K. pneumoniae, in contrast to the large amount of different K-antigens, only 11 different O-antigens types have been found and described (O1, O2a, O2ac, O2afg, O2aeh (also called O9), O3, O4, O5, O7, O8, and O12) (Follador et al., 2016; Trautmann et al., 2004). O1, O2, O3, and O5 are the most prevalent serotypes as they account for approximately 90 percent of the K. pneumoniae strains (Choi et al., 2020).

O-antigens are composed of repeating units of one or more sugar residues. In O1 and O2 strains, O-antigens are comprised of D-galactans and in O3 and O5 of mannans (Follador et al., 2016). The O1 and O2 antigen polysaccharide chains both have repeating-units of d-galactan I, but the distinction is that the O1 antigen has an additional d-galactan II capping unit and the O2 antigen not (E et al., 2002). The wbbY-wbbZ region is required for d-galactan II synthesis (P. F. Hsieh et al., 2014). This is particularly interesting as a wbbY mutant showed higher levels of serum killing than the wild-type NTUH-K2044 strain. Furthermore, plasmids containing either wbbY or the whole wbbY-wbbZ region efficiently restored serum resistance in the wbbY mutant strain (P. F. Hsieh et al., 2014). Another study showed that O2 strains were substantially more sensitive to human serum killing than strains with an O1 serotype (Pennini et al., 2017). Conversion of an O1 strain to O2 by deletion of d-galactan II units resulted in a severe loss in serum resistance, whereas conversion of an O2 strain to O1 by insertion of these genes resulted in a rise in serum resistance (Pennini et al., 2017). These findings point to the significance of d-galactan II in increasing complement resistance.

Recently, d-galactan III has been found in ST258, a O2-type clone that is widely distributed and highly drug resistant. D-galactan III is formed through the conversion of d-galactan I, which is encoded by gmlABC. Modification of an O2a clinical isolate to express d-galactan III increased its survival in serum, indicating that d-galactan III contributes to serum resistance. (V et al., 2016). D-galactan III is also expressed within the O1 serotype as 40% of O1 serotype clinical isolates carry the gmlABC genes. (Stojkovic et al., 2017). Both d-galactan I and d-galactan III are capped by D-galactan II, with both considered O1 serotypes. Unfortunately, the mechanisms behind the difference in complement resistance in various O-types have yet to be studied. It will be interesting to see how C3b/C5-C9 deposition and MAC insertion in the outer membrane differs between O-types.

In the latest TraDIS research, when O-antigen genes were mutated, the majority of them showed a decrease in serum survival. (Short et al., 2020). However, like cps genes, the number of O-antigen genes involved in complement resistance and the extent of the serum survival, differs between strains. In conclusion, the composition of LPS, especially the O-antigen, has a key function in modulating complement activation.

Deletion of outer membrane proteins increases complement susceptibility

Aside from capsule formation and O-antigen composition, outer membrane proteins (OMPs) play a role in complement resistance. OMPs have a role in the stability of the cell envelope. TraDIS experiments identified that some of the outer membrane proteins are involved in complement resistance (P.-F. Hsieh et al., 2013; Short et al., 2020). One of these proteins is murein lipoprotein (Lpp), a peptidoglycan-linked outer membrane protein which is one of the most abundant OMPs of K. pneumoniae. Lpp is not surface exposed and it is the only OMP that covalently crosslinks the outer membrane and peptidoglycan. (Mathelié-Guinlet et al., 2020). The deletion of the Lpp gene in various complement resistant K. pneumoniae strains resulted in an increase in complement susceptibility. (P.-F. Hsieh et al., 2013; Short et al., 2020). Lpp mutant colonies are flat and unstructured, but they still produce capsule (Short et al., 2020). However, the amount of cell-associated capsule produced by Lpp mutants is moderately reduced. In comparison to the wild type, Lpp mutants bind considerably more C3b and C5b-9. However, the survival rate in human serum of Lpp mutants compared to wild type is only moderately decreased.

