Verwondering is het begin van wijsheid.

(Socrates)

5 Genetic variation of innate immune response genes in invasive pneumococcal and meningococcal disease applied to the pathogenesis of meningitis

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ABSTRACT

The susceptibility, severity and prognosis of infectious diseases depend on the ability of the host immune system to respond to pathogens. Genetic variation of immune response genes is associated with susceptibility to and severity of infectious diseases.

Bacterial meningitis (BM) is a serious and life-threatening infectious disease of the central nervous system (CNS). Despite adequate antibiotic treatment and immunization strategies, mortality remains high, especially in developing countries. *Streptococcus pneumoniae* and *Neisseria meningitidis* are the two most common causative microorganisms of BM worldwide.

The pathogenesis of BM starts with mucosal bacterial colonization, followed by invasion and survival of bacteria in the bloodstream, crossing of the blood–brain barrier, finally causing infection in the CNS, where host defense is less adequate. Host defense to BM starts with a complex cascade of pathogen recognition and subsequent intracellular signaling causing transcription of genes leading to the production of inflammatory mediators. Although this immune reaction is essential for killing microbes, it is also associated with damage to healthy cells and thus adverse disease outcome.

This review provides an overview of the pathogenesis of invasive pneumococcal disease and invasive meningococcal disease related to the influence of genetic variation in genes involved in innate immunity, focusing on BM.

INTRODUCTION

Bacterial meningitis (BM) is a life-threatening infectious disease of the central nervous system (CNS) accounting for an estimated annual 170 000 deaths worldwide. Despite the availability of antibiotics, universal immunization strategies, and continuing improvement of supportive care, mortality remains 4 to 10% in children in industrialized countries and is even higher in the developing world [1]. The overall incidence of meningitis in developed countries is about 2 to 10 cases per 100 000 people per year. In neonates, the attack rate is about 400 per 100 000, in children between 1 month and 2 years of age the attack rate is 20 per 100 000 and in adults 1 to 2 per 100 000 [2]. The most common pathogens causing BM beyond the neonatal period worldwide are *Streptococcus pneumoniae* (SP, 53%) and *Neisseria meningitidis* (NM, 19%) [2,3]. Survivors of BM have a high risk to develop neurological sequelae, ranging from subtle learning and behavioral disorders in over 20% of cases, to deafness, paresis and severe encephalopathy in 10–15% [2,4,5].

Bacterial infections of the CNS are very often preceded by bacteremia [6]. The clinical course of BM depends on the causative microorganism, the mode of acquisition and the immunological response of the affected patient. An acquired pathogen may lead to bacterial colonization but may also result in meningitis with severe neurological sequelae. For example, genetically determined host–bacteria interactions determine the immune response in case of severe meningococcal disease (MD), defined as sepsis and/or meningitis [7,8]. For several infectious diseases it is known that genetic variation determines susceptibility to develop disease on acquisition of a certain pathogen [9]. Defects of the innate immune system, affecting host susceptibility, have been described in both pneumococcal and meningococcal infections within families [10]. Single-nucleotide polymorphisms (SNPs) in immune response genes have been shown to be involved in the susceptibility, severity and outcome of severe infections, including BM [11-13]. Several SNPs are associated with susceptibility to invasive pneumococcal disease (IPD) and invasive meningococcal disease (IMD) [10,14]. For SP, associations are described with genetic variation in genes involved in intracellular innate immune cell signaling and complement. For NM, convincing association with genetic variation has been found in cell-surface molecule genes, surfactant protein (SP) genes, complement genes and cytokine genes [10].

We found no papers that exclusively studied the role of SNPs in the development of BM. We recently published a paper on the role of Toll-like receptor (TLR) 9 SNPs affecting susceptibility to meningococcal meningitis (MM) [12].

Here, we summarize studies that describe associations with SNPs in large cohorts of patients with IPD and IMD, including those with BM. The proportion of BM patients in these cohorts was approximately 10%. For this review, we focus on the

essential steps in the development of BM: epithelial colonization and disruption, infection of the blood stream, crossing the endothelial blood–brain barrier (BBB) and finally infection of the CNS. We review how genetic variation is involved in pathogen acquisition and epithelial interactions, mechanisms that predispose for bloodstream infection and how genetic variation affects pathogen recognition and the subsequent inflammatory response, both in the bloodstream and inside the CNS.

In concordance with a recent systematic review on IPD and IMD, we will summarize studies on genes involved in adhesion to epithelial surfaces, pathogen recognition, complement and cytokines.

PATHOGENESIS OF BACTERIAL MENINGITIS

The sequential steps in the pathogenesis of BM from the pathogen's perspective are: (1) nasopharyngeal colonization with bacteria that have the potential to cause BM; (2) epithelial disruption by bacterial components, enabling these bacteria to enter the bloodstream where they replicate and cause bacteremia [15,16]; (3) pathogen specific passage of the BBB and bacterial multiplication inside the subarachnoidal space; (4) bacterial recognition inside the CNS by microglia and astrocytes and by non-neural structures in direct contact with the cerebrospinal fluid (CSF), such as dendritic cells and macrophages, all expressing pathogen recognition receptors (PRRs) including TLRs and nucleotide-binding oligomerization domain (NOD) proteins. PRR activation triggers an intracellular signaling cascade resulting in (5) the transcription of pro-inflammatory cytokines and chemokines inside the CNS. Cytokine induced increased permeability of the BBB and chemokine induced influx of inflammatory cells from the bloodstream into the CNS result in enhancement of the local inflammatory response inside the brain. The clinical consequence is brain edema, raised intracranial pressure, infarction and neuronal injury [6]. The ability of a host to sense CNS invasion by microbes and to respond appropriately to control the local infection is essential for killing microbes but the inflammatory response also results in the production of several cytotoxic mediators responsible for damage to healthy neuronal cells and thus for adverse disease outcome [6]. *Figure 1* summarizes the pathogenesis of BM for SP (a) and NM (b).

Next, we will discuss the various steps in the pathogenesis of BM in more detail and discuss the SNPs in genes involved in each of these steps.

Nasopharyngeal colonization and mucosal invasion

Nasopharyngeal colonization precedes invasion but identifiable disease occurs only in a small percentage of persons who are colonized. SP and NM are common inhabit-

ants of the nasopharyngeal cavity but are also capable of causing invasive disease such as meningitis [15,16]. Nasopharyngeal colonization of SP and NM is possible because of their ability to inactivate the host's local antibody defense by producing immunoglobulin A proteases, which inactivate neutralizing immunoglobulin A antibodies in the epithelial cells [2]. Conversion of asymptomatic colonization to invasive disease may be enhanced by local generation of inflammatory factors, as seen in the presence of viral infections [17].

Surfactant protein (Sp)-A and Sp-D are also implied in the first line of defense against nasopharyngeal and respiratory colonization [18,19]. Sp-A and Sp-D belong to a family of proteins called collectins. They act as pattern recognition molecules and activate phagocytosis and inflammation on binding of bacterial capsular sugars.

SP is a Gram-positive coccus and a prototypic extracellular bacterial pathogen. The external polysaccharide capsule determines the antigenic differences and > 90 serotypes have been described. The pneumococcal cell wall consists of peptidoglycan and lipoteichoic acid (LTA), which are of particular importance in SP virulence. The capsule prevents entrapment in the nasopharyngeal mucus and also inhibits effective opsonophagocytosis. Pneumococcal enzymes, such as neuraminidases (NanA and B), cleave glycoproteins and oligosaccharides and enhance colonization by decreasing the viscosity of the mucus [18,20]. Pneumococcal immunoglobulin A1 protease supports nasopharyngeal colonization by inactivating human secretory immunoglobulin A and promotes attachment to host cells [21]. Phosphorylcholine (ChoP) is an adhesin on the pneumococcal surface that mediates adherence to the receptor for platelet activating factor and activates host cell signaling [22]. The ability to invade the mucosa correlates with the presence of the polymeric immunoglobulin receptor on the human mucosal cell surface and choline-binding protein (CbpA) on the pneumococcus [23]. This binding facilitates transcytosis and enhances bacterial traversal of the mucosa. Another important property of the pneumococcus is autolysis, which is essential for pneumolysin release. Pneumolysin, a pore-forming toxin common for all SP serotypes, is thought to be a multi-effective virulence factor following pneumococcal infection. Its role in mucosal colonization and BM remains contradictory [24]. Pneumococcal colonization and mucosal invasion are summarized in *Figure 1a*.

