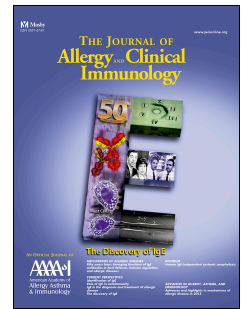


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The complement system in the airway epithelium – an overlooked host defense mechanism and therapeutic target?

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Title: The complement system in the airway epithelium – an overlooked host defense mechanism and therapeutic target?

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ABBREVIATIONS:

BAL	Bronchoalveolar lavage
C3	Complement protein C3
C3a	Anaphylatoxin from C3
C3b	Part of C3 left after cleavage to release C3a
iC3b	inactivated C3b (relative to hemolytic activity)
C5	Complement protein C5
C5a	Anaphylatoxin from C5
C5aR1	C5a receptor 1, also known as CD88
C5aR2	C5a receptor 2, also known as C5L2
CD46	Cluster of differentiation 46, also known as MCP (membrane cofactor protein)
CD55	Cluster of differentiation 55, also known as DAF (decay accelerating factor)
CF	Cystic fibrosis
CFP	Properdin gene
CIP	Complement inhibitory proteins
EMT	Epithelial-mesenchymal transition
hBD2	Human beta-defensin 2
IFX-1	a specific monoclonal anti-human C5a antibody
IgE	Immunoglobulin E
IL-1 α	Interleukin-1 alpha
IL-1 β	Interleukin-1 beta
IL-4	Interleukin-4
IL-5	Interleukin-5
IL-13	Interleukin-13
IL-17a	Interleukin-17a
IL-33	Interleukin-33
IPF	Idiopathic pulmonary fibrosis
LTx	Lung transplant
MMP-9	Matrix metalloproteinase 9
MUC5B	Mucin 5B
MUC5AC	Mucin 5AC
OB	Obliterative bronchiolitis
PARP	Poly-ADP ribose polymerase
SAEC	Small airway epithelial cells
TGF β	Transforming growth factor

TEXT:

The airway epithelium – from the nasal cavity to the alveoli – persistently encounters toxins and pathogens. If the invader clears the mucus and breaches the epithelium, it must be contained by an innate immune system while awaiting the arrival of the adaptive immune system. Hence, we need a biological process to immediately recognize, engage and destroy a pathogen. The complement cascade is the prototype of such an innate process. It quickly amplifies based upon cleavage of its most abundant complement protein, C3, to C3a and C3b (Figure 1A). C3a is a vasodilator and a chemoattractant for neutrophils and monocytes, while C3b is an opsonin for pathogens and debris. This cleavage (and thus, activation) is primarily triggered in three ways: antibody binding to antigen (classical pathway); mannose-binding lectin attaching to oligosaccharides/sugars (lectin pathway); or engagement of a feedback loop to deposit C3b on a target (alternative pathway).¹

Complement activation is traditionally viewed as occurring in the blood, owing to abundant hepatic production and its secretion of most of its components. However, multiple cell types are also known to synthesize complement proteins.¹ Those synthesized by resident and invading immune cells have recently been summarized.² In contrast, less is known about production and activation of complement in local tissues in contact with the environment, like the airway. Moreover, whether local complement activation worsens or mitigates injury has been inadequately investigated in most organ systems. In this mini-review, we appraise the literature published in the last three years on locally synthesized complement proteins in the airway and their potential role in respiratory disease.

Allergic rhinitis and asthma

Unbiased transcriptomic approaches have provided insights into complement expression following environmental exposures. Patients with allergic rhinitis who underwent nasal allergen challenge with grass pollen demonstrated upregulated transcripts of complement protein properdin (*CFP*) and C5a receptor (*C5aR1*) in their nasal mucosal specimens, along with interleukin-1 (IL-1) family of cytokines (IL-1 α , IL-1 β), Th2 cytokines (IL-4, IL-5 and IL-13) and interferons.³ Prednisone downregulated *CFP* and *C5aR1* in some, but failed in 7 of 19 individuals, which highlights the variation in the immune response from patient-to-patient in those with allergic rhinitis and asthma. These patients also had variable baseline *IL33* mRNA levels. Thus, the underlying genetic predisposition, as well as baseline levels of cytokines, may influence complement gene expression, and account for why patients had a variable response to prednisone.

