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UPDATE

Targeting the complement system in bacterial meningitis

Diederik L.H. Koelman, Matthijs C. Brouwer and Diederik van de Beek

Bacterial meningitis is most commonly caused by *Streptococcus pneumoniae* and *Neisseria meningitidis* and continues to pose a major public health threat. Morbidity and mortality of meningitis are driven by an uncontrolled host inflammatory response. This comprehensive update evaluates the role of the complement system in upregulating and maintaining the inflammatory response in bacterial meningitis. Genetic variation studies, complement level measurements in blood and CSF, and experimental work have together led to the identification of anaphylatoxin C5a as a promising treatment target in bacterial meningitis. In animals and patients with pneumococcal meningitis, the accumulation of neutrophils in the CSF was mainly driven by C5-derived chemotactic activity and correlated positively with disease severity and outcome. In murine pneumococcal meningitis, adjunctive treatment with C5 antibodies prevented brain damage and death. Several recently developed therapeutics target C5 conversion, C5a, or its receptor C5aR. Caution is warranted because treatment with C5 antibodies such as eculizumab also inhibits the formation of the membrane attack complex, which may result in decreased meningococcal killing and increased meningococcal disease susceptibility. The use of C5a or C5aR antagonists to specifically target the harmful anaphylatoxins-induced effects, therefore, are most promising and present opportunities for a phase 2 clinical trial.

Amsterdam UMC, University of Amsterdam, Department of Neurology, Amsterdam Neuroscience, Meibergdreef 9, 1105 AZ, Amsterdam, The Netherlands

Correspondence to: Prof. Dr Diederik van de Beek
Department of Neurology
Amsterdam UMC, University of Amsterdam
Amsterdam Neuroscience
PO Box 22660
1100DD Amsterdam, The Netherlands
E-mail: d.vandebeek@amsterdamumc.nl

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Abbreviations: MAC = membrane attack complex; MBL = mannose-binding lectin

Introduction

Bacterial meningitis is a life-threatening infection of the CNS. Bacteria enter the CSF in the subarachnoid space either by crossing the blood–CNS barrier (either the blood–brain barrier through the brain parenchymal microvasculature or the blood–CSF barrier through the choroid plexus or the pial or arachnoidal microvasculature) or from a contiguous site of infection (Mook-Kanamori *et al.*, 2011; Coureuil *et al.*, 2017). Bacterial pathogen-associated molecular patterns and the resulting damage-associated molecular patterns in the CSF provoke a massive and often uncontrolled inflammatory response leading to high rates of complications, morbidity, and mortality in patients (Mook-Kanamori *et al.*, 2011; van de Beek *et al.*, 2016*a*).

Most common pathogens causing community-acquired bacterial meningitis are currently *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Listeria monocytogenes*,

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accounting for 70%, 10%, and 5% of cases, respectively (van de Beek *et al.*, 2004*b*; Bijlsma *et al.*, 2016). Pneumococcal meningitis is also one of the most common forms, after listerial meningitis, the form with the highest mortality rate (12–18%) (van de Beek *et al.*, 2006; Bijlsma *et al.*, 2016; Polkowska *et al.*, 2017). The introduction of pneumococcal conjugate vaccination has resulted in a decline in incidence as reported by cohorts from several geographical areas (Hsu *et al.*, 2009; Alari *et al.*, 2016; Bijlsma *et al.*, 2016; Ruiz-Contreras *et al.*, 2017). Nevertheless, reported effectiveness of the 7- and 13-valent pneumococcal conjugate vaccines differed per region, and subsequent increase of invasive pneumococcal disease due to non-vaccine pneumococcal serotypes has been reported (Weinberger *et al.*, 2011; Gladstone *et al.*, 2015; Weiss *et al.*, 2015; Brouwer and van de Beek, 2018; Vadlamudi *et al.*, 2019). It is therefore likely that pneumococcal meningitis will remain a major health challenge (Koelman *et al.*, 2019).

Over the past 15 years, the implementation of anti-inflammatory treatment with adjunctive dexamethasone therapy to dampen the inflammatory response has resulted in an absolute decrease of mortality by 10% (de Gans *et al.*, 2002; van de Beek *et al.*, 2004*a*; Brouwer *et al.*, 2010). Nevertheless, mortality and morbidity are still too high with death occurring in ~20% of patients, inability to live an independent life in 20%, and (neuro)psychological sequelae in 50% (Schmidt *et al.*, 2006; Hoogman *et al.*, 2007; Bijlsma *et al.*, 2016; Lucas *et al.*, 2016). Therefore, new adjunctive therapies are needed (Davis and Greenlee, 2003; van de Beek, 2012). Further, dampening the host inflammatory response with such new adjunctive treatments is the promising approach in bacterial meningitis (van de Beek *et al.*, 2012).

The complement system plays a key role in the innate immune system, through facilitating the clearing of pathogens and damaged cells by immune cells, direct bacterial killing by the pore-forming membrane attack complex (MAC), but also by promoting the inflammatory response through the production of anaphylatoxins (Murphy and Weaver, 2017). Diseases directly linked with the complement system include paroxysmal nocturnal haemoglobinuria (PNH), age-related macular degeneration, atypical haemolytic uraemic syndrome (aHUS), hereditary angioedema, and C3-glomerulopathies (Morgan and Harris, 2015). The complement system also appears to play an important role in the pathogenesis of multiple neurological diseases, resulting in a strong increase in pathophysiological studies and clinical trials investigating complement system intervention over the past 15 years (Morgan, 2015). Because of the multiplicity of complement system activation routes and the various components, proteases, convertases, anaphylactic peptides and receptors involved, the complement system includes numerous points to intervene (Fig. 1) (Morgan and Harris, 2015). Bacterial meningitis is one of the diseases in which the complement anaphylatoxin-induced cellular immune response seems to have profound detrimental effects on the brain and blood compartment.

In this comprehensive update, we will systematically evaluate evidence gained from genetic variation studies, complement level measurements in blood and CSF, and experimental work that have together identified the complement system as a promising treatment target in bacterial meningitis. We will conclude with an up-to-date summary of the therapeutic options available to move complement system intervention forward to clinical translation in bacterial meningitis.