Although variable, carriage of SP shows an increase before the age of 2 years and the peak incidence of colonization is 55% at the age of 3 years [15]. Colonization rates decline to < 10% in adults [22] and increase during respiratory infections to 22–45%, which might implicate greater adherence during (viral) infections [15]. Carriage is mostly asymptomatic, can be simultaneous and serial by multiple strains and lasts for a few weeks to a few months. Nasopharyngeal carriage shows quite similar

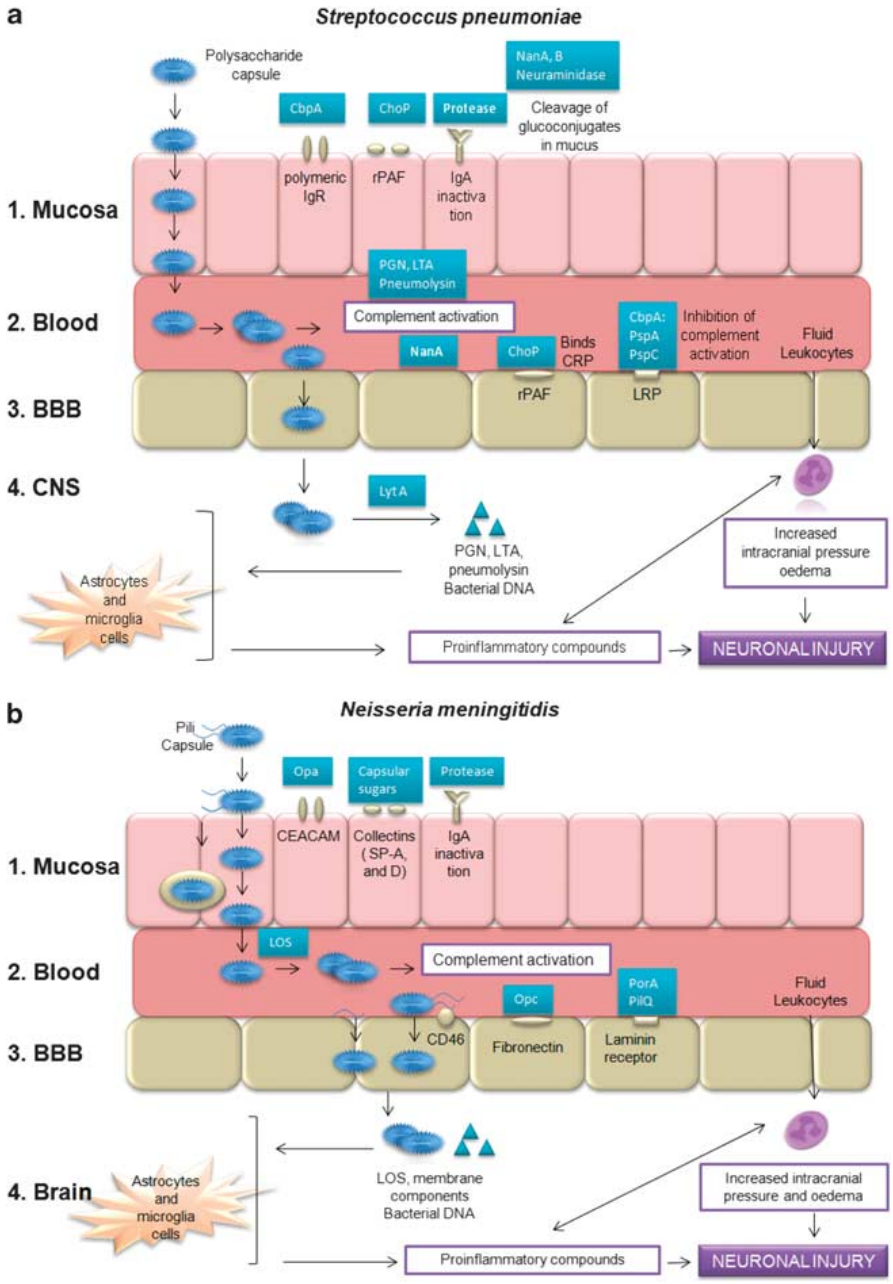

Figure 1 . Schematic representation of the sequential steps in the pathogenesis of pneumococcal and meningococcal meningitis

(a) Schematic representation of the sequential steps in the pathogenesis of pneumococcal meningitis.
(1) Colonization: the pneumococcal polysaccharide capsule prevents entrapment in the mucus and inhibits phagocytosis. Pneumococcal neuraminidases (NanA and B) cleave glycocproteins and oligosaccharides in mucus enhancing colonization by decreasing the viscosity. Pneumococcal immunoglobulin A (IgA)I protease inactivates local secretory IgA and further facilitates colonization. Phosphorylcholine (ChoP) on the pneumococcal surface binds to the receptor for platelet-activating factor (rPAF) and activates cell signaling. Mucosal invasion is facilitated by pneumococcal choline-binding proteins (CbpA) to the immunoglobulin receptor (IgR). SP then traverses the mucosa by transcytosis.
(2) Intravascular bacterial survival and multiplication. Pneumococcal ChoP binds to C-reactive protein (CRP) and enhances complement activation. Pneumococcal surface proteins PspA and PspC inhibit complement activation by interfering binding of SP to complement factor C3 and H, respectively.
(3) Attachment of BMECs by cholin binding surface proteins (Cbpa) to the laminin receptor protein (LRP) and, ChoP to rPAF followed by transcellular live bacterial traversal of the BBB.
(4) Bacterial invasion of the meninges and replication in the CNS.
LytA is responsible for autolysis and release of subcapsular components, recognized by immunocompetent cells that trigger cytokine and chemokine production leading to increased BBB permeability and pleocytosis, in turn enhancing the local inflammatory response with subsequent increased intracranial pressure and edema, ultimately resulting in neuronal injury
(b) Schematic representation of the sequential steps in the pathogenesis of MM.
(1) Colonization: the polysaccharide capsule enables attachment to nasopharyngeal mucosal cells of outer membrane proteins and pili with carcinoembryonic antigen cell adhesion molecules (CEACAMs). Capsular saccharides bind to collectins, and inactivate IgA by protease. Bacteria then cross the mucosa by transcytosis or probably through phagocytosis in a 'Trojan horse' manner.
(2) Intravascular bacterial survival and multiplication; lipooligosaccharide (LOS) contribute to a high-degree of bacteremia, and complement activation.
(3) Attachment of BMECs by bacterial pili to CD46, opacity-associated adhesion protein (Op) binding to fibronectin, and PorA and PilQ binding to laminin receptor protein (LRP). Attachment of these bacterial epitopes contribute to live bacterial transversal of the BBB by endoctyosis or transcytosis after disorganizing the cell polarity via binding to type IV pili.
(4) Bacterial invasion of the meninges and replication in the CNS triggering the production of cytokines and chemokines by immunocompetent cells leading to increased BBB permeability and pleocytosis, in turn enhancing the local inflammatory response with subsequent increased intracranial pressure and edema, ultimately resulting in neuronal injury.

serotype distributions in Europe and the United States. The serotypes *6B*, *14*, *19F* and *23F* are the most common causative types in BM [15].

NM is a Gram-negative bacterium. Most meningococci express a polysaccharide capsule, the basis of the serogroup typing system [25]. Virulence determinants include the polysaccharide capsule, outer membrane proteins including pili, porin A and B (PorA and B), opacity-associated adhesion protein (Op), iron sequestration mechanisms and endotoxin [26]. Adhesion to epithelial surfaces is a crucial step in meningococcal acquisition. NM attaches to the mucosa by pili. Human carcinogenic embryonic antigen cell adhesion molecules (CEACAMs), also known as CD66, are cell-surface molecules in nasopharyngeal epithelial cells and neutrophils that

interact with Op *type a* (Opa) of NM [27]. Upregulation of CEACAM expression in vitro leads to increased uptake of meningococci [28]. Besides transcytosis, [29,30] NM can cross the epithelium directly following damage to the monolayer integrity [29]. It has recently been suggested that pairing of Opa and CEACAMs results in cellular infiltration by bacteria, which are carried across epithelial and endothelial barriers inside phagocytic cells as a Trojan-horse [16,17]. Although the vasculature is considered the primary route to the brain, NM has recently shown to be able to pass directly from nasopharynx to the meninges via the olfactory nerve [31]. Meningococcal colonization and mucosal invasion are summarized in *Figure 1b*.

NM colonizes 8–25% of healthy individuals [26]. In approximately 25% of individuals, meningococcal acquisition in the upper respiratory tract results in prolonged carriage during several months. In 35% of individuals carriage is brief (days or weeks) and in the remaining 40% carriage is transient or infrequent [32]. Carriage is less frequent in children under the age of 10 (< 3%) than in adults (approximately 10%) and highest in adolescents (7–37%) [26]. Colonization is an important immunizing process, because bactericidal antibodies are acquired through meningococcal carriage. Otherwise, colonization is also a prerequisite for invasive disease [32]. Strains of NM can be divided in 13 serogroups; invasive meningococcal isolates most often express capsules of serogroups *A*, *B*, *C*, *Y* and sometimes *W-135* [26]. Epidemiological studies have revealed that only around 50% of colonizing meningococci express a capsule, while those of patients are almost always encapsulated [33].

Single nucleotide polymorphisms in genes involved in adhesion to epithelial surfaces

No SNPs have been reported in genes involved in adhesion to epithelial surfaces in relation to SP infection, in contrast to NM infections.

Genetic diversity in the CEACAM genes influences the affinity of epithelial cells to adhere to epitopes of the meningococcus. Dose-dependent associations of three CEACAM haplotypes with MD were observed. The effect of carrying these haplotypes is amplified in homozygous individuals. Two haplotypes (in CEACAM3 and CEACAM6) were protective while another haplotype in CEACAM6 was associated with a twofold increase in disease susceptibility [34].

A SNP resulting in the substitution of glutamine with lysine at residue 223 in the carbohydrate recognition domain of Sp-A2 increases susceptibility to MD, as well as the risk of death [18].