Properdin, a positive regulator of the alternative pathway (Figure 1A), is elevated in the bronchoalveolar lavage (BAL) of asthmatic patients. The pathophysiological correlation in a mouse model has been described elsewhere.^{S44} C5aR1 binds C5a, the latter being generated by the cleavage of C5 downstream of C3 activation (Figure 1A). While there are no clinical trials yet targeting C5aR in asthma, pre-clinical studies showed that C5aR1 antagonism decreased BAL and parenchymal inflammatory cells, IL-13 levels and Th2 cytokine gene expression, without reducing serum IgE levels, in OVA-sensitized and challenged mice.⁴ Hence, properdin and C5aR1 should be further evaluated as potential therapeutic targets for difficult-to-treat allergic rhinitis and asthma.

Cystic fibrosis

Complement proteins have also shown to be produced and secreted by airway epithelial cells. C3 was identified as one of the most abundant proteins, along with MUC5B, MUC5AC and fibronectin, in apical secretions from cultures of bronchial epithelial cell lines derived from patients with cystic fibrosis (CF).⁵ Factor B was increased, but Factor I was decreased in the CF secretome, as detected by mass spectrometry-based proteomics. Factor B is a critical enzymatic component of the alternative pathway and promotes inflammation (Figure 1A), whereas Factor I is a fluid-phase serine protease that cleaves C3b to iC3b, and thus, mitigates inflammation (Figure 1B). Hence, low levels of Factor I could lead to poorly controlled C3 convertase formation, excessive cascade activation and potentially a heightened airway inflammatory response. Supporting this concept, there was significantly more C3 activation in the CF secretome as detected by immunoblot.⁵ Proteases such as MMP-9, cathepsins B and D were also elevated. Whether these proteases are directly involved in cleaving C3 is unknown; however, it suggests that the CF airway epithelial microenvironment may facilitate C3 cleavage *in vivo*.

Obliterative bronchiolitis

Obliterative bronchiolitis (OB) is a form of chronic airway epithelial cell injury that is a predominant manifestation of chronic rejection in post-lung transplant (LTx) recipients and a major impediment to long-term graft survival. Epithelial injury resulting in OB post-LTx has been associated with complement-dependent cytotoxicity; however, its precise mechanism is unclear. C3a and C5a upregulate proinflammatory mediators associated

with airway epithelial cell injury (Figure 1A).^{S34} Conversely, CD55 (decay accelerating factor) is downregulated on airway epithelial cells of patients with severe asthma in response to injury (viral infection or ozone).^{S1} CD55 (decay accelerating factor) is an ubiquitously expressed membrane inhibitor that prevents binding of Factor B to the membrane and facilitates decay of the C3 convertase (Figure 1B). CD46 is also a widely expressed membrane regulator and a cofactor for Factor I to inactivate C3b (Figure 1B).¹ Thus, downregulation of these complement inhibitory proteins (CIPs) could lead to poorly controlled airway inflammation and its downstream consequences such as fibrosis.

Accordingly, reduced expression of CD55 and CD46 was implicated in driving OB following lung transplantation. Both were decreased on the epithelium of patients with OB.⁶ These recipients had elevated C3a levels in their BAL, indicating C3 activation. **In a mouse model** where OB developed at day 21 post-LTx, CD55 and Crry (murine functional homolog of CD46) were decreased in the allograft at day 1, establishing that these changes occurred early. When CD3⁺ T cells were co-cultured with allograft-specific antigen presenting cells, C3a drove up IL-17a production, which subsequently downregulated Crry. Additionally, treatment with an anti-C5 antibody mitigated OB in the mice. This report proposed a cross-talk between IL-17a and C3a generation that downregulated CD55 and CD46 expression, thereby increasing the risk of developing epithelial injury and OB post-LTx.