Another OMP involved in complement resistance is peptidoglycan-associated lipoprotein (Pal), which is a component of the Tol–Pal protein complex (Godlewska et al., 2009; P.-F. Hsieh et al., 2013). The Tol–Pal complex is comprised of five core proteins (TolQ, TolR, TolA, TolB and Pal) that join together to create a multiprotein complex that spans the membrane. Pal also interacts with other OMPs like outer membrane protein A (OmpA) and Lpp. Deletion of the gene encoding for the Pal protein increased serum susceptibility substantially in the NTUH-K2044 strain (P.-F. Hsieh et al., 2013). Furthermore, a recent study discovered that deletion of not only Pal, but also of TolR, TolA, and TolB resulted in an increase in complement susceptibility (Short et al., 2020). This emphasizes the significance of the entire Tol–Pal complex in complement resistance. In E. coli, Pal proteins play a role in the translocation of the surface O-antigen subunits through the OM, modulating the O-antigen structure (ED et al., 2005). As a result, the role of Pal in complement resistance may be mediated indirectly through the loss of the O-antigen. It should be highlighted for both Pal and Lpp that these OMPs are highly conserved and essential for overall survival in many K. pneumoniae strains/isolates (P.-F. Hsieh et al., 2013). Pal or Lpp mutations disrupt the OM permeability barrier, allowing periplasmic proteins to escape. Furthermore, isolates of K. pneumoniae lacking Pal or Lpp are hypersensitive to bile salts and SDS. Pal and Lpp likely play a role in complement resistance by stabilizing the cell membrane. However, because these proteins are highly conserved, they do not play as important a role in complement resistance as the capsule and LPS.

While deletion of the outer membrane proteins Pal and Lpp reduce complement resistance, the loss of OmpA has no effect on complement susceptibility (P.-F. Hsieh et al., 2013). Despite the fact that OmpA is a prevalent outer membrane protein, removing it did not increase serum susceptibility. This implies that the presence of the Pal and/or Lpp proteins in the outer membrane is sufficient for membrane integrity and, as a result, complement resistance.

OmpK36 is one of the major outer membrane porins of Klebsiella pneumoniae (Tsai et al., 2011). Unlike Pal and Lpp, OmpK36 is surface exposed and thereby directly accessible to complement proteins. C1q can directly bind to outer membrane protein K36 (OmpK36) of K. pneumoniae to initiate the classical pathway, which leads to deposition of C3b and C5b-9 and complement-mediated killing (Albertí et al., 1993). In a serum susceptibility assay the OmpK36 mutant strain showed an increase in serum resistance after 1 hour (Chen et al., 2010). However, the OmpK36 mutant strains were all killed in serum, demonstrating that complement activation is still present. This could be due to delayed classical pathway activation as C1q can still be deposited on antibodies that bind to other OMPs and/or surface exposed structures of K. pneumoniae. Aside from the classical pathway, the lectin and/or alternate pathway may be activated, also resulting in complement-mediated killing.

To summarize, outer membrane proteins play an role in complement resistance, but not as significant as the capsule and LPS. Outer membrane proteins that are exposed on the surface, such as OmpK36, have a direct effect on survival. However, the loss of these proteins is not required for survival in all strains/isolates of K. pneumoniae. Loss of more conserved genes, such as Pal and Lpp, disrupts the outer membrane and makes the bacteria more susceptible to complement-mediated killing. The exact mechanism by which these genes protect against complement activation remains unknown.

Other genes involved in complement resistance in K. pneumoniae

K. pneumoniae establishes complement evasion primarily by modifying the capsule, LPS, or outer membrane proteins. Furthermore, there are several genes involved in complement resistance that do not fully fit into these three categories. For example, mutation of genes encoding for non-outer membrane proteins involved in cell membrane or cell wall structure and function show an increase in complement susceptibility. TraDIS studies revealed some of these genes (Short et al., 2020). One of these genes is dacA, which is involved in cell wall synthesis and maintenance by the mediation of peptidoglycan crosslinking, structural stabilization and cell wall modification (Short et al., 2020; Yang et al., 2018). In E. coli, deletion of dacA disrupts the peptidoglycan network, resulting in an incomplete cell wall and an increase in cell outer membrane permeability (Yang et al., 2018). Because this TraDIS study only looked at serum survival, little is known regarding C3b deposition and/or MAC formation. Another gene linked to complement resistance is dedA, an inner membrane protein and is thought to operate as membrane transporter (Iqbal et al., 2021; Short et al., 2020). Interestingly, dedA is also linked to colistin resistance in K. pneumoniae (Jana et al., 2017). K. pneumoniae also requires dedA for virulence by ATCC-43816 strain lung infection (Paczosa et al., 2020). All of this makes dedA a promising target for synergistically combating complement and antibiotic resistance.

Metabolic genes, mainly those involved in pyrimidine metabolism and carbohydrate metabolism, are also linked to complement resistance (Short et al., 2020). The majority of these genes have an indirect effect since they are involved in the biosynthesis of capsule and LPS precursor molecules. One of these genes is manA, encoding for mannose 6-phosphate isomerase (MPI). MPI converts mannose-6-phosphate to fructose 6-phosphate, which can then be used in a variety of metabolic processes, including glycolysis and capsular polysaccharide biosynthesis (Li et al., 2020). Deletion of galE also decreased complement resistance in some strains. GalE encodes for UDP-galactose 4-epimerase, which is an enzyme that catalyses the interconversion of UDP-glucose and UDP-galactose (Li et al., 2020). UDP-galactose is used for the biosynthesis of galactose-containing carbohydrate polymers, such as LPS and the capsular polysaccharide (Fry et al., 2000). In serotype O1:K20 of K. pneumoniae, d-galactan I synthesis was dependent on galE (Clarke et al., 1992). This emphasizes the significance of O-antigen composition in complement resistance, as strains with an O1 type are more complement resistant. Two other genes producing metabolic enzymes are implicated in complement resistance (pgi and pgm) (Short et al., 2020). The pgi gene codes for glucose-6-phosphate isomerase, while the pgm gene codes for phosphoglucomutase. Both enzymes contribute to the biosynthesis of capsule and LPS precursor molecules.