Table 1 summarizes the clinical relevant SNPs affecting epithelial adhesion, predisposing to MM.

Table 1. SNPs in genes affecting the susceptibility to develop BM in case invasive pneumococcal or meningococcal infections

Pneumococcal disaese								*Meningococcal disease*						
Gene	SNPs	Cases/controls	Effects	P	OR (95% CI)	Ethnic group	Refs	SNPs	Cases/ controles	Effects	P	OR (95% CI)	Ethnic group	Refs
Epithelial adhesion														
CEACAM3	_							Haplotype C	384/190	ProMD	NA	0.52 (0.35-0.75)	UK(white)	34
CEACAM6	_							Haplotype B	384/190	ProMD	NA	0.29 (0.14-0.61)	UK (white)	34
	_							Haplotype C	384/190	SuMD	NA	2.01 (1.13-3.6)	UK (white)	34
SP-A2	_							+6319 C>G (rs165234)	303/222	SuMD	0.016	6.7 (1.4-31.5)	UK	18
Complement														
MBL2	+154 C>T (rs5030737)	229/353	SuIPD	0.002	2.6 (1.4-4.8)	UK (white)	59	+154C>T (rs 5030737)	88/110	SuMD	<0.001	NA	Europe (white)	63
	+161 G>A (rs1800450)	140/250	SuPD	NS	2.4 (0.9-6.6)	Denmark (mixed)	60	+161 A>G (rs1800450)	194/272	SuMD	NA	6.5 (2.0-27.2)	England (mixed)	64
									72/110	SuMD	NA	4.5 (0.9-29.1)	England (white)	
	+170 G>A (rs1800451)	63/162	SuPD	NA	2.8 (0.2-1.8)	Belgium (white)	61	+170 A>G (rs1800451)	50/31	SuBM	<0.001	NA	Turkey	62
CFD	_							+638 T>G (rs34337649)	Case report	SuMM	_	_	The Netherlands	36

								+640 T>C (rs11558092)	Case report	SuMD	_	_	Turkey	66
CFH	_							-496 C>T (rs3753394)	157/147	SuMD	0.001	2.0 (1.3-3.2)	UK (white)	38
								rs10489456	1443/6079	SuMM	1.7×10^{-9}	0.67 (0.59-0.76)	Europe (mixed)	14
								rs11799395	1443/6079	SuMD	1.4×10^{-10}	0.66 (0.58-0.75)	Europe (mixed)	14
								rs742855	1443/6079	SuMD	2.5×10^{-10}	0.66 (0.58-0.75)	Europe (mixed)	14
								rs1065489	1443/6079	SuMD	2.2×10^{-11}	0.64 (0.56-0.73)	Europe(mixed)	14
								rs11582939	1443/6079	SuMD	3.7×10^{-10}	0.66 (0.58-0.75)	Europe (mixed)	14
Pathogen recognition														
IRAK4	5 SNPs Case reports		SuPD	_	_	_	82.83, 86, 87	_						
NEMO	6 SNPs Case reports		SuPD	_	_	_	83,88	_						
TLR4	+596 A>G (rs4986790) 85/409	ProPD	<0.05	0.3 (0.1-1)		Australia	91	+596 A>G	197/214	SuMD	NA	1.5 (0.9-2.7)	Europe (white)	101
	_							Rare coding variants	197/238	SuMD	0.02	8.2(NA)	UK (white)	95
								Rare coding variants	230/421	SuMD	2×10^{6}	24.0 (NA)	UK, The Netherlands US (white)	95

	CD14	-260 C>T (rs25691909) 85/409	SuPD	<0.05	1.7 (1-2.8)	Australia	91	-260 C>T	197	MortMD	0.021	3.3 (1-10)	Europe (white)	102
	0.006	6.6 (2-26)												
TLR9	_							+2848 G>A (rs352140)	380/392	ProMM	<0.01	0.6 (0.4-0.9)	The Netherlands (white)	12
TIRAP	+537 C>T (rs8177374)	36/199	ProPD	0.003	0.7 (0.4-1)	UK	92	_						
NFkBIA	-837 T>C (rs3138053)	226/766	ProPD	0.0003	0.6 (0.5-0.8)	UK (white)	93	_						
NFkBIA	-818 C>T (rs2233406)	260/762	ProPD	1×10^{5}	0.6 (0.4-0.7)	UK (white)	93	_						
NFkBIA	-2844 G>A (rs529948)	288/756	SuPD	0.001	1.4 (0.73-2.78)	UK (white)	93	_						
Cytokines														
IL-6	-174 G>C (rs 13447445)	100/50	ProEPD	0.04	0.26 (0.01-0.9)	Germany (Caucasian)	123							
MIF	Rs5844572	15PM/93PD	SuPM	0.02	3.3 (1.3-8.3)	Germany, USA	125							
IL1RN								VNTR 2/2	183/389	SuMD	0.003	NA	Ireland (white)	126
								+2018 CC (rs2234663)	285/481	SuMD	0.008	2.0 (1.1-3.4)	Central Europe (white)	129

Abbreviations: BM, bacterial meningitis; CD14, cluster of differentiation 14; CEACAM, carcinoantigen cell adhesion molecules; CF, complement factor D; CFH, complement factor H; CI, confidence interval; EPD, extrapulmonary pneumococcal disease; IL, interleukin; IL1RN, interleukin 1 receptor antagonist; IRAK, interleukin-1 receptor-associated kinase; IPD, invasive pneumococcal disease; MD, meningococcal disease; MBL, mannose-binding lectin: MIF, macrophage migration inhibitory factor: MM, meningococcal meningitis; Mort, mortality: NA, not available: NEMO, nulear factor kappa B essential modulator protein; NFkB, nuclear factor kappa B; OR, odds ratio; PD, pneumococcal disease; Pro, protective; SP-A2, surfact protein A2; SNP, single-nucleotide polymorphism; Su, susceptibility; TLR, Toll-like receptor; TIRAP, Toll IL-1 receptor (TIR)domain-containing adaptor protein; VNTR, variable number of tandem repeats.

Invasive disease and complement evasion

Disruption of colonized nasopharyngeal epithelium enables bacteria to enter the bloodstream where they can multiply, resulting in bacteremia, often a prerequisite for the development of meningitis. Intravascular invasion by traversing the endothelium is established in a pathogen-specific way.

The critical stimuli for the inflammatory response on invasion with SP are peptidoglycan, LTA and pneumolysin, 35 but not the polysaccharide capsule, which lacks inflammatory potential but inhibits phagocytosis and complement-mediated bactericidal activity [22]. A higher incidence of infections with encapsulated bacteria, especially meningococci, is observed in people with deficiencies in all three pathways (the classical, the alternative and the lectin-mediated pathway) of the complement system [36-39].

C-reactive protein binds specifically to ChoP of SP and next, interacts with complement component C1q to activate the classical pathway of complement [22]. Pneumococcal surface protein (Psp) A and PspC are involved in the inhibition of complement activation by interfering binding of SP with complement factor C3 (classical pathway) and factor H (alternative pathway) respectively [22].

Inside the CNS, complement proteins are important for the innate immune response but they are nearly absent under physiological conditions. However, their concentration increases during BM but will always remain below blood levels [40]. All classical and alternative complement components can be produced in the CNS [41]. The critical role of the complement system for innate immune responses in case of pneumococcal meningitis (PM) and MM is well illustrated in experimental meningitis studies and by case reports in people with specific mutations causing deficiencies in complement components, which will both be discussed here.

Rise of complement proteins seems to be essential for limiting pneumococcal outgrowth within the CNS. Tuomanen *et al.* [42] provided the first evidence for a functional role of the complement system in limiting PM. In rabbits depleted of C3, intracisternal inoculation of SP resulted in higher bacterial titers than in complement sufficient controls. Using mice, deficient in the complement components C1q, lacking the classical pathway, or deficient of C3, lacking all three pathways, Rupprecht *et al.* [40] concluded that the complement system limits PM via all three pathways, although it is unable to eradicate the pathogen. C3 deficiency led to diminished CNS inflammation (higher bacterial titers in the CNS, but reduced CSF leukocyte counts) and CNS complications, while survival was decreased, presumably due to worse systemic complications. Earlier, our group demonstrated classical complement pathway activation in PM in rats. C1 inhibitor reduced outgrowth of pneumococci in the brain and resulted in reduced clinical illness, a less pronounced inflammatory infiltrate around the meninges, and lower brain levels of pro-inflammatory cytokines

and chemokines [43]. Data on 10 children with PM demonstrated that the total hemolytic activity of both the classical and the alternate pathways were reduced in one patient, determination of individual complement components indicated predominant activation of the alternate pathways [44]. In four case reports, low C3 levels are associated with recurrent PM [45-48].

As NM is a strictly human pathogen, [49] our knowledge on the role of complement in MM comes from human data, mostly case reports. A study of 35 children with MM showed transiently reduced total hemolytic activity of the classical pathway in one case [44]. In another study, the functional activity of the classical and alternative pathways of the complement system and the levels of C3, C4, and factor B were determined in 10 children with MM. It seems that the alternative pathway is preferentially activated, probably due to the greater ability of endotoxin to activate this pathway in vivo [50]. Four case reports associated frequent episodes of MM with C5, C6 or C7 deficiency [51-54]. Single episodes of MM were also described in C7 deficiencies [55,56]. Two mutations causing deficiency of C8 were diagnosed in an adult following three episodes of MM [57]. A complement factor D (CFD) deficiency was found in one case of MM [36].