Complement variation studies

The complement system is often regarded as a double-edged sword (Morgan, 2015). Deficiencies in the complement system are well-known to increase the risk of bacterial infections (Brouwer *et al.*, 2009). Terminal pathway deficiencies that inhibit MAC formation, for instance, are associated with increased susceptibility to Gram-negative bacteria such as *Neisseria* species. The cell wall of these bacteria is thin in comparison to Gram-positive bacteria such as *S. pneumoniae*, making it vulnerable for the MAC (Skarnes and Watson, 1957). Conversely, an overactive complement system, although limiting susceptibility, could result in an uncontrolled inflammatory response in patients once infected, and lead to an unfavourable outcome. It was noted more than 30 years ago that patients with invasive meningococcal disease who had a late complement deficiency had a lower mortality rate (Ross and Densen, 1984). Several studies have investigated this double-edged sword hypothesis for other complement gene variations by investigating its associations with disease course and outcome of invasive pneumococcal and meningococcal disease (Table 1) (Hibberd *et al.*, 1999; Kronborg and Garred, 2002; Perez-Castellano *et al.*, 2006; Faber *et al.*, 2007; Endeman *et al.*, 2008; Garcia-Laorden *et al.*, 2008, 2013; Woehrl *et al.*, 2011; Garnacho-Montero *et al.*, 2012; Adriani *et al.*, 2013; Brouwer *et al.*, 2013; Lundbo *et al.*, 2014; Bradley *et al.*, 2015; Mills *et al.*, 2015; Kasanmoentalib *et al.*, 2017). Overall, patients with genetic variations associated with complement deficiencies seemed to have higher susceptibility, but inversely improved rate of favourable outcome. Results of the different reports are, however, not always similar and may differ per pathogen (Hibberd *et al.*, 1999; Faber *et al.*, 2007; Garnacho-Montero *et al.*, 2012; Brouwer *et al.*, 2013), homozygous and heterozygous deficiencies (Hellemann *et al.*, 2007), and clinical situation (Garred *et al.*, 2003; Fidler *et al.*, 2004). Pathogens differ in their way of complement activation. For *S. pneumoniae*, mannose-binding lectin (MBL) does not function as complement activator (Ali *et al.*, 2012). A factor compromising the interpretation of genetic variations with outcome is that a linkage