TraDIS research has discovered an increasing number of complement resistance genes in K. pneumoniae. However, there still is a great deal of variation amongst strains/isolates. In the future, TraDIS research should explore a broader range of strains/isolates to identify new and overlapping complement resistance genes. The majority of the identified genes are connected to either LPS or CPS synthesis, indicating the importance of LPS and CPS in complement resistance.

Discussion

Klebsiella pneumoniae is considered a major public health threat due to the rise of multidrug-resistant and hypervirulent strains. K. pneumoniae has developed a variety of virulence traits and mechanisms in order evade the complement system. The thick capsule and the long O-antigens are the most important evasion mechanism of K. pneumoniae.

The capsule is seen as a physical barrier for complement deposition, as poorly encapsulated and nonencapsulated mutant bacteria have more C3b deposition than K. pneumoniae strains with more extensive capsules. Furthermore, C5b-9 is deposited within the capsule in some complement resistance strains, but the MAC cannot form in the outer membrane. Likely this is due to the fact that the that the MAC cannot reach the outer membrane.

The O-antigen of LPS can also prevent complement-mediated killing, as smooth LPS strains with an O-antigen are more resistant to serum killing than rough LPS strains without an O-antigen. The evasion mechanism is similar to that of the capsule, as the O-antigen can suppress MAC formation either by preventing the MAC from reaching the outer membrane or by decreasing complement deposition of C1q and C3b. Recent research has focused on the effect of O-antigen composition on complement resistance. O2 strains are substantially more sensitive to human serum killing than strains with an O1 serotype. The O1 and O2 antigen both have repeat-units of d-galactan I, but the O1 antigen has an additional d-galactan II capping unit (E et al., 2002). The wbbY-wbbZ region is required for d-galactan II synthesis (P. F. Hsieh et al., 2014). Interestingly, a wbbY mutant showed higher levels of serum killing than the wild-type NTUH-K2044 strain. Furthermore, plasmids containing either wbbY or the whole wbbY-wbbZ region efficiently restored serum resistance in the wbbY mutant strain (P. F. Hsieh et al., 2014; Pennini et al., 2017). These studies indicate that serum killing resistance is linked to d-galactan II synthesis (P. F. Hsieh et al., 2014). However, they do not give a clear explanation why this is the case. Longer O-antigen side chains result in C3b deposition farther away from the surface, hindering the MAC from properly depositing on the bacterial membrane (Merino et al., 1992). As a result, pore formation and bacterial killing is decreased. This could explain why O1-type strains with longer O-antigens are more resistant to complement than O2-type strains with shorter O-antigens. Furthermore, d-galactan II is a high molecular weight polymer in contrast to d-galactan I, which is a low molecular weight polymer(P. F. Hsieh et al., 2014). It would be interesting to see if the increased serum resistance of the O1 serotype over the O2 serotype is entirely due to the longer O-antigen or if it is related to the composition of d-galactan II. Immunoelectron microscopy research should disclose how C3b/C5-C9 deposition and MAC insertion differ between O-types.

Even in serum resistant K. pneumoniae there is large variation in the evasion mechanisms. Some strains completely avoid complement recognition, whereas other strains survive in serum despite high deposition of C5b-C9. TraDIS studies have revealed a growing number of genes involved in complement resistance in K. pneumoniae. These screenings, highlighted the role of cell envelope integrity proteins like Pal and Lpp in complement resistance. Pal and Lpp mutations both enhanced outer membrane permeability, which likely explain why complement susceptibility is increased. However, because these proteins are present in

practically all K. pneumoniae strains/isolates, these genes are likely to be hits in other, non-complement specific, screens. As a result, their contribution to complement resistance is less specific and important than that of capsule and O antigen-related genes.

TraDIS studies also revealed dacA, dedA, hrtA, manA, galE, pgi and pgm genes to be implicated in complement evasion, but the mechanism is still unknown. Extensive research focusing on one or two of these genes will give us with a better understanding of K. pneumoniae's interaction with the complement system. While there are currently many individual genes known to contribute to complement resistance, little is known about how these genes might interact with each other. Some of the genes may be involved in the same pathway. It is also possible that when one gene is knocked out, other genes with similar functions take over. Dual knockout of complement resistant genes might give us more insight in the interaction of these genes.