Single nucleotide polymorphisms in complement genes

Three SNPs in the mannose-binding lectin (MBL)2 gene result in three variant structural alleles (protein B, C and D), which are associated with decreased MBL concentrations [58]. Roy *et al.* [59] reported that individuals with homozygote mutants for MBL codon variants are at increased risk of IPD. In contrast, Kronborg *et al.* [60] found only a non-significant increased risk between the three MBL structural codon variants and IPD. Moens *et al.* [61] also described a non-significant increased risk of severe pneumococcal infection but combined with the data of Kronborg they described a small but significantly increased risk to develop IPD. Importantly, pooling the data of these three studies revealed a significant increased prevalence of the mutant alleles in the patient groups. A small Turkish study found that the +154 C>T SNP in the MBL gene may have a role in susceptibly to purulent BM in children (31 patients with CSF findings suggestive for BM, 4 positive cultures: 2 SP, 1 NM, 1 *Staphylococcus aureus*) [62].

The role of MBL SNPs in NM infection is assessed in two studies [63,64]. Variant alleles were more frequent in patients with MD as compared with healthy controls. Three SNPs in the MBL2 gene result in three variant structural alleles and are associated with low serum MBL levels as compared with the wild types but was not associated with nosocomial sepsis or infections with NM or SP in neonates [65]. Another study suggests that *MBL2* variants are significantly associated with susceptibility to childhood IMD in an age-dependent manner. The overall frequency of this genotype

was significantly higher in patients infected with NM than in controls (31.8 vs 8.2%). For children under the age of 1 year this association was even stronger [63].

Factor D is an essential factor for C3 convertase complex formation in the alternative complement pathway. Factor D deficiency was found in several patients with MD and factor D-deficient serum appeared to be hyporesponsive to NM in vitro. In two reports, SNPs associated with meningococcal infection are found in the CFD gene [36,66].

Factor H is a complement-regulatory protein in plasma and acts as a co-factor for factor I in the classical and alternative pathways. The complement factor H (*CFH*) -496C>T SNP affects its activity: the -496 C/C genotype was associated with higher concentrations of factor H and reduced bactericidal activity against NM. The -496 C/C genotype was significantly associated with meningococcal infection [38]. Recently, Davila *et al*. [14] performed a genome-wide association study and found in a United Kingdom (UK) population SNPs in *CFH* associated with susceptibility to meningococcal disease. They confirmed these results in two independent cohorts from Western Europe and Southern Europe. They conclude that host genetic variation in these regulators of complement activation play a role in the occurrence of invasive disease upon pathogen acquisition.

Table 1 summarizes the clinical relevant SNPs in complement genes, predisposing for BM.

Crossing the blood-brain barrier and bacterial recognition inside the central nervous system

In order to enter the CNS, bacteria must attach to and finally cross the BBB, a structural and functional barrier that is formed by the brain microvascular endothelial cells (BMECs). SP and NM have shown to be able to invade the BBB within a membrane-bound vacuole [67,68] as live bacteria [69]. This process is enhanced by cytokine release, leading to an increased BBB permeability during meningitis [6]. Pneumococci use endothelial cells that express the platelet-activating factor and cross the endothelial layer via the platelet-activating factor-recycling pathway [68,70]. The binding of pneumococcal ChoP to the platelet-activating factor enables this pathway. Pneumococcal CbpA, via the laminin receptor protein [71] and NanA [72] has also shown to promote the passage of rodent and human BMECs. Using a microvascular endothelial cell culture model it was shown that pneumococci, expressing pneumolysin, were able to breach the endothelial cells, whereas mutant pneumococci, deficient in pneumolysin, were unable to penetrate the cell barrier [24]. Hyaluronidase facilitates pneumococcal invasion by degrading connective tissue. Pneumococcal strains with higher hyaluronidase activity breach the BBB and disseminate more effectively [73]. Strains causing meningitis have significantly higher

hyaluronidase activity than strains causing otitis media. SP has been shown to be nearly twice as efficient at invading cerebral compared with peripheral endothelium. Low polysaccharide content of the capsule and high amount of CbpA and LTA in SP increases the ability of invasion of the BBB [74].

Meningococci traverse the endothelium of the BBB by transcytosis via endocytosis in membrane-bound vacuoles, 67 and recent data also suggest paracelllar penetration [75]. NM, via type IV pili-mediated adhesion to human BMECs, can disorganize cell–cell junctions and polarity, opening the paracellular route to invade the meninges [75]. NM has been shown to use several BBB receptors in order to invade the CNS [69,76]. NM pili bind to CD46, a human cell surface protein, richly expressed at BMECs [77], promoting passage of the BBB in human CD46 transgenic mice [78]. NM contains Opc, binding to endothelial fibronectin and anchoring NM to the integrin a5b1 receptor on BMECs [69]. PorA and PilQ bind to laminin receptor protein and contribute to live traversal of the BBB by transcytosis [71] (*Figure 1b*).

After bacteria have crossed the BBB they are recognized inside the CNS by PRRs on residing cells. TLRs are a major group of PRRs and are also expressed by cells in the CNS, especially on microglia and astrocytes, endogenous cells in the CNS and key players in the immune response in this compartment [79]. Microglia are myeloid derived cells, able to recruit and activate peripheral immune cells, such as monocytes and T-lymphocytes, leading to cellular influx in the CSF. These recruited cells also express TLRs and are able to produce pro-inflammatory cytokines as signaling mediators. Studies in mice with deficiencies in TLRs demonstrated that TLR activation is a key event in meningeal inflammation and for meningitis-associated tissue damage [80].

Many studies showed the importance of TLR2 and TLR4 in PM. TLR activation leads to Myeloid differentiation primary response gene (MyD)-88-dependent production of IL-1 family cytokines via caspase 1, which in turn forms a positive feedback loop that boosts the MyD88-dependent production of inflammatory mediators [80]. NM strains showed activation of TLR2, TLR4 and *TLR9* by genetic complementation of *HEK293* cells [81]. When bacterial components inside the CNS trigger the inflammatory cascade in the host, various cells like macrophages, (monocyte derived) microglia, meningeal and endothelial cells, produce pro-inflammatory cytokines like tumor necrosis factor (TNF) and interleukins (ILs) on microbial invasion of the CNS. Cytokines and chemokines (proteins inducing migration of inflammatory cells to the site of infection) lead to influx of neutrophils from the bloodstream into the CNS [6]. This enhances the local inflammatory response, further increases the permeability of the BBB and leads to increased intracranial pressure and edema, ultimately resulting in neuronal injury.

Altogether, the CNS is a compartment with suppressed but inducible immune reactivity.

Bacterial recognition and the local inflammatory response cascade are summarized in more detail in *Figures 2a* and *b* for SP and NM, respectively.

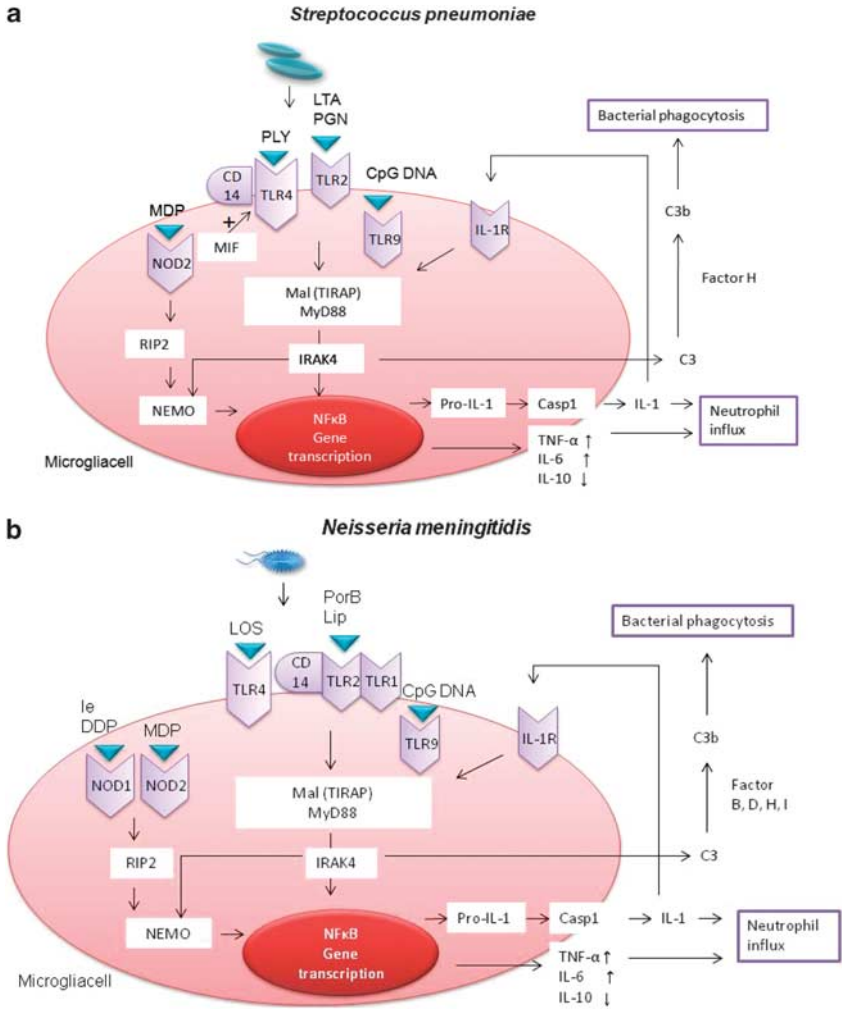

Figure 2. Sequential steps in the local innate immune response inside the CNS in case of pneumococcal (a) and meningococcal (b) meningitis