Interstitial lung disease

Concomitantly, patients with idiopathic pulmonary fibrosis (IPF) had elevated C3a and C5a in plasma and lung homogenates, leading to the speculation that complement is both systemically and locally activated in this irreversible lung disease.⁷ In small airway

epithelial cells (SAECs), fibrogenesis mediator TGF- β induced a dose-dependent downregulation of CD55 and CD46 expression. This was associated with increased cleaved PARP (poly-ADP ribose polymerase) expression, a marker of injury, and a concurrent decrease in E-cadherin, a marker of epithelial-to-mesenchymal transition (EMT), both hallmarks of IPF. Treatment of SAECs with the anaphylatoxins C3a and C5a at concentrations similar to those identified in IPF patient lungs also resulted in a loss of CD55 and CD46, implicating that this downregulation independently occurred with both anaphylatoxins and TGF- β .

The argument for exuberant complement activation in IPF was strengthened by finding elevated soluble C5b-9 levels in lung lavage fluid from these patients, suggesting that terminal membrane attack complex formation may mediate cell damage (Figure 1A).⁸ **In a mouse model** of bleomycin-induced lung injury, inhibiting C3aR or C5aR with systemically administered antagonists arrested fibrosis by downregulating TGF- β /bone morphologic protein signaling pathways and reducing local complement activation. Of note, this work predominantly focused on C5aR1. While TGF- β upregulated C5aR2 in SAECs, its role in IPF is still unclear.

These recent reports renew the importance of local complement activation in the respiratory tract. Complement proteins produced by human airway epithelial cells are listed in Table 1. Additionally, the complement system does not act independently in the lung microenvironment. There is a bidirectional interaction between complement proteins and other components of the innate immune system such as antimicrobial peptides (neutrophil-derived α -defensins, β -defensin hBD2) that facilitates host defense at the air-liquid interface.⁹ An emerging concept for lung disease pathogenesis is that external stress (e.g., hypoxia, allergen challenge) or the inherent proinflammatory environment

(e.g., CF, IPF) increases local synthesis of complement proteins. At the same time, mediators activating the complement cascade also downregulate CIPs, thus putatively propagating ongoing epithelial injury. Why CIP downregulation is a part of the airway epithelial response is puzzling. We speculate that one reason may be that in an acute insult (for example, pneumonia), the airway requires a proinflammatory status to upregulate the complement cascade and downregulate CIPs for the rapid clearing of pathogen or debris. However, chronic complement activation becomes deleterious if this proinflammatory status is prolonged, resulting in irreversible lung disease. Unfortunately, there is no clear evidence that CH50, AH50 and MBL levels are diagnostic biomarkers in any pulmonary disease. To mitigate dysregulation of the complement system in disease, novel therapies targeting the locally active complement proteins need to be designed and tested in these difficult-to-treat respiratory diseases.

While there are no clinical trials currently targeting locally generated complement proteins in the lung, complement inhibitors are now being evaluated for respiratory diseases. These include the use of intravenous C1-inhibitor to reduce house-dust mite-plus lipopolysaccharide-induced bronchial inflammation (NCT03051698, Phase IV, recruiting) and the use of IFX-1, a monoclonal anti-human C5a antibody for treating early severe sepsis or septic shock displaying at least one newly developed organ dysfunction and showing clinical evidence of pulmonary or abdominal infection (NCT02246595, Phase II, completed). A recent review provides a detailed description of historical and current complement-modulating therapies being evaluated in clinical trials.¹⁰ Additionally, locally delivered complement inhibitors (such as lampalizumab, a specific inhibitor of Factor D in age-related macular degeneration, NCT02288559) are now being tested. Hence, one approach would be to consider locally delivering already approved specific inhibitors such as C1-inhibitors and anti-C5 monoclonal antibodies to the lungs (via

nebulizers). Additionally, a relatively unexplored area is enhancing complement regulatory proteins to reduce inflammation, especially in diseases such as IPF and OB that have a known imbalance in regulators. While recombinant and soluble forms of CD46 and CD55 have halted at the pre-clinical stage in non-pulmonary disease, soluble CR1 was tested in acute lung injury; but remains to be evaluated in chronic, fibrotic lung injury.^{S45} This is an exciting but challenging and rapidly expanding field that now more than ever, needs to be better understood and tapped for mitigating lung diseases.