Another limitation is the use of the type of isolates and strains. While strains like NTUH-K2044 and ATCC-43816 are useful for studying complement resistance mechanisms, they are not totally representative for clinical isolates. Furthermore, there is a lot of variability in strains and isolates. In a study that examined four different K. pneumoniae strains, researchers only discovered three overlapping complement resistance genes. It is hardly surprising that researchers find a wide range of genes, given there are numerous strategies to avoid complement killing like interference with recognition, C3b deposition, MAC insertion, and membrane damage susceptibility. For example, in some strains, if one gene is missing, complement can still blocked by another method, giving the impression that this gene does not play a role, while this does not have to be the case. Therefore, many additional TraDIS studies with multiple isolates and strains are required to identify more overlapping complement resistance genes. Because of the wide variety of clinical isolates, finding overlapping complement resistance genes is critical.

To summarize, K. pneumoniae primarily uses the capsule and the O-antigen of LPS to avoid complement-mediated killing. This is mainly achieved by decreasing complement deposition of C1q and C3b and by preventing the MAC from reaching the outer membrane. However, the fundamental mechanism by which this occurs, remains somewhat unknown. Understanding how complement and K. pneumoniae interact is critical for developing therapies to enhance complement-mediated killing of these bacteria. Discovering overlapping complement resistance genes is important since this could lead to the universal treatment options for various K. pneumoniae strains.

Layman's summary

Klebsiella pneumoniae is een bacterie die wordt beschouwd als een grote bedreiging voor de volksgezondheid vanwege een grote toename in antibiotica resistentie. Normaal gesproken wordt K. pneumoniae gedood door het immuun systeem, maar in personen met een zwakkere gezondheid vaak niet. K. pneumoniae heeft namelijk verschillende mechanismen ontwikkeld om het immuun systeem van mensen te ontwijken.

Een belangrijk onderdeel van het immuunsysteem is het complement systeem en dit dient als een soort van verdedigingslinie voor bacteriën zoals K. pneumoniae. Het complementsysteem bestaat uit een netwerk van eiwitten die op een gecoördineerde manier samenwerken. Het systeem wordt geactiveerd na herkenning van bepaalde patronen op de bacterie. Vervolgens wordt er een kettingreactie veroorzaakt die er uiteindelijk voor kan zorgen dat de bacteriën gedood worden. De bacterie kan op twee verschillende manieren gedood worden. Ten eerste kunnen er bepaalde complement eiwitten op de bacterie worden geplakt. Deze eiwitten kunnen worden herkent door immuun cellen, waarna deze cellen de bacteriën 'opeten'. De andere manier is dat er vijf verschillende complement eiwitten een complex gaan vormen op het membraan van de bacterie. Dit complex maakt een gat in het membraan van de bacterie, waardoor deze uit elkaar valt en uiteindelijk dood gaat. Echter heeft K. pneumoniae verschillende mechanismen ontwikkelt waardoor deze bacterie het complement systeem kan ontwijken. Het doel van deze studie is om een uitgebreid overzicht te geven van de diverse complement-ontwijkingsstrategieën van K. pneumoniae.

De belangrijkste strategieën zijn het hebben van een capsule en LPS. De capsule is een dikke suiker laag die om de bacterie heen zit. Deze laag zorgt ervoor dat de complement eiwitten niet goed kunnen binden op het membraan van de bacterie. Hierdoor kan er geen porie worden gevormd in het membraan van de bacterie en dus wordt deze niet gedood. LPS is een belangrijk bestanddeel van de buitenmembraan van K. pneumoniae. LPS bestaat uit drie verschillende delen, waaronder het O-antigeen. Er zijn tweede types LPS, LPS met O-antigeen LPS zonder O-antigeen. Over het algemeen worden bacteriën zonder O-antigen moeilijker gedood door het complement systeem dan bacteriën met O-antigeen. Het O-antigeen zorgt er namelijk voor de complement eiwitten te ver van het buitenmembraan binden en daardoor kan de porie niet in het membraan gevormd worden.

K. pneumoniae gebruikt dus voornamelijk de capsule en LPS om doding door het complementsysteem te voorkomen. De capsule en LPS zorgen er enerzijds voor dat er mindercomplement eiwitten kunnen binden en anderzijds dat de complement eiwitten te ver van het membraan binden, waardoor de porie niet in het membraan gevormd kan worden. De exacte mechanismen blijver echter enigszins onbekend. Het is belangrijk om er achter te komen hoe K. pneumoniae het complement systeem ontwijkt. Hierdoor kunnen we therapieën ontwikkelen om doding van deze bacteriën door het complement systeem te verbeteren.

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