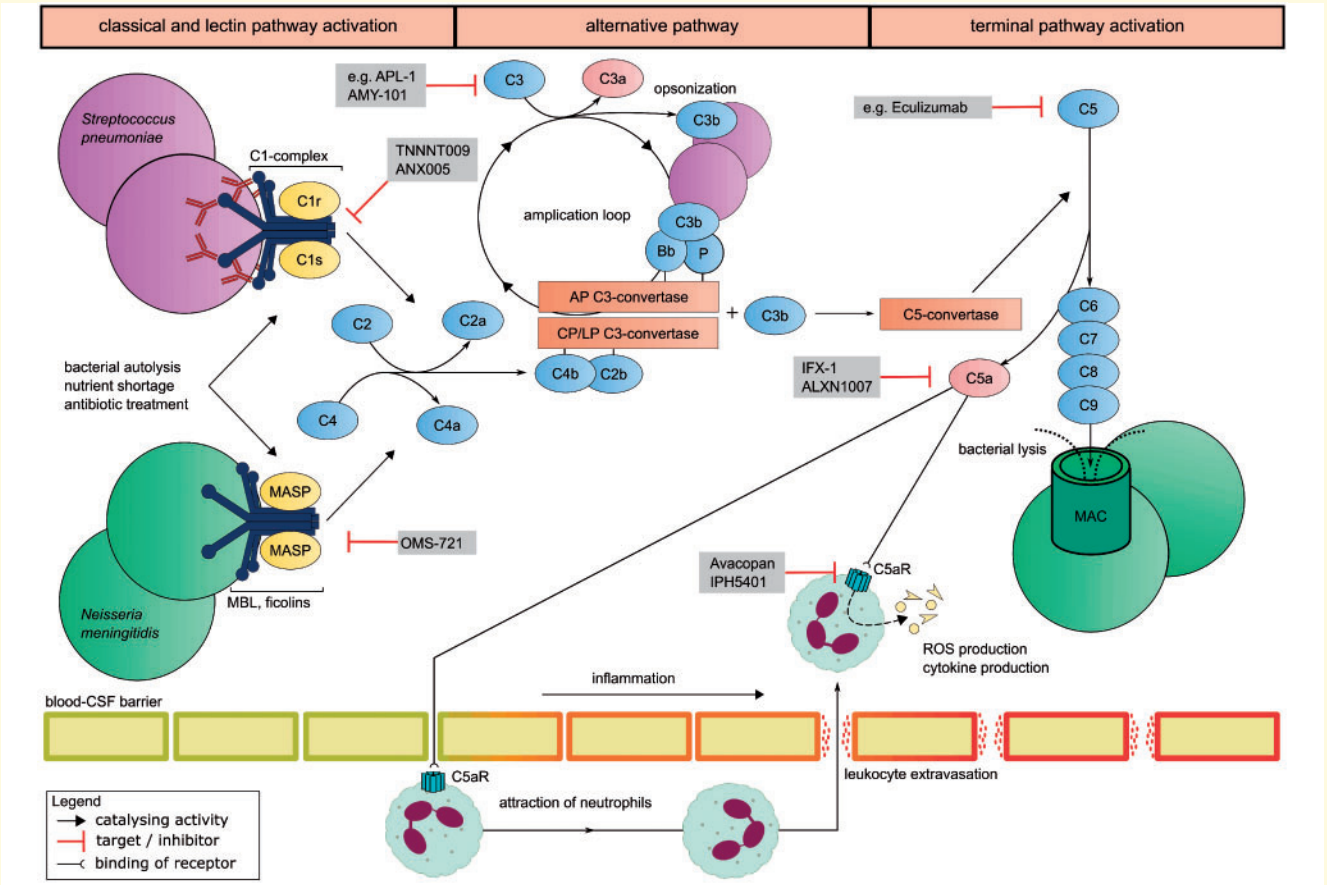

Figure 1 Complement system and therapeutic targets in bacterial meningitis. The complement system is activated via multiple pathways: the classical pathway, the lectin pathway, and the alternative pathway. The classical pathway starts with binding of C1q to immune complexes such as non-specific IgM that binds to the pneumococcal C polysaccharide. The lectin pathway is activated through direct binding of collectins, such as mannose-binding lectin (MBL) and ficolins, to sugars on the bacterial surface. This results in the binding with the corresponding serine proteases [C1r and C1s, and MBL-associated serine proteases (MASP), respectively], to form complexes that facilitate cleavage of C2 and C4. This forms C3-convertase (C4bC2b), which catalyses the conversion of C3 to C3a and C3b. The alternative pathway is activated when C3b, either produced due to spontaneous hydrolysis or initiating pathway activation, binds to a microbe. This allows C3b to bind with factor B. Subsequently, factor B is cleaved into Ba and Bb by factor D. Bb remains bound to C3 and forms a complex (C3bBb). The serum protein properdin binds this complex to make it more stable. C3bBb(P) acts as another C3-convertase further promoting C3 conversion, thus amplifying complement system activation. There are several natural inhibitors of the alternative pathway including complement receptor 1, factor H and complement protease complement factor I. C3b is an opsonin that facilitates phagocytosis. When C3b binds with the C4b2b complex or to a C3bBb complex, it forms C5 convertase (C4b2b3b and C3bBb3b respectively). Because of the catalysing activity of C5 convertase, C5 is cleaved to C5a and C5b. C5b is the first complement component of the MAC complex. Simplified, C5b consecutively binds C6, C7, C8, which induced the binding and subsequent polymerization of 10 to 16 C9 molecules, creating the pore-forming structure known as the MAC. The pores formed by the MAC complex enable molecules to diffuse freely in and out of the cell. If enough pores form, the cell will no longer be viable. C3a and C5a are anaphylatoxins that are produced during complement system activation both in order of production (from early to late) as in order of potency (from weak to active). Anaphylatoxins upregulate the inflammatory response by binding to its specific receptor C5aR, which are mainly expressed by immune cells, and result in increased blood–CSF barrier permeability and the accumulation of polymorphonuclear leucocytes in the CSF. Various complement therapeutics are available targeting C1s [TNNNT009 (True North), C1q (ANX005 (Annexon)], MASP-2 and 3 [OMS-721 and OMS906, respectively (Omeros)], C2 [PRO-02 (Prothix/Broteio)], C3b [H17 (Elusys); S77 (Genentech), factor B (bikaciomab (Novelmed)], factor D [lampalizumab (Genentech); ACH-4471 (Achillion)], properdin [CLG561 (Novartis)], C3 [compstatin family: AMY-101 (Amyndas), APL-1 and APL-2 (Apellis)], C5 [soliris/eculizumab (Alexion); ALXN1210 (Alexion); tesidolumab/LFG316 (Novartins/Morphosys); SKY59/ RO7112689 (Chugai and Roche); REGN3918 (Regeneron); ABP 959 (Amgen); Coversin (Akari); Zilucoplan/RA101495 (Ra Pharma); Zimura (Ophtotech); ALN-CC5 (Alnylam)], C5a [IFX-1 (InflaRx); ALXN1007 (Alexion)], and C5aR [Avacopan/CCX168 (Chemocentryx); IPH5401 (Innate Pharma)].

disequilibrium may exist (Fijen *et al.*, 1999; Spath *et al.*, 1999). The most striking genetic association identified was that of a genetic variation in the C5 encoding region (rs17611), which was significantly associated with unfavourable outcome of pneumococcal meningitis, even when corrected for multiple testing, or in a multivariate regression model corrected for risk factors for poor outcome (Woehrl *et al.*, 2011).

Table 1 Associations of complement gene variants and severity of invasive bacterial disease

Article	Complement component	Measurement	Results
All bacteria			
Woehrl, 2011 Meningitis	Seven complement genes (*C3*, *C5*, *C6*, *C7*, *C8B*, *C9*, *CFH*)	Unfavourable outcome, CSF C5a, CSF MAC	Genetic variants in *C5*, *C8B* and *CFH* were associated with mortality in univariate, but not in multivariate analysis
Adriani, 2013 Meningitis	*C3*	CSF C3, C3a, iC3b, C5a, MAC level	*C3* gene variant significantly associated with low C3 in CSF
Neisseria meningitidis			
Hibberd, 1999 Invasive disease	*MBL2*	Mortality, PRISM score, ICU admission	NS
Faber, 2007 Invasive disease	*MBL2*	Mortality, GMSPS	NS
Bradley, 2015 Invasive disease	*CFH*	Bacterial load	NS
Streptococcus pneumoniae			
Kronborg, 2002 Invasive disease	*MBL2*	Mortality	NS
Perez-Castellano, 2006 Pneumonia	*MBL2*	Serum MBL, serum CRP, bacteraemia, fine scale	*MBL2* gene variant associated with higher MBL in blood
Endeman, 2008 Pneumonia	*MBL2*	Mortality, ICU admission, bacteraemia, length of hospital stay	NS
Garcia-Laorden, 2008 Pneumonia	Two complement genes (*MBL2* and *MASP2*)	Severe sepsis, ICU admission, ARF, 90-day mortality	MBL deficiency predisposed for worse disease, irrespective of causative pathogen
Woehrl, 2011 Meningitis	*C5*	Unfavourable outcome, CSF leucocyte count	Genetic variant in *C5* was associated with and decreased CSF leucocyte count, and associated with unfavourable outcome, both corrected for multiple testing and in a multivariable model with important risk factors for unfavourable outcome
Garnacho-Montero, 2012 Sepsis	*MBL2*	90-day mortality	Gene variant significantly associated with mortality in multivariate analysis
García-Laorden, 2013 Pneumonia	*MBL2*	ICU admission, MODS, septic shock, PSI IV-V	Gene variant significantly associated with increased rate of ICU admission, MODS, septic shock, and PSI IV-V
Brouwer, 2013 Meningitis	*MBL2*	Mortality, CSF MBL	*MBL2* variant associated with lower CSF MBL concentration
Adriani, 2013 Meningitis	*C3*	CSF C3, C3a, iC3b, C5a, MAC	*C3* variant associated with lower CSF C3 (0.6 versus 1.7 μg/ml $P = 0.001$), C5a (15 versus 28 μg/ml, $P = 0.019$) and MAC (2.3 versus 3.5 μg/ml, $P = 0.037$)
Lundbo, 2014 Meningitis	*MBL2*	30-day mortality	NS
Mills, 2015 Pneumonia	*MBL2*	Mortality	NS
Kasanmoentalib, 2017 Meningitis	*MASP2*	Unfavourable outcome	NS

ARF = acute renal failure; CAP = community-acquired pneumoniae; CFH = complement factor H; CRP = C-reactive protein; GMSPS = Glasgow meningococcal sepsis prognostic score; MASP = MBL-associated serine protease; MODS = multiple organ dysfunction syndrome; NS = no significant associations; PRISM = Paediatric Risk of Mortality Score; PSI = pneumoniae severity index.