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FIGURE LEGENDS:

Figure 1A: Assembly, activation, amplification and attack: The complement system is commonly activated by one of three triggers. **The lectin pathway (LP)** is triggered when lectins bind carbohydrates (oligosaccharides) on the pathogen surface. Next, mannose-associated serine proteases (MASPs) are activated and they cleave C4 and C2. **The classical pathway (CP)** is triggered by antigen-antibody complexes interacting with the C1 complex [C1(qr₂s₂)]. This activates C1s to cleave C4 and C2. The **alternative pathway (AP)** is constitutively active at low levels as C3 metabolizes to C3(H₂O) at ~1% per hour. It is amplified when C3(H₂O) binds to Factor B, and is enhanced with the binding of positive regulator properdin (P). Factor B is cleaved by Factor D to Bb, forming C3bBbP. This forms an autocrine feedback loop that can be amplified. **All three proteases are denoted in red.** The three pathways converge to form the C3 convertases (formed by enzyme subunits - either C4bC2a, for the classical and lectin pathways, or C3bBbP for the alternative pathway). The C3 convertases cleave C3 to C3a and C3b. Attachment of C3b to C3 convertases changes their substrate specificity, allowing them to become C5 convertases (C4bC2aC3b or C3bBbC3bP). The C5 convertases cleave C5 to C5a and C5b. C5b then forms the membrane-attack complex with C6, C7, C8 and C9, resulting in cell perturbation. C3a and C5a bind to their G-protein coupled receptors C3aR and C5aR1, respectively, leading to chemotactic, vasodilatory, phagocyte activating and immunomodulatory effects.

Figure 1B: Complement regulation. The complement cascade is tightly regulated by membrane-associated and plasma proteins to prevent excessive inflammation. **CD46** (known as MCP, membrane cofactor protein) and **CD55** (known as DAF, decay-accelerating factor) are **membrane regulators**, while Factor H, Factor I and C4BP (C4-binding protein) are **fluid-phase regulators**. CD55 irreversibly decays (dissociates) the C2a from C4b, as well as the Bb from C3b to inactivate C3 and C5 convertases. Not shown in the figure is the ability of CD55 to competitively inhibit binding of Bb to C3b, and C2a to C4b. **Factor H** also decays the C3b-containing convertases, while C4BP decays the C4b-containing convertases. **Factor I** proteolytically degrades C3b or C4b, but only in the presence of the appropriate cofactors. **Complement receptor one (CR1 or CD35)** is another membrane-associated receptor and regulator, almost exclusively present on hematopoietic cells, which has both decay-accelerating and cofactor activity. **CD59** is a membrane regulator that functions independent from CD46 and CD55. It prevents insertion of C8 and C9 into the membrane, thus, inhibiting formation of the membrane-attack complex and preventing cell perturbation.