(a) Sequential steps in the local innate immune respons inside the CNS in pneumococcal meningitis SP is able to activate antigen-presenting cells such as microglia and astrocytes through TLR2, TLR4, *TLR9*, and nucleotide oligomerizatioa domain protein 2 (NOD2), TLR2 recognizes LTA and pneumococcal peptidoglycan (PGN), TLR4 and its co-receptor CD14 recognize pneumolysin (PLY). TLR4 expression is upregulated by macrophage migration inhibitory factor (MIF). *TLR9* and NOD2 are intracellular receptors recognizing pneumococcal DNA and internalized muramyl dipeptide, respectively. NOD2 induces RIP2-dependent signaling. Activation of other receptors induces a signaling cascade via Mal (TIRAP) and MyD88 resulting in NFkB activation in the nucleus and subsequent transcription of proinflammatory cytokine genes, and in induction of complements factor 3 (C3) expression via IRAK4. C3b enhances phagocytosis, by microglia but also contributes to the expression of IL-1 family cytokines, promoting neutrophil influx into the CSF.

(b) Sequential steps in the local innate immune response inside the CNS in MM. MM is able to activate antigen-presenting cells such as microglia and astrocytes. TLR2 recognize porin B (PorB) and Lip with co-receptors TLR1 and CD14. TLR4 recognizes lipooligosaccharide (LOS). *TLR9*, nucleotide oligomerization domain protein (NOD)I and NOD2 are intracellular receptors recognizing meningococcal DNA, internalized muramyl dipeptide, and dipeptide meso-diaminopimelic acid (iE-DDP), respectively. NODs induce RIP2-dependent signaling. Activation of TLR 1, 2 , 4 and 9 induces a signaling cascade via Mal (TIRAP) and MyD88 resulting in NFkB activation in the nucleus and subsequent transcription of pro- and anti-inflammatory cytokine genes, and induction C3 expression via IRAK4. C3b contributes to phagocytosis of bacteria by but also contributes to the expression of IL-1 family cytokines, promoting neutrophil influx into the CSF.
Figure adapted from Koedel et al.[80]

Single nucleotide polymorphisms in genes involved in pathogen recognition

For severe infections with SP, defects in innate immunity were first discovered by studies on extreme phenotypes, such as recurrent or familial infections. For example, studies in family members with recurrent IPD discovered SNPs in the IL-1 receptor-associated kinase 4 (IRAK4) gene and the nuclear factor kappa B (NFkB) essential modulator protein (NEMO) gene [82,83]. MyD88 has an important role in immunity against PM as shown by MyD88 KO mice, which displayed diminished inflammatory host response in the CNS, as evidenced by reduced CSF pleocytosis, and expression of cytokines, chemokines and complement factors, but also a worsening of disease that seemed to be attributable to severe bacteremia [84]. Human studies on MyD88, show nine children with MyD88 deficiency suffering from recurrent pyogenic infections including IPD, while they were otherwise healthy with normal resistance to microbes [85].

IRAK4 is an important enzyme in TLR-mediated pathogen recognition and its downstream signaling to activate the inflammatory response. NEMO is a regulatory protein downstream of TLR4 and NOD proteins. SNPs in either of these genes were associated with impaired pathogen recognition and in vitro unresponsiveness to LPS and clinically associated with recurrent IPD [83,86-88].

Another approach to study SNPs is by selection of candidate genes on the basis animal models or systems biology and compare them in a case–control design. We summarize polymorphism studies on TLR2, TLR4, CD14, Toll IL-1 receptor domain-containing adaptor protein (TIRAP/Mal) and NFkB inhibitor genes.

SP generally has the potential to activate immune cells through TLR1/2, TLR4, *TLR9*, NOD2, and presumably as yet unidentified PRRs and some of these PRRs appear to work synergistically [89]. Moens *et al.* [90] did not find an association between *TLR2* +1736 G>A, +1892 C>A and +2257 G>A and *TLR4* +896 A>G (Asp299Gly) SNPs and infection with SP. However, based on murine data they hypothesized that TLR2 and TLR4 were associated with increased susceptibility to develop SP infections. It should, however, be mentioned that neither the controls, nor the patients in their study contained homozygous mutant individuals. Yuan *et al.* [91] compared SNPs in pathogen recognition genes between children with IPD and healthy blood donors and concluded that genetic variability in the *TLR4* +896 A>G and *CD14* –260 C>T genes is associated with an increased risk of developing invasive disease in patients who are infected with SP. They found a lower incidence of the *TLR4* +896 A>G polymorphism and a higher incidence of the *CD14* –260 SNP in patients. No differences were found in incidences of the *TLR2* +2257 G>A polymorphism.

TIRAP is an essential adaptor protein for the inflammatory signaling cascade downstream of TLR2 and TLR4. A recently discovered SNP in *TIRAP* that changes serine to a leucine residue on position 180 (Ser180-Leu; C539T) impairs TLR2-mediated NFkB signaling in reconstitution experiments [92]. Moreover, the 180L variant was less able to bind TLR2 in comparison with the 180S variant. The heterozygous variant was associated with protection to pneumococcal bacteremia [92].

TLR-mediated pathogen recognition induces intracellular signaling leading to the activation of NFkB in the nucleus and subsequent transcription of pro-inflammatory cytokine genes. NFkB inhibitors, coded in NFKBIA, NFKBIB and NFKBIE inhibit this activation. Chapman *et al.* [93] found two *NFKBIA* SNPs to be associated with protection against infection with SP and a *NFKBIE* SNP associated with increased susceptibility. *NFKBIB* SNPs were not associated with susceptibility to severe PD.

Meningococcal LOS interacts with TLR4, while Neisserial DNA activates *TLR9*. TLR2 recognizes outer membrane proteins porin B and Lip with its co-receptors TLR1 and CD14. In addition to muramyl dipeptide, the cell wall of NM contains dipeptide meso-diaminopimelic acid, having potentials activating NOD2 and NOD1, respectively [80]. Candidate gene studies on innate immunity genes found relevant polymorphisms in TLR2 and TLR4 genes.

One study on *CD14* -159 C>T polymorphisms in 185 surviving IMD patients, did not find an association with susceptibility, neither for *TLR4* Asp299Gly SNPs [94].

One study in patients with severe infection with NM studied TLR2 and suggested a protective effect of the *TLR2* +1892 C>A polymorphism but only a non-significant higher frequency of this variant was found in control patients [95].

C3H/HeJ mice have an intrinsic point mutation in TLR4 that abolishes LPS responses. These mice are hyporesponsive to gram-negative infections [96]. Arbour *et al.* [97] demonstrated the importance of TLR4 by finding an association of two *TLR4* SNPs with hyporesponsiveness to inhaled LPS in alveolar macrophages and epithelial cells. These two important SNPs of the TLR4 gene (+896 A>G and +1196 C>T) have been intensively studied. *TLR4* +896 A>G was determined in a study on meningitis exclusively, but there was no association with the susceptibility to group A meningococcus during epidemics in the Gambia [98]. Most studies have demonstrated that these two *TLR4* polymorphisms confer an increased risk to infections, but this finding could not be observed consistently [99]. Agnese *et al.* [100] did find a significantly higher incidence of Gram-negative infection among patients with mutations in *TLR4* compared with the wild-type population. Several genetic studies have examined whether there is an association between *TLR4* +896 A>G and bacterial infections. Faber *et al.* [101,102] showed age-dependent associations with susceptibility to IMD in children up to 1-year old and mortality in these children up to 2 years old. Smirnova *et al.* [95] investigated rare *TLR4* SNPs that are highly variable in humans and animals. They found that *TLR4* polymorphisms were obviously more present in patients with severe meningococcal infections. However, the exact functional consequences of these SNPs remain unknown.

The promoter region of the CD14 gene contains a SNP at position -260 C>T that affects the binding of transcription factors. The result of a SNP in the CD14 gene is an elevated expression of CD14 in membrane form on monocytes and neutrophils and in a soluble form in serum (sCD14) [103]. Genetically determined variation in CD14 serum levels may have functional consequences. Recently, we detected a protective SNP in *TLR9* +2848 leading to a decreased susceptibility to MM [12].

Table 1 summarizes the clinical relevant SNPs in pathogen recognition genes affecting the susceptibility to develop BM.

Local inflammatory response inside the central nervous system: cytokines and chemokines

Once inside the CNS, bacteria multiply in the SAS and are recognized by innate immune receptors on microglia and astrocytes. The activation of these receptors triggers an intracellular signaling cascade resulting in the nuclear transcription of pro-inflammatory cytokines and chemokines. These small messenger proteins then enhance increased permeability of the BBB (cytokines) and influx of inflammatory cells, mainly granulocytes, from the bloodstream into the CNS (chemokines) [104,105].