Complement activation in bacterial meningitis

Pathogens that cross the blood–CNS barrier are able to multiply freely in the CSF as the effectiveness of the host defence is limited in the subarachnoid space (Mook-Kanamori *et al.*, 2011). In non-infected healthy individuals, complement levels in the CSF are 100 to a 1000-fold lower compared to serum concentrations; too low for any significant antibacterial activity (van de Beek *et al.*, 2016*a*). Historically, the CNS was considered immunologically privileged. Before the invention of antibiotics when bacterial meningitis was almost universally fatal,

patients were even occasionally (and sometimes successfully) treated with intrathecally administered serum or complement to make-up for the lack of CSF bactericidal activity (M'Kenzie and Martin, 1908; Finland *et al.*, 1938; Coleman, 1940; Domingo *et al.*, 2019). The adjuvant use of intrathecal serum or complement in patients treated with antibacterial sulphonamides in the late 1930 to 1940s did, not, however, seem to beneficially affect the disease course and may have led to increased mortality in meningococcal meningitis patients [17% (374/2139) versus 9% (482/5221)] (Coleman, 1940; Domingo *et al.*, 2019). Studies in the 1910–1940s that identified complement activity in the CSF of a small proportion of cases with acute inflammatory conditions thought it was the sole result of extravasation of complement components from the blood related to increased blood–CSF barrier permeability (Kolmer *et al.*, 1918; Fothergill, 1935). However, since the 1990s, it has been known that several cells in the CNS have an immune-regulatory function, and that astrocytes, but also microglia, ependymal cells, oligodendrocytes and neurons all express complement proteins resulting in a complete and functional complement system (Morgan and Gasque, 1996; Stahel *et al.*, 1997).

The host immune response in the CSF is activated through surface-bound or intracellular pattern recognition receptors including different Toll-like receptors (TLRs) and NOD-like receptors (NLRs) (Mook-Kanamori *et al.*, 2011), and pattern-recognition molecules including collectins and complement component C1q, especially when pathogens die because of stress conditions (e.g. nutrient shortage, antibiotic administration) (Hajishengallis and Lambris, 2016; van de Beek *et al.*, 2016*a*). The activation of these TLRs and NLRs leads to the activation of inflammatory transcription factors, in particular the nuclear factor NF-kB, which amongst others may lead to increased complement protein production through TLR-complement crosstalk, though this remains largely unexplored (Mook-Kanamori *et al.*, 2011; Lian *et al.*, 2015; Hajishengallis and Lambris, 2016).

Several studies included blood and/or CSF complement measurements and correlated results to disease severity and outcome (Table 2) (Greenwood *et al.*, 1976; Whittle and Greenwood, 1977; Zwahlen *et al.*, 1982; Stahel *et al.*, 1997; Goonetilleke *et al.*, 2010, 2012; Woehrl *et al.*, 2011; Adriani *et al.*, 2013; Brouwer *et al.*, 2013; Lucas *et al.*, 2013; Mook-Kanamori *et al.*, 2014; Kasanmoentalib *et al.*, 2017). The techniques used for measuring complement levels have varied over time and studies included different distributions of causative pathogens. Despite this heterogeneity among publications, it remains evident that massive complement system activation occurs in the CSF of patients with bacterial meningitis. Concentrations of C1q, MBL, MBL-associated serine protease 2 (MASP2), factor B, factor H, C3a, iC3b, C5a, and MAC in the CSF of the diagnostic lumbar puncture were all significantly elevated compared to control CSF (for instance of patients with thunderclap headache) (Stahel *et al.*, 1997; Brouwer *et al.*, 2013; Mook-Kanamori *et al.*, 2014). Although many patients with bacterial meningitis have bacteraemia or sepsis, data on serum complement levels in bacterial meningitis patients are scarce, and only two studies have assessed paired complement levels in serum and in the CSF (Zwahlen *et al.*, 1982; Goonetilleke *et al.*, 2012). To date, studies including serial blood sampling to determine complement activation profiles of patients with acute bacterial meningitis are lacking. Similar to in bacterial meningitis, focus of research in sepsis shifted from the pathogenicity of the causative pathogen towards the dysregulated hosts' systemic inflammatory and immune response (Hotchkiss *et al.*, 2016). Differences in complement activation between patients with uncomplicated sepsis and patients who eventually died were already identified over 40 years ago (McCabe, 1973). An increased degree of complement activation is also associated with unfavourable outcome in bacterial meningitis. High concentrations of C1q, MASP2, C5a and MAC in the CSF of the diagnostic puncture have been significantly associated with mortality in pneumococcal meningitis (Goonetilleke *et al.*, 2010; Woehrl *et al.*, 2011; Mook-Kanamori *et al.*, 2014; Kasanmoentalib *et al.*, 2017). High levels of C5a in the CSF were also associated with development of the detrimental complication of delayed cerebral thrombosis later in the disease course (Lucas *et al.*, 2013). Patients with this devastating complication suddenly deteriorate after initial recovery, developing headache, fever, a decreased level of consciousness, brainstem signs, or hemiparesis due to inflammation and brain infarction (Schut *et al.*, 2009). Low CSF C3 levels indicating complement consumption due to massive complement activation have also been associated with mortality (Zwahlen *et al.*, 1982; Goonetilleke *et al.*, 2010, 2012). Differences are described between causative pathogens. In contrast to pneumococcal meningitis, high MAC levels in meningococcal meningitis were associated with favourable outcome (Mook-Kanamori *et al.*, 2014). In addition, CSF C5a and MAC levels were significantly higher in patients with pneumococcal meningitis than in patients with meningococcal meningitis, even when corrected for age, immunocompromised state, and level of consciousness (Mook-Kanamori *et al.*, 2014).