276 Table 1: Complement components produced by airway epithelial cells

Components	Evidence	Primary Cell Data	Tissue Data
Classical Pathway			
C1q	Not expressed ^{S1}		
C1r	mRNA ^{S2-5} Protein ^{S6}	Zhou et al ^{S2}	
C1s	mRNA ^{S3,S7} Protein ^{S6}		
C4	mRNA ^{S8-10} Protein ^{S6} Supernatant ^{S11,12}	Scheetz et al ^{S9} Walters et al ^{S10} Peters-Hall et al ^{S11} Brass et al ^{S12}	
C2*	mRNA ^{S8}		
Lectin Pathway			
MBL	mRNA ^{S5,S13}		Vandermeer et al ^{S13}
H-ficolin	mRNA ^{S14} Protein ^{S14,S15}		Akaiwa et al ^{S14}
Alternative Pathway			
C3	mRNA ^{S7,S8,S16-18} Protein ^{S6, S18,S19} Supernatant ^{S1,S16,S20,S21}	Ali et al ^{S20} Pillai et al ^{S21}	Schlosser et al ^{S17} Lane et al ^{S18}
Factor B (FB)	mRNA ^{S8,S13,S16,S17,S22} Protein ^{S6} Supernatant ^{S11,S12,S16,S21}	Peters-Hall et al ^{S11} Brass et al ^{S12} Pillai et al ^{S21} Cooper et al ^{S22}	Vandermeer et al ^{S13} Schlosser et al ^{S17}
Factor D (FD)	No data and not expected to occur (primary source: adipocytes)		
Properdin (P)*	mRNA ^{S13,S18,S23}		Vandermeer et al ^{S13} Lane et al ^{S18}
Terminal Complement Complex			
C5	mRNA ^{S7,S8,S17} Protein ^{S6} Supernatant ^{S24}		Schlosser et al ^{S17}
C6,C7,C8,C9*	mRNA ^{S6,S17,S25} Protein ^{S6}		Schlosser et al ^{S17} Ying et al ^{S25}
Regulators, Fluid-phase			
C1INH	No data		
C4BP*	Supernatant ^{S26,27}		
Factor H (FH)	mRNA ^{S13,S28} Protein ^{S6} Supernatant ^{S21,S28}	Pillai et al ^{S21}	Vandermeer et al ^{S13}
Factor I (FI)	mRNA ^{S13} Protein ^{S6} Supernatant ^{S21,S26}		
Clusterin	mRNA ^{S5,S29} Supernatant ^{S11}	Peters-Hall et al ^{S11} Hackett et al ^{S29}	
Regulators, Membrane-bound			
CD46 (MCP)	mRNA ^{S26,S30} Protein ^{S1,S31-33}		Suzuki et al ^{S32} Varsano et al ^{S33}
CD55 (DAF)	mRNA ^{S34-37} Protein ^{S1,S32,S33,S35,S37} Supernatant (shedding) ^{S36}	Agrawal et al ^{S34} Pandya et al ^{S35}	Suzuki et al ^{S32} Varsano et al ^{S33} Vainer et al ^{S37}
CD59	mRNA ^{S4,S38} Protein ^{S1,S33,S39} Supernatant (shedding/exosomes) ^{S12,S20,S40}	Brass et al ^{S12} Ali et al ^{S20} Castillon et al ^{S39} Kesimer et al ^{S40}	Varsano et al ^{S33}
Receptors			
C3aR1	mRNA ^{S41} Protein ^{S41-43}	Drouin et al ^{S41}	Drouin et al ^{S41}
C5aR1	mRNA ^{S29,S41} Protein ^{S41-43}	Hackett et al ^{S29} Drouin et al ^{S41}	Fregonese et al ^{S42} Gu et al ^{S43}

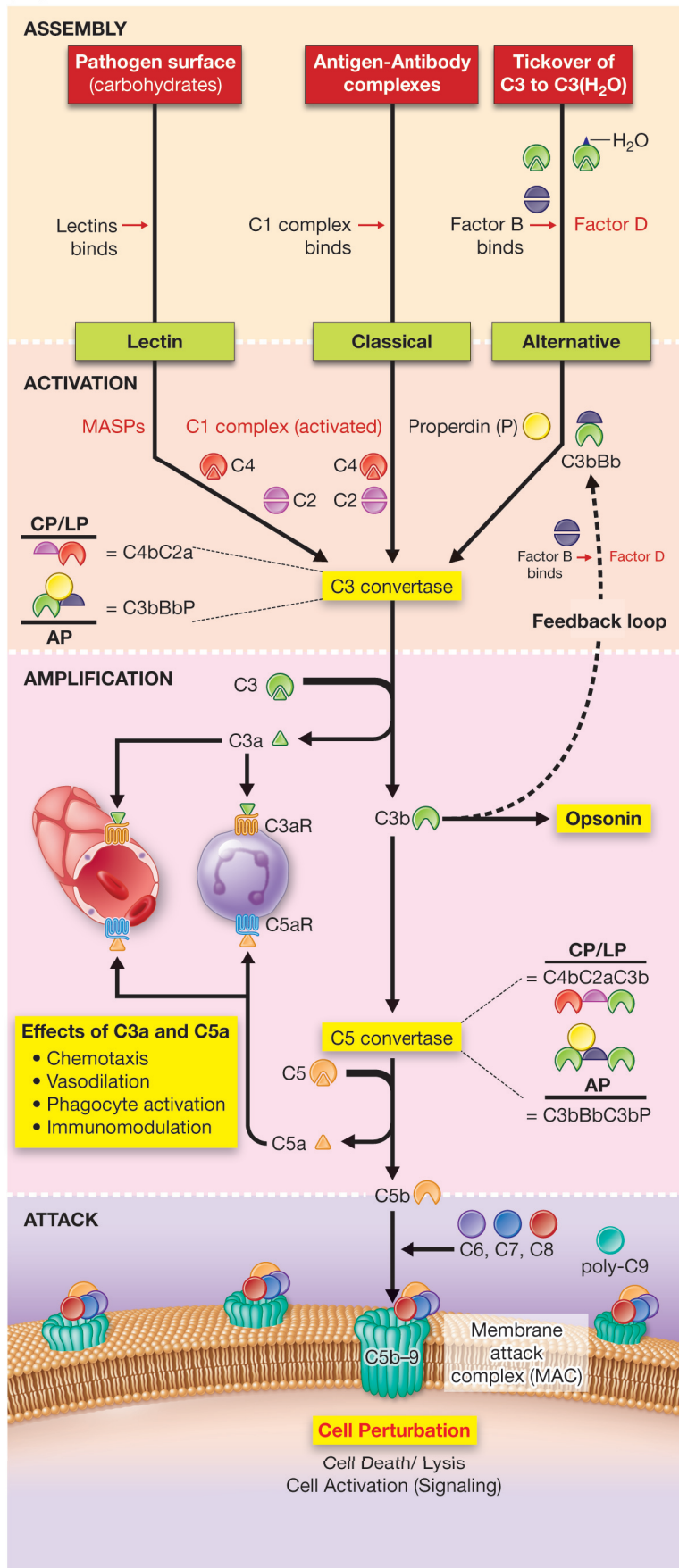
<i>C5aR2</i>	<i>No data</i>
<i>CR1 (CD35)</i>	<i>Not expressed</i> ^{S33}