Pneumococcal DNA loads are associated with high plasma cytokine concentrations. In children with PM, median CSF cytokine concentrations were significantly higher than plasma cytokine concentrations [106].

TNF-α is produced by a wide variety of cells, including microglial cells, in response to pneumococcal cell wall in vitro. [107,108] In rats, intracisternal treatment with TNF-α alone does only cause minimal inflammatory changes, whereas combined with SP components it resulted in a maximal inflammatory response with high intracranial pressure and brain edema [109]. TNF-α has also been suggested to be involved in the breakage of the BBB during SP bacteremia in mice [110].

IL-1b is one of the early key inflammation-initiating cytokines during PM. CSF bacterial loads in children with PM were associated with CSF IL-1b [106]. Levels of IL-1a and IL-1b mRNA and protein levels are upregulated in the brains of mice with PM [111]. The absence of an intact IL-1 signal in *IL1R* -/- mice was associated with a higher susceptibility to PM, impaired bacterial clearance, decreased brain cytokine and higher and earlier mortality [111]. Klein *et al.* [112] intracisternally infected mice with PM and observed markedly elevated levels of IL-1b.

IL-6 is produced by microglial cells in response to SP [108] and is elevated in CSF during PM [113]. IL-6 enhances immune responses and might have a role in the disruption of the BBB but also seems to have anti-inflammatory effects in PM [114]. Comparing wild-type mice with *IL6* -/- mice, Paul *et al.* [114] concluded that IL-6 acts as an anti-inflammatory cytokine by suppressing the migration of leukocytes into the SAS but has a major role in the increase in vascular permeability, causing brain edema and increase in intracranial pressure. TNF-α and IL-1b levels in brain tissue of infected *IL-6* -/- mice were increased compared with infected WT controls.

IL-8 has an important role in the recruitment of leukocytes during PM. In rabbits infected with SP, intravenous, but not intracisternal treatment with anti-IL-8 attenuated pleocytosis significantly [115].

Microglial cells produce IL-12 in response to SP [108]. This induces the production of interferon (IFN)-γ with TNF-α as a co-stimulator.

IL-18 is upregulated during PM in mice and contributes to an unfavorable inflammatory response during meningitis. IL-18 does not seem to affect susceptibility to PM, yet *IL-18* -/- mice showed a suppressed inflammatory response and a prolonged survival, as reflected by lower concentrations of cytokines in brain tissue and a less profound inflammatory infiltrate around the meninges [116].

A deletion of the TGF-b receptor II on leukocytes is found to enhance recruitment of neutrophils to the site of infection and to promote bacterial clearance in mice with PM. Moreover, this improved immunity was associated with an almost complete prevention of meningitis induced vasculitis and endogenous TGF-b suppressed the innate immune response [117].

IFN-γ is produced by microglia during PM. IFN-γ presence during PM modulated the patterns of LPS induced cytokine release in a dose-dependent, potent and complex manner. Although amounts of TNF-α and IL-6 remained nearly unchanged, IFN-γ enhanced the production of IL-12 [118].

In vitro studies show that leukocytes stimulated by outer membrane vesicles of NM produce TNF-α, IL-1b and IL-8, which was enhanced in the presence of IFN-γ [119].

TNF-α and its soluble receptors are also intrathecally produced in case of MM [120]. IL-1b and IL-1 receptor antagonist (IL-1Ra) are increased in CSF of MM patients, as is the level of IL-1 soluble receptor type II (IL-1sRII) in CSF. The pattern in plasma is different, indicating that the inflammatory response is differentially regulated [121]. IL-8 levels are higher in CSF of MM patients compared with controls [122]. IL-10 inhibits TNF-α, IL-1b and IL-8 production triggered by NM [119]. Mogensen *et al.* [81] observed that different strains of NM differed in their ability to induce cytokine expression.

Single nucleotide polymorphisms in cytokine genes

After NFkB is activated on bacterial recognition, proinflammatory cytokines are released (IL-1, IL-6, TNF-α) followed by anti-inflammatory cytokines (IL-10 and soluble cytokine agonists such as IL-1 receptor antagonist (IL1RA) and soluble TNF receptors). *IL6* –174 [123] *IL10* –1082, *TNFA* -308 and lymphotoxin a (*LTA*) +252 [124] SNPs were not associated with susceptibility to pneumococcal infection, but *IL6* GG carriers were less likely to develop extrapulmonary infection including meningitis [123]. An association was found between a high-expression macrophage migration inhibitory factor (*MIF*) -794 CATT allele and susceptibility to PM [125]. MIF upregulates TLR4 expression by macrophages.

Several studies on the role of cytokine gene SNPs in meningococcal infection have been performed, especially on IL1 genes. Balding *et al.* [126] associated *IL1RA* variable number of tandem repeats SNPs with susceptibility to MD (including one-third meningitis cases), but studies by Carrol *et al.* [127] and a meta-analysis of Brouwer *et al.* [10] did not confirm this relation. Read *et al.* [128] did not find an association of two *IL1* SNPs with susceptibility to MD, but patients carrying the common allele at *IL1B* –511 were more likely to survive and significantly less likely to survive if they also carried the rare allele at the IL-1 receptor antagonist gene *IL1RN* +2018. In another study, this SNP was also associated with susceptibility and mortality of MD, [129] while no association was found in five other SNPs in *IL1A* and *IL1B*. Severity and mortality of MD was associated with *IL6* -174 G/G and *IL10* -1082 A/A, but not with *LTA* +252, *TNF* -308, *IL10* -592, or *IL1B* SNPs [126].

Significant associations of cytokine genes with susceptibility to meningococcal infections are summarized in *Table 1*.

DISCUSSION

Only for the *TLR9* +2848 and *MIF* –794 SNPs, specific associations were found with meningitis [12,125]. For IPD, the strongest relation with genetic variation was found in genes involved in innate immune cell signaling and in complement genes. TLR2 and TLR4 are important cell surface receptors and animal data have shown their subtle role in the response to PM: deficiency leads to reduced bacterial clearing from the CNS [130,131]. The recognition by TLR4 of pneumolysin in the pneumococcal cell wall is crucial in mounting this response [132]. Although *TLR2* and *TLR4* SNPs have been shown to be associated with human diseases such as tuberculosis, lues and Lyme's disease, [133] no association was found between *TLR2* and *TLR4* SNPs and IPD in a cohort of severely ill patients where SP was cultured from blood, CSF or joint fluid [90]. Their cohort did not include any homozygous mutants for *TLR2*, which might have shown larger differences but they concluded that variations in TLR genotype is probably a minor cause of increased susceptibility to IPD. Homozygosity of the *TIRAP* Ser180Leu allele, shown to be associated with IPD, leads to defect MyD88-dependent signaling downstream of TLR2 and TLR4 [92]. Heterozygosity, however, protects against IPD because this leads to attenuated signaling but reduced NFkB activation. It seems that there is a delicate balance between pathogen recognition, inflammation and bacterial clearance: no recognition fails to clear a pathogen but too much inflammation damages the host itself [134]. This observation is supported by the protective role described of the *NFKBIA* and *NFKBIE* SNPs and IPD [93].

Strong relations were described for susceptibility of severe meningococcal infections and genetic variation in cell-surface molecule genes, BM genes and cytokine genes. Cell surface proteins in the human nasopharyngealepithelium (CEACAMs) adhere to meningococcal outer membrane proteins and are important in pathogen acquisition. Genetic diversity of these proteins was described to be associated with susceptibility to MD [34]. As nasopharyngeal pathogen acquisition is an important step in the pathogenesis of BM, we extrapolate this result as important genetic determinant for susceptibility to meningitis as well.

The complement system is important in early pathogen recognition and uses opsonization to clear microbes. MBL, a protein belonging to the collectin group, binds to MBL serine proteases, which activate the complement cascade and opsonize bacteria by means of surface oligosaccharides [59]. MBL deficiency, caused by several SNPs, leads to reduced opsonization in early infection, leading to longer initial survival of SP, thus enhancing the possibility of invasion and subsequent BM. MBL deficiency is not associated with susceptibility for meningococcal infection. CFH is responsible for downregulation of complement activation and polymorphisms in *CFH* are indepen-

dently associated with MD. Individuals with the C496T CC genotype have increased levels of CFH and have reduced bactericidal activity against meningococci, [38] thus predisposing for MM.

CONCLUSIONS

All together we were able to summarize the literature on SNPs that affect the susceptibility to IPD and IMD. Taken into account the several pathophysiological steps to develop BM we focused on SNPs that very likely predispose to the development of BM by these microorganisms. We advocate that multidisciplinary efforts are needed in order to reveal the exact role of host genetic factors in severe infections including meningitis, which will require large numbers of patients and controls, with the ultimate goal to invent better effective treatment and prevention strategies for severe infections.