Data on the deposition of complement components in the brain is limited as it has not been thoroughly evaluated by any of the few case series of autopsied bacterial meningitis patients (Engelen-Lee *et al.*, 2016). Strong and specific staining of the C3a receptor was seen in astrocytes, microglia and infiltrating cells, macrophages, and neutrophils in brain tissue of bacterial meningitis patients, as was true for the C5a receptor for non-bacterial meningitis brain inflammation (Gasque *et al.*, 1998).

Experimental rationale for complement system targets

The effects of complement system intervention on bacterial killing and spurring inflammation have been investigated in

Table 2 Complement levels in blood and CSF in patients with bacterial meningitis

Blood				
Studies per measured complement component	**Causative pathogen**	**Sample size**	**Level in serum**	**Associations**
C3				High levels of C3 in serum associated with:
Greenwood, 1976	NM	198	115%$^{\uparrow}$ (R)	Negative meningococcal antigen (119% versus 99%, $P = 0.01$)
Zwahlen, 1982	All	27	121%$^{\uparrow}$ (R)	NS
Goonetilleke, 2012	SP	80	1.49μg/ml (P)	NS
CSF				
Studies per measured complement component	**Causative pathogen**	**Sample size**	**Level in CSF**	**Associations**
C1q				High levels of C1q in CSF associated with:
Goonetilleke, 2010	SP	20	Not specified (M)	Mortality
Mook-Kanamori, 2014	SP	269	188 ng/ml$^{\uparrow}$	NS
Mook-Kanamori, 2014	NM	41	226 ng/ml$^{\uparrow}$	NS
MBL				High level of MBL in CSF associated with:
Brouwer, 2013	SP	155	13.6 ng/ml$^{\uparrow}$	NS
Mook-Kanamori, 2014	SP	269	13 ng/ml$^{\uparrow}$	NS
Mook-Kanamori, 2014	NM	41	16 ng/ml$^{\uparrow}$	NS
MASP-2				High levels of MASP2 in CSF associated with:
Kasanmoentalib, 2017	SP	307	4.77 ng/ml$^{\uparrow}$	Unfavourable outcome
C3				High levels of C3 in CSF associated with:
Whittle, 1977	NM	38	8,8% (R)	High CSF protein levels
Zwahlen, 1982	All	2	2% (R)	High CSF CMOA, complete recovery
Stahel, 1997	All	18	48 μg/ml$^{\uparrow}$	NS
Goonetilleke, 2010	SP	20	Not specified (M)	Survival
Goonetilleke, 2012	SP	80	0.13μg/ml$^{\uparrow}$ (W)	Survival
Adriani, 2013	All	344	1.5μg/ml (L)	Low levels of C3a, C5a and C5b-9
Mook-Kanamori, 2014	SP	269	1.2μg/ml^{NS} (L)	NS
Mook-Kanamori, 2014	NM	41	1.6μg/ml^{NS} (L)	NS
C3a				High levels of C3a in CSF associated with:
Adriani, 2013	All	344	0.48 μg/ml	NS
Mook-Kanamori, 2014	SP.	269	0.56 μg/ml$^{\uparrow}$	NS
Mook-Kanamori, 2014	NM	41	0.48 μg/ml$^{\uparrow}$	NS
iC3b				High level of iC3b in CSF associated with:
Adriani, 2013	All	344	19.8 ng/ml	NS
Mook-Kanamori, 2014	SP	269	23 ng/ml$^{\uparrow}$	NS
Mook-Kanamori, 2014	NM	41	17 ng/ml$^{\uparrow}$	NS
Factor B				High levels of Factor B in CSF associated with:
Stahel, 1997	All	18	16 μg/ml$^{\uparrow}$	NS
CFH				High levels of CFH in CSF associated with:
Mook-Kanamori, 2014	SP	269	11.4 μg/ml$^{\uparrow}$	NS
Mook-Kanamori, 2014	NM	41	12.7 μg/ml$^{\uparrow}$	NS
C5a				High levels of C5a in CSF associated with:
Woehrl, 2011	All	204	Not specified	CSF wbc count $>1000/mm^3$, unfavourable outcome, mortality
Adriani, 2013	All	344	13.4 ng/ml	NS
Lucas, 2013	All	299	Not specified	Delayed cerebral thrombosis
Mook-Kanamori, 2014	SP	269	17 ng/ml$^{\uparrow}$	Mortality
Mook-Kanamori, 2014	NM	41	4 ng/ml$^{\uparrow}$	NS
MAC				High levels of MAC in CSF associated with:
Woehrl, 2011	All	204	Not specified	Unfavourable outcome, mortality
Adriani, 2013	All	344	1.9 μg/ml	NS
Lucas, 2013	All	299	Not specified	Delayed cerebral thrombosis
Mook-Kanamori, 2014	SP	269	2.3 μg/ml$^{\uparrow}$	Mortality
Mook-Kanamori, 2014	NM	41	1.8 μg/ml$^{\uparrow}$	Survival

Complement components were measured using enzyme-linked immunosorbent assay (ELISA) unless specified otherwise: R = radial immunodiffusion; P = particle-enhanced turbidimetric immunoassay; M = mass spectrometry; W = western blot; L = Luminex; and values are expressed as means or medians except for measurements by radial immunodiffusion, which are expressed as percentage of pooled normal serum. If CSF complement levels in patients with bacterial meningitis were compared with control CSF; NS = a non-significant result; the arrows indicate a significantly higher (↑) or lower (↓) level in the CSF of bacterial meningitis patients. CFH = complement factor H; CMOA = complement mediated opsonic activity; MAC = membrane attack complex; MASP = MBL-associated serine protease; NM = *N. meningitidis*; SP = *S. pneumoniae*; NS = no significant association; wbc = white blood cell.