Abbreviations: C1INH: C1-inhibitor; C4BP: C4b-binding protein; DAF: decay-accelerating factor; FB: Factor B; FD: Factor D; FH: Factor H; FI: Factor I; MBL: mannose-binding lectin; MCP: membrane cofactor protein; mRNA: messenger RNA.

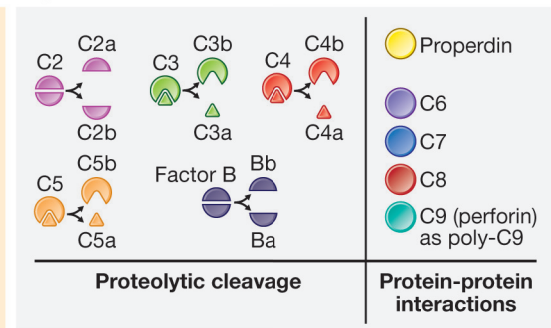
*These represent studies that are from a single research group, or conflicting evidence exists in the literature. The components that are not expressed or have no data supporting their production by airway epithelial cells are italicized.

Evidence for 'protein' content was based on immunoblotting, flow cytometry or mass spectrometry of the cells/cell lysate. 'Supernatant' denotes that the cells secreted the protein, unless otherwise specified (in which case it was either shed or secreted as exosomes).

(1A) Assembly, activation, amplification and attack

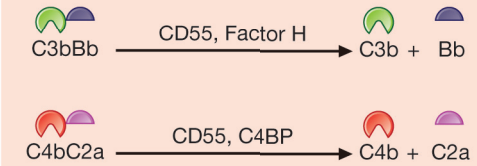


Key



(1B) Regulation

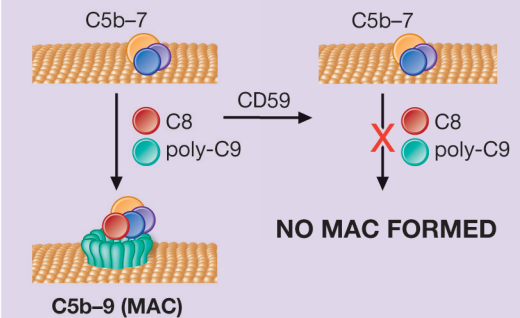
1 Accelerating decay of the convertases:



2 Cofactor activity:



3 Inhibition of MAC formation:



Supplementary References

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