REFERENCES

1. Chavez-Bueno S, McCracken Jr GH. Bacterial meningitis in children. Pediatr Clin North Am 2005; 52: 795–810, vii.
2. Mace SE. Acute bacterial meningitis. Emerg Med Clin North Am 2008; 26: 281–317, viii.
3. van der Ende A, Spanjaard L. Bacterial meningitis in the Netherlands, annual report 2009, Netherlands reference laboratory for bacterial meningtis (AMC/RIVM). Amsterdam; 2009. Report No.: 2009.
4. Koomen I, Grobbee DE, Roord JJ, Donders R, Jennekens-Schinkel A, van Furth AM. Hearing loss at school age in survivors of bacterial meningitis: assessment, incidence, and prediction. Pediatrics 2003; 112: 1049–1053.
5. Koomen I, Raat H, Jennekens-Schinkel A, Grobbee DE, Roord JJ, van Furth AM. Academic and behavioral limitations and health-related quality of life in school-age survivors of bacterial meningitis. Qual Life Res 2005; 14: 1563–1572.
6. Kim KS. Pathogenesis of bacterial meningitis: from bacteraemia to neuronal injury. Nat Rev Neurosci 2003; 4: 376–385.
7. Brouwer MC, Read RC, van de Beek D. Host genetics and outcome in meningococcal disease: a systematic review and meta-analysis. Lancet Infect Dis 2010; 10: 262–274.
8. Haralambous E, Weiss HA, Radalowicz A, Hibberd ML, Booy R, Levin M. Sibling familial risk ratio of meningococcal disease in UK Caucasians. Epidemiol Infect 2003; 130: 413–418.
9. Schroder NW, Schumann RR. Single nucleotide polymorphisms of Toll-like receptors and susceptibility to infectious disease. Lancet Infect Dis 2005; 5: 156–164.
10. Brouwer MC, de Gans J, Heckenberg SG, Zwinderman AH, van der Poll T, van de Beek D. Host genetic susceptibility to pneumococcal and meningococcal disease: a systematic review and meta-analysis. Lancet Infect Dis 2009; 9: 31–44.
11. Netea MG, Van der Meer JW. Immunodeficiency and genetic defects of pattern-recognition receptors. N Engl J Med 2011; 364: 60–70.
12. Sanders MS, van Well GT, Ouburg S, Lundberg PS, van Furth AM, Morre SA. Single nucleotide polymorphisms in *TLR9* are highly associated with susceptibility to bacterial meningitis in children. Clin Infect Dis 2011; 52: 475–480.
13. Texereau J, Chiche JD, Taylor W, Choukroun G, Comba B, Mira JP. The importance of Toll-like receptor 2 polymorphisms in severe infections. Clin Infect Dis 2005; 41 (Suppl 7): S408–S415.
14. Davila S, Wright VJ, Khor CC, Sim KS, Binder A, Breunis W *et al.* Genome-wide association study identifies variants in the CFH region associated with host susceptibility to meningococcal disease. Nature Genetics 2010; 42: 772–779.
15. Bogaert D, de Groot R, Hermans PW. *Streptococcus pneumoniae* colonisation: the key to pneumococcal disease. Lancet Infect Dis 2004; 4: 144–154.
16. Carbonnelle E, Hill DJ, Morand P, Griffiths NJ, Bourdoulous S, Murillo I *et al.* Meningococcal interactions with the host. Vaccine 2009; 27 (Suppl 2): B78–B89.
17. Virji M. Pathogenic neisseriae: surface modulation, pathogenesis and infection control. Nat Rev Microbiol 2009; 7: 274–286.
18. Jack DL, Cole J, Naylor SC, Borrow R, Kaczmarski EB, Klein NJ *et al.* Genetic polymorphism of the binding domain of surfactant protein-A2 increases susceptibility to meningococcal disease. Clin Infect Dis 2006; 43: 1426–1433.
19. Crouch E, Wright JR. Surfactant proteins a and d and pulmonary host defense. Annu Rev Physiol 2001; 63: 521–554.

20. Manco S, Hernon F, Yesilkaya H, Paton JC, Andrew PW, Kadioglu A. Pneumococcal neuraminidases A and B both have essential roles during infection of the respiratory tract and sepsis. Infect Immun 2006; 74: 4014–4020.
21. Weiser JN, Bae D, Fasching C, Scamurra RW, Ratner AJ, Janoff EN. Antibody-enhanced pneumococcal adherence requires IgA1 protease. Proc Natl Acad Sci USA 2003; 100: 4215–4220.
22. Kadioglu A, Weiser JN, Paton JC, Andrew PW. The role of *Streptococcus pneumoniae* virulence factors in host respiratory colonization and disease. Nat Rev Microbiol 2008; 6: 288–301.
23. Zhang JR, Mostov KE, Lamm ME, Nanno M, Shimida S, Ohwaki M *et al*. The polymeric immunoglobulin receptor translocates pneumococci across human nasopharyngeal epithelial cells. Cell 2000; 102: 827–837.
24. Hirst RA, Kadioglu A, O'Callaghan C, Andrew PW. The role of pneumolysin in pneumococcal pneumonia and meningitis. Clin Exp Immunol 2004; 138: 195–201.
25. Stephens DS. Biology and pathogenesis of the evolutionarily successful, obligate human bacterium *Neisseria meningitidis*. Vaccine 2009; 27 (Suppl 2): B71–B77.
26. Stephens DS, Greenwood B, Brandtzaeg P. Epidemic meningitis, meningococcaemia, and *Neisseria meningitidis*. Lancet 2007; 369: 2196–2210.
27. Virji M, Makepeace K, Ferguson DJ, Watt SM. Carcinoembryonic antigens (CD66) on epithelial cells and neutrophils are receptors for Opa proteins of pathogenic neisseriae. Mol Microbiol 1996; 22: 941–950.
28. Bradley CJ, Griffiths NJ, Rowe HA, Heyderman RS, Virji M. Critical determinants of the interactions of capsule-expressing *Neisseria meningitidis* with host cells: the role of receptor density in increased cellular targeting via the outer membrane Opa proteins. Cell Microbiol 2005; 7: 1490–1503.
29. Hill DJ, Griffiths NJ, Borodina E, Virji M. Cellular and molecular biology of *Neisseria meningitidis* colonization and invasive disease. Clin Sci (Lond) 2010; 118: 547–564.
30. Sim RJ, Harrison MM, Moxon ER, Tang CM. Underestimation of meningococci in tonsillar tissue by nasopharyngeal swabbing. Lancet 2000; 356: 1653–1654.
31. Sjolinder H, Jonsson AB. Olfactory nerve-A novel invasion route of *Neisseria meningitidis* to reach the meninges. PLoS One 2010; 5: e14034.
32. Tzeng YL, Stephens DS. Epidemiology and pathogenesis of *Neisseria meningitidis*. Microbes Infect 2000; 2: 687–700.
33. Claus H, Maiden MC, Maag R, Frosch M, Vogel U. Many carried meningococci lack the genes required for capsule synthesis and transport. Microbiology 2002; 148 (Part 6): 1813–1819.
34. Callaghan MJ, Rockett K, Banner C, Haralambous E, Betts H, Faust S *et al*. Haplotypic diversity in human CEACAM genes: effects on susceptibility to meningococcal disease. Genes Immun 2008; 9: 30–37.
35. Hirst RA, Gosai B, Rutman A, Guerin CJ, Nicotera P, Andrew PW *et al*. *Streptococcus pneumoniae* deficient in pneumolysin or autolysin has reduced virulence in meningitis. J Infect Dis 2008; 197: 744–751.
36. Biesma DH, Hannema AJ, van Velzen-Blad H, Mulder L, van ZR, Kluijt I *et al*. A family with complement factor D deficiency. J Clin Invest 2001; 108: 233–240.
37. Fremeaux-Bacchi V, Le CA, Blouin J, Kazatchkine MD, Weiss L. Partial properdin deficiency revealed by a septicemia caused by *Neisseria meningitidis*. Presse Med 1995; 24: 1305–1307.
38. Haralambous E, Dolly SO, Hibberd ML, Litt DJ, Udalova IA, O'dwyer C *et al*. Factor H, a regulator of complement activity, is a major determinant of meningococcal disease susceptibility in UK Caucasian patients. Scand J Infect Dis 2006; 38: 764–771.