various experimental studies. Intervening early in the complement cascade by targeting the initiating pathways has the potential benefit of limiting the production of anaphylatoxin C3a, but may impair its opsonophagocytic function. This is illustrated by various *in vitro* opsonophagocytosis assays, showing reduced opsonization and phagocytosis of *S. pneumoniae* in classical (C1q) and lectin pathway (MASP-2) deficient mice and human serum, and may lead to increased bacterial outgrowth in *in vivo* models (Brown *et al.*, 2002; Yuste *et al.*, 2008; Ali *et al.*, 2012). Targeting the alternative pathway does not alter opsonization as significantly. Opsonization of *S. pneumoniae* was less intense for sera of factor B-deficient mice (Brown *et al.*, 2002), and only limited for some *S. pneumoniae* strains in human factor B-depleted serum resulting in only mild impairment of phagocytosis (Yuste *et al.*, 2008). Properdin to stabilize C3-convertase significantly improved opsonization of both *S. pneumoniae* and *N. meningitidis* in serum (Ali *et al.*, 2014). Targeting C3 itself, as illustrated in rats and rabbits that were enzymatically depleted of C3 following intraperitoneal injection of cobra venom factor, a proteolytic activator of C3 and C5, almost completely abolishes opsonizing function resulting in an increased bacterial outgrowth (Crosson *et al.*, 1976; Tuomanen *et al.*, 1986). Targeting C5 conversion, C5a, or the C5a receptor has the benefit of not limiting opsonization, while still targeting the production of the most potent anaphylatoxin C5a. The chemotactic activity of C5a in the accumulation of polymorphonuclear leucocytes has been recognized for a long time (Ernst *et al.*, 1984). Intrathecally administered human C5a in rabbits resulted in a rapid leucocytosis (Kadurugamuwa *et al.*, 1989).

Several models of experimental bacterial meningitis have been used, either investigating the difference between wild-type and complement-deficient mice (Rupprecht *et al.*, 2007; Woehrl *et al.*, 2011; Kasanmoentalib *et al.*, 2017), or using adjunctive complement-targeted therapies (Table 3) (Crosson *et al.*, 1976; Tuomanen *et al.*, 1986; Zwijnenburg *et al.*, 2007; Woehrl *et al.*, 2011; Kasanmoentalib *et al.*, 2015, 2017; Klein *et al.*, 2018). Most models investigated pneumococcal meningitis. The development of experimental meningococcal meningitis models has been hindered by the exclusivity of this pathogen to humans. Classical pathway deficiency (C1q) was associated with lower rates of intracranial complications and lower CSF leucocyte count, but higher mortality rates due to increased bacterial outgrowth and septicaemia (Rupprecht *et al.*, 2007). Adjunctive treatment with a classical pathway inhibitor (C1-INH, inhibitor of C1r and C1s) also attenuated CSF leucocyte count and cytokine and chemokine response and reduced clinical illness measures (Zwijnenburg *et al.*, 2007). Surprisingly, C1-INH treated rats and mice had reduced bacterial outgrowth, possibly related to increased CR3-receptor expression, improving phagocytic function in spite of impaired opsonization. Lectin pathway deficiency (MASP-2) improved survival, and adjunctive treatment with MASP-2 antibodies in adjunction to standard care with dexamethasone and antibiotics significantly reduced progression of clinical severity scores and non-significantly lowered mortality rates (Kasanmoentalib *et al.*, 2017). C3-deficient mice had increased bacterial outgrowth and increased mortality (Rupprecht *et al.*, 2007). Whilst different from treatment in meningitis, C3-inhibition by compstatin in a baboon *E. coli* sepsis model had organ-protective effects, even when administered after sepsis induction (Silasi-Mansat *et al.*, 2010; Mastellos *et al.*, 2015). Most clear is the evidence resulting from experimental models investigating targeting C5a. Mice with C5a receptor deficiency had reduced inflammation and improved clinical scores (Woehrl *et al.*, 2011). Adjunctive treatment with C5-antibody therapy, preventing C5 conversion to C5a, was significantly associated with decreased mortality, improved neuroscore and clinical score, and less frequent cerebral haemorrhages (Woehrl *et al.*, 2011). A subsequent investigator-blinded experimental model showed that combined treatment with dexamethasone and C5 antibodies significantly reduced mortality when compared to placebo, and also when compared with dexamethasone and C5-antibody therapy alone (Kasanmoentalib *et al.*, 2015). A recently published experimental pneumococcal meningitis model comparing the use of ceftriaxone with different combinations of dexamethasone, daptomycin, IL1-antibody, roscovitine and C5a antibodies in mice, showed most favourable results for the combination of daptomycin and anti-C5 antibody (Klein *et al.*, 2018). Mice treated with adjunctive dexamethasone and C5-antibody had similar clinical scores and survival (all mice survived), but had higher hearing thresholds and CSF leucocyte count (Klein *et al.*, 2018). An experimental study comparing different complement interventions in the same model has not been performed, while this would overcome the limitation of comparing heterogeneous models, using different bacterial strains with varying pathogenicity (for instance *S. pneumoniae* strain ATCC 6303 has shown to be more lethal than D39 in wild-type mice) (Kostyukova *et al.*, 1995; Lim *et al.*, 2007).

Therapeutic options

Targeting the complement system in bacterial meningitis has the goal to decrease complications deriving from the uncontrolled, massive inflammatory response in the CNS, mainly driven by anaphylatoxin C5a production. To date, only two complement-targeted drugs, eculizumab (Alexion) and C1-INH [Cinryze (Shire), Berinert (CSL Behring), Cetor (Sanquin), Ruconest/conestat alfa (Pharming)], have received approval for its use in clinical practice, but dozens are currently investigated in one or multiple clinical trials covering almost the whole complement cascade, each with its own benefits and caveats (Ricklin *et al.*, 2017; Harris, 2018).

The main detrimental effects of complement activation are considered to be caused by the spurring inflammatory

Table 3 Complement intervention and complement deficiency studies in experimental bacterial meningitis

Complement intervention studies				
Article	**Intervention**	**Model**	**Sample size**	**Intervention associated with:**
Crosson, 1976	CVF i.p.	Rats inoculated intranasally with 2×10^7 CFU/ml *H. influenzae*	19 versus 19 saline	Mortality Incidence and magnitude of bacteraemia
			18 versus 12 saline	Low CSF leucocyte count
Tuomanen, 1986	CVF i.p.	Rabbits inoculated intracisternally with 10^3 cells *S. pneumoniae* strain A_{II} or 8249	8 versus 8 non-treated	Mortality Lower lethal dose Increased CSF bacterial outgrowth Increased time to leucocytosis
Zwijnenburg, 2007	C1-inhibitor i.t.	Rats inoculated intracisternally with 5×10^6 CFU *S. pneumoniae* ST 6A	8 versus 8 PBS	Lower C4b/c in plasma and CSF Lower clinical illness score[a] Decreased bacterial outgrowth in CSF and blood Decreased meningeal inflammatory infiltrate Low CSF leucocyte count Increased CR3 expression
		Mice inoculated intranasally with 8×10^4 CFU *S. pneumoniae* ST 6A	10 versus 16 saline	Decreased bacterial outgrowth in CSF Decreased meningeal inflammatory infiltrate Low cytokine and chemokine expression in brain homogenates
Woehrl, 2011	C5 mAb i.p	Mice inoculated intracisternally with 1.5×10^5 CFU *S. pneumoniae* ST 2, *strain* D39, and treated with ceftriaxone	10 versus 21 IgG	Survival Low MAC in brain homogenates Low CSF leukocyte count Low clinical status score[a] Low neuroscores[a] Less cerebral haemorrhages
Kasanmoentalib, 2015	C5 mAb i.p.	Mice inoculated intracisternally with 10^4 CFU *S. pneumoniae* ST3, strain ATCC6303, and treated with ceftriaxone	31 versus 16 saline	Survival
			30 versus 15 in adjunction to DXM	Lower clinical severity score[a]
Kasanmoentalib, 2017	MASP-2 mAb i.p.	Mice inoculated intracisternally with 10^4 CFU *S. pneumoniae* ST3, strain ATCC6303, and treated with ceftriaxone	22 versus 23 isotype and 22 saline	Lower clinical severity score[a]
			18 versus 18 saline	Low MAC level in plasma and brain homogenates. Low TNF-α in plasma
Klein, 2018	C5 mAb i.p.	Mice inoculated intracisternally with 1.5×10^5 CFU *S. pneumoniae* ST 2, *strain* D39, and treated with ceftriaxone	9 versus 9 in adjunction to daptomycin	Lower clinical score[a]
			10 versus 13 in adjunction to DXM	Lower clinical score[a]
Complement deficiency studies				
Article	**Deficiency**	**Model**	**Sample size**	**Deficiency associated with:**
Rupprecht, 2007	*C1qa*$^{-/-}$	Mice inoculated intracisternally with 1.5×10^5 CFU *S. pneumoniae* ST 3	14 versus 13 WT	Mortality Increased bacterial outgrowth in blood and CSF Low CSF leucocyte count Increased cytokine level in blood
	C3$^{-/-}$	[b]	13 versus 13 WT	Mortality Increased bacterial outgrowth in blood and CSF Low CSF leucocyte count Decreased cytokine expression in brain homogenates Increased cytokine expression in blood
Woehrl, 2011	*C5aR1*$^{-/-}$	Mice inoculated intracisternally with 1.5×10^5 CFU *S. pneumoniae* ST 2, strain D39.	9 versus 10 WT	Low CSF leuckocyte count Low clinical status score[a] Lower intracranial pressure Low neuroscore[a] Decreased cytokine and chemokine expression in brain homogenates.
	C6$^{-/-}$	[b]	14 versus 20 WT	Mortality Increased blood–brain barrier permeability
	C3aR1$^{-/-}$	[b]	12 versus 12 WT	NS
Kasanmoentalib, 2017	*Masp2*$^{-/-}$	Mice inoculated intracisternally with 10^4 CFU *S. pneumoniae* ST3, strain ATCC6303, and treated with ceftriaxone	12 versus 12 WT	Survival Low clinical severity score[a]
	Masp2$^{-/-}$	[b]	24 versus 24 WT	Decreased cytokine and chemokine expression in brain homogenates.

$^{-/-}$ = deficient; CFU = colony forming units; CR3 = complement receptor 3; CVF = cobra venom factor; DXM = dexamethasone; i.p. = intraperitoneal; i.t. = intrathecal; IgG = immunoglobulin; mAb = monoclonal antibody; PBS = phosphate-buffered saline; ST = serotype; WT = wild-type; NS = no significant association.

[a]Low score indicates favourable disease course.

[b]Indicates that the model was the same for the different interventions or deficiency studies in the same article.

effects of the complement system further upstream. The caveat of targeting the initiating pathway is the resulting opsonization deficit that may result in decreased bacterial killing (Brown *et al.*, 2002), neither is complement activation in bacterial meningitis restricted to a single pathway. A potential advantage of intervening early in the complement system is the inhibition of the anaphylatoxin C3a produced early in the complement cascade, but no data to date support the importance of this less potent anaphylatoxin in the pathophysiology of bacterial meningitis (Woehrl *et al.*, 2011; Mook-Kanamori *et al.*, 2014). C4a does not share the inflammatory activities of C3a and C5a, and therefore should not be considered as an anaphylatoxin (Barnum, 2015). Targeting the amplification loop or C3 has the benefit of reducing complement amplification and concurrent massive anaphylatoxin production irrespective of the initiation pathway, but was not found to be beneficial in experimental meningitis models (Table 3). None of these models included antibiotic treatment, which may have over-emphasized the impaired opsonophagocytosis. Nevertheless, a C3-bypass mechanism has been shown with thrombin to be able to act as a potent C5 convertase in the absence of C3, in a dose-dependent manner (Huber-Lang *et al.*, 2006). This suggests conversion to C5a can still occur in patients despite total blockage of C3, further limiting it as a good treatment option in bacterial meningitis.

Experimental evidence makes targeting C5a, the anaphylatoxin most broadly associated with disease severity and clinical outcome, the most promising therapeutic intervention to add to the current treatment regimen for bacterial meningitis patients. Three strategies are available to target C5a production. This includes targeting C5 to prevent conversion to C5a and C5b, specifically targeting C5a, or targeting the C5a receptor (C5aR). A multitude of therapeutic options has been developed to accomplish this (Supplementary Table 1). Eculizumab is a monoclonal C5 antibody registered for PNH, aHUS and generalized myasthenia gravis (Hillmen *et al.*, 2006; Legendre *et al.*, 2013; Howard *et al.*, 2017). Functional C5a generation *ex vivo* despite eculizumab treatment in extreme complement activation situations has, however, been reported (Harder *et al.*, 2017). Also in patients with aHUS, C5a was only partly suppressed to normal range or above (Wehling *et al.*, 2017).

The major problem with targeting C5 conversion is the inhibition of MAC formation. The MAC complex is not considered as a modulator of the inflammatory response, although blockage is considered harmful as it may limit bacterial killing, especially in patients with meningococcal meningitis. In these patients, low concentration of the MAC complex in CSF was significantly associated with unfavourable clinical outcome despite subsequent administration of antibiotics, warranting caution (Mook-Kanamori *et al.*, 2014). Exemplary, eculizumab, abrogates meningococcal killing in whole blood (Konar and Granoff, 2017), and patients treated with eculizumab are at high risk of developing invasive meningococcal disease even when vaccinated (McNamara *et al.*, 2017).

An alternative approach might be to use anti-C5a or C5a receptor antagonists, which are more selective than eculizumab, and have been shown to inhibit the potentially harmful effects of *N. meningitidis*-induced C5a formation, at least *in vitro*, while preserving complement-mediated meningococcal killing via MAC (Sprong *et al.*, 2003; Herrmann *et al.*, 2018). It is therefore assumed that specific C5a targeting will be safe, even for patients with meningococcal meningitis. Of note, the effect of anti-C5 antibodies in bacterial meningitis other than pneumococcal meningitis is unclear and needs to be carefully evaluated. Two monoclonal antibodies against C5a exist to date, IFX-1 (InflaRx) and ALXN1007 (Alexion) (Supplementary Table 1). Targeting the C5a receptor with Avacopan/CCX168 (Chemocentryx) or IPH5401 (Innate Pharma) is another option (Supplementary Table 1).

The blood–CNS barrier (either the blood–brain barrier or the blood–CSF barrier) protects and regulates the homeostasis of the brain. However, this barrier also limits the access of drugs to the brain, posing—in spite of increased permeability due to inflammation—several challenges for C5-targeted therapies to cross the blood–CNS barrier, which to date have only partially been unravelled (Nau *et al.*, 2010; Mook-Kanamori *et al.*, 2011; Carpanini *et al.*, 2019; Tattevin *et al.*, 2019): monoclonal antibodies such as eculizumab and IFX-1 do not cross the blood–brain barrier, though ~0.1% of circulating antibodies penetrate in the CNS with a hypothetically increasing proportion due to inflammation (Freskgård and Urich, 2017). Eculizumab reduced the attack frequency in AQP4-positive neuromyelitis optica spectrum disorders, a disease where the blood–CNS barrier is disrupted (Pittock *et al.*, 2013; Carpanini *et al.*, 2019).

Small-molecules (<400 Da) can passively diffuse into the CNS if they are lipophilic (Freskgård and Urich, 2017). Small peptides (Zilucoplan) have the advantage of increased blood–CNS barrier penetration, stressing the importance of these treatments in neurological disease without blood–CNS barrier breaching, such as Alzheimer's disease (Craik *et al.*, 2013; Baig *et al.*, 2018). In addition, several new techniques are in development to accomplish brain delivery, such as receptor-mediated transport and transcytosis to shuttle therapeutics into the brain, or manipulating the blood–CNS barrier through signalling cascades, and barrier gene expression (Oller-Salvia *et al.*, 2016; Greenwood *et al.*, 2017).

It is also important to acknowledge that effectivity of complement inhibition may differ between patients. Patients with more pronounced complement activation due to genetic variations will likely benefit more from complement intervention (Harris *et al.*, 2012; Kavanagh *et al.*, 2015). Moreover, some patients may not respond to certain treatments because of mutations, as is seen with eculizumab (Nishimura *et al.*, 2014). Some patients are therefore likely to require a tailored approach. The acute disease course of

bacterial meningitis limits the options for extensive workup before enrolling patients in a clinical trial to reduce the risk of false-negative trial designs. Timely bedside genetic biomarkers are currently not available. Another important factor for trial design in bacterial meningitis is the variety in causative pathogens. Patients with pneumococcal meningitis have significantly more pronounced complement activation in the CSF and are therefore more likely to benefit from complement inhibition. Previously, the benefit of dexamethasone was shown to be most pronounced in patients with pneumococcal meningitis (de Gans *et al.*, 2002; Bijlsma *et al.*, 2016). CSF Gram staining or bedside PCR may identify the causative pathogen relatively quick compared to CSF culture, but will inevitably lead to treatment delays. As complement inhibition is considered most beneficial when started early, it makes sense to treat all patients with bacterial meningitis till the causative pathogen is identified.

Conclusion

There is a multitude of evidence that confirms the importance of complement system activation in bacterial meningitis. Patients with genetic complement deficiencies, and/or lower complement levels in the CSF tend to have favourable outcome. Experimental animal studies have increasingly shown the important regulatory function of the complement system in spurring the inflammatory response in bacterial meningitis. Amongst all complement components, C5a was most significantly associated with unfavourable outcome, both the genetic variation in complement component 5 gene and the anaphylatoxin C5a concentration in the CSF. The experimental results of targeting anaphylatoxin C5a production have been very promising. Three separate experimental studies significantly favoured treatment with C5 monoclonal antibodies, one of which was a randomized investigator-blinded trial. C5 monoclonal antibodies also showed to be beneficial when given in adjunction to the standard treatment regimen with antibiotics and dexamethasone as proposed in the current ESCMID guideline for acute bacterial meningitis (van de Beek *et al.*, 2016*b*). Specific targeting of the anaphylatoxin C5a or its receptor C5aR, therefore, emerges as the most promising treatment target. Targeting the initiating pathways, C3 or C5 all have their caveats, mainly due to the opsonophagocytosis deficits, complement bypass routes, and/or inhibiting MAC formation. The promising results of the experimental models warrant the initiation of a randomized clinical phase IIb or III trial with concomitant genetic profiling of host and pathogen to determine its interactions with complement intervention to allow for future patient stratification. Possible candidates for clinical trials are currently IFX-1 (InflaRx), ALXN1007 (Alexion), Avacopan/CCX168 (Chemocentryx), and IPH5401 (Innate Pharma). The initiation of clinical trials and the clinical use of complement-targeted therapies for bacterial meningitis may be further spurred in the future when more economical therapeutic options have become available.

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Competing interests

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Supplementary material

Supplementary material is available at *Brain* online.

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