39. Schneider MC, Exley RM, Ram S, Sim RB, Tang CM. Interactions between *Neisseria meningitidis* and the complement system. Trends Microbiol 2007; 15: 233–240.
40. Rupprecht TA, Angele B, Klein M, Heesemann J, Pfister HW, Botto M *et al.* Complement C1q and C3 are critical for the innate immune response to *Streptococcus pneumoniae* in the central nervous system. J Immunol 2007; 178: 1861–1869.
41. Barnum SR. Complement biosynthesis in the central nervous system. Crit Rev Oral Biol Med 1995; 6: 132–146.
42. Tuomanen E, Hengstler B, Zak O, Tomasz A. The role of complement in inflammation during experimental pneumococcal meningitis. Microb Pathog 1986; 1: 15–32.
43. Zwijnenburg PJ, van der Poll T, Florquin S, Polfliet MM, van den Berg TK, Dijkstra CD *et al.* C1 inhibitor treatment improves host defense in pneumococcal meningitis in rats and mice. J Infect Dis 2007; 196: 115–123.
44. Ernst T, Spath PJ, Aebi C, Schaad UB, Bianchetti MG. Screening for complement deficiency in bacterial meningitis. Acta Paediatr 1997; 86: 1009–1010.
45. Totan M. Recurrent pneumococcal meningitis in homozygous C3 deficiency. Indian J Pediatr 2002; 69: 625–626.
46. Singh DK, Rai R. Recurrent meningitis secondary to isolated C3 deficiency. Indian J Pediatr 2009; 76: 95–96.
47. Farhoudi A, Bazargan N, Pourpak Z, Mahmoudi M. Two related cases of primary complement deficiencies. Iran J Allergy Asthma Immunol 2003; 2: 69–74.
48. Bhide SS. Recurrent meningitis in a family with C3 deficiency. Indian Pediatr 2006; 43: 269–270.
49. Granoff DM, Welsch JA, Ram S. Binding of complement factor H (fH) to *Neisseria meningitidis* is specific for human fH and inhibits complement activation by rat and rabbit sera. Infect Immun 2009; 77: 764–769.
50. Roxo JP, Ferriani VP, Teixeira JE, Barbosa JE. Complement levels in Brazilian children during and after meningococcal meningitis. Clinics (Sao Paulo) 2005; 60: 127–130.
51. Lopez-Lera A, Garrido S, de la Cruz RM, Fontan G, Lopez-Trascasa M. Molecular characterization of three new mutations causing C5 deficiency in two non-related families. Mol Immunol 2009; 46: 2340–2347.
52. Delgado-Cervino E, Fontan G, Lopez-Trascasa M. C5 complement deficiency in a Spanish family. Molecular characterization of the double mutation responsible for the defect. Mol Immunol 2005; 42: 105–111.
53. Nishizaka H, Horiuchi T, Zhu ZB, Fukumori Y, Nagasawa K, Hayashi K *et al.* Molecular bases for inherited human complement component C6 deficiency in two unrelated individuals. J Immunol 1996; 156: 2309–2315.
54. Schirinzi R, Lantin JP, Fremeaux-Bacchi V, Schifferli JA, Trendelenburg M. Combined-heterozygous deficiency of complement C7 in a patient with recurrent meningitis. Med Klin (Munich) 2006; 101: 655–658.
55. Barroso S, Rieubland C, Jose´ a´lvarez A, Lopez-Trascasa M, Bart PA, Nunez-Roldan A *et al.* Molecular defects of the C7 gene in two patients with complement C7 deficiency. Immunology 2006; 118: 257–260.
56. Nishizaka H, Horiuchi T, Zhu ZB, Fukumori Y, Volanakis JE. Genetic bases of human complement C7 deficiency. J Immunol 1996; 157: 4239–4243.

57. Arnold DF, Roberts AG, Thomas A, Ferry B, Morgan BP, Chapel H. A novel mutation in a patient with a deficiency of the eighth component of complement associated with recurrent meningococcal meningitis. J Clin Immunol 2009; 29: 691–695.
58. Thiel S, Frederiksen PD, Jensenius JC. Clinical manifestations of mannan-binding lectin deficiency. Mol Immunol 2006; 43: 86–96.
59. Roy S, Knox K, Segal S, Griffiths D, Moore CE, Welsh KI *et al.* MBL genotype and risk of invasive pneumococcal disease: a case-control study. Lancet 2002; 359: 1569–1573.
60. Kronborg G, Weis N, Madsen HO, Pedersen SS, Wejse C, Nielsen H *et al.* Variant mannose-binding lectin alleles are not associated with susceptibility to or outcome of invasive pneumococcal infection in randomly included patients. J Infect Dis 2002; 185: 1517–1520.
61. Moens L, Van Hoeyveld E, Peetermans WE, De Boeck C, Verhaegen J, Bossuyt X. Mannose-binding lectin genotype and invasive pneumococcal infection. Hum Immunol 2006; 67: 605–611.
62. Vardar F, Pehlivan S, Onay H, Atlihan F, Guliz N, Ozkinay C *et al.* Association between mannose binding lectin polymorphisms and predisposition to bacterial meningitis. Turk J Pediatr 2007; 49: 270–273.
63. Faber J, Schuessler T, Finn A, Murdoch C, Zenz W, Habermehl P *et al.* Age-dependent association of human mannose-binding lectin mutations with susceptibility to invasive meningococcal disease in childhood. Pediatr Infect Dis J 2007; 26: 243–246.
64. Hibberd ML, Sumiya M, Summerfield JA, Booy R, Levin M. Association of variants of the gene for mannose-binding lectin with susceptibility to meningococcal disease. Meningococcal Research Group. Lancet 1999; 353: 1049–1053.
65. van der Zwet WC, Catsburg A, van Elburg RM, Savelkoul PH, Vandenbroucke-Grauls CM. Mannose-binding lectin (MBL) genotype in relation to risk of nosocomial infection in pre-term neonates in the neonatal intensive care unit. Clin Microbiol Infect 2008; 14: 130–135.
66. Sprong T, Roos D, Weemaes C, Neeleman C, Geesing CL, Mollnes TE *et al.* Deficient alternative complement pathway activation due to factor D deficiency by 2 novel mutations in the complement factor D gene in a family with meningococcal infections. Blood 2006; 107: 4865–4870.
67. Nikulin J, Panzner U, Frosch M, Schubert-Unkmeir A. Intracellular survival and replication of *Neisseria meningitidis* in human brain microvascular endothelial cells. Int J Med Microbiol 2006; 296: 553–558.
68. Ring A, Weiser JN, Tuomanen EI. Pneumococcal trafficking across the blood-brain barrier. Molecular analysis of a novel bidirectional pathway. J Clin Invest 1998; 102: 347–360.
69. Kim KS. Acute bacterial meningitis in infants and children. Lancet Infect Dis 2010; 10: 32–42.
70. Cundell DR, Gerard NP, Gerard C, Idanpaan-Heikkila I, Tuomanen EI. *Streptococcus pneumoniae* anchor to activated human cells by the receptor for platelet-activating factor. Nature 1995; 377: 435–438.
71. Orihuela CJ, Mahdavi J, Thornton J, Mann B, Wooldridge KG, Abouseada N *et al.* Laminin receptor initiates bacterial contact with the blood brain barrier in experimental meningitis models. J Clin Invest 2009; 119: 1638–1646.
72. Uchiyama S, Carlin AF, Khosravi A, Weiman S, Banerjee A, Quach D *et al.* The surface-anchored NanA protein promotes pneumococcal brain endothelial cell invasion. J Exp Med 2009; 206: 1845–1852.

73. Kostiukova NN, Volkova MO, Kvetnaia AS, Ivanova VV, Markov OP. The pathogenicity factors of *Streptococcus pneumoniae* strains causing meningitis. Zh Mikrobiol Epidemiol Immunobiol 1996; 3: 47–49.
74. Gillespie SH, Balakrishnan I. Pathogenesis of pneumococcal infection. J Med Microbiol 2000; 49: 1057–1067.
75. Coureuil M, Mikaty G, Miller F, Lecuyer H, Bernard C, Bourdoulous S *et al.* Meningococcal type IV pili recruit the polarity complex to cross the brain endothelium. Science 2009; 325: 83–87.
76. Kim KS. Mechanisms of microbial traversal of the blood-brain barrier. Nat Rev Microbiol 2008; 6: 625–634.
77. Shusta EV, Zhu C, Boado RJ, Pardridge WM. Subtractive expression cloning reveals high expression of CD46 at the blood-brain barrier. J Neuropathol Exp Neurol 2002; 61: 597–604.
78. Johansson L, Rytkonen A, Bergman P, Albiger B, Kallstrom H, Hokfelt T *et al.* CD46 in meningococcal disease. Science 2003; 301: 373–375.
79. Jack CS, Arbour N, Manusow J, Montgrain V, Blain M, McCrea E *et al.* TLR signaling tailors innate immune responses in human microglia and astrocytes. J Immunol 2005; 175: 4320–4330.
80. Koedel U. Toll-like receptors in bacterial meningitis. Curr Top Microbiol Immunol 2009; 336: 15–40.
81. Mogensen TH, Paludan SR, Kilian M, Ostergaard L. Live *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitidis* activate the inflammatory response through Toll-like receptors 2, 4, and 9 in species-specific patterns. J Leukoc Biol 2006; 80: 267–277.
82. Picard C, Puel A, Bonnet M, Ku CL, Bustamante J, Yang K *et al.* Pyogenic bacterial infections in humans with IRAK-4 deficiency. Science 2003; 299: 2076–2079.
83. Ku CL, Picard C, Erdos M, Jeurissen A, Bustamante J, Puel A *et al.* IRAK4 and NEMO mutations in otherwise healthy children with recurrent invasive pneumococcal disease. J Med Genet 2007; 44: 16–23.
84. Koedel U, Rupprecht T, Angele B, Heesemann J, Wagner H, Pfister HW *et al.* MyD88 is required for mounting a robust host immune response to *Streptococcus pneumonia*e in the CNS. Brain 2004; 127 (Part 6): 1437–1445.
85. von Bernuth H, Picard C, Jin Z, Pankla R, Xiao H, Ku CL *et al.* Pyogenic bacterial infections in humans with MyD88 deficiency. Science 2008; 321: 691–696.
86. Ku CL, von Bernuth H, Picard C, Zhang SY, Chang HH, Yang K *et al.* Selective predisposition to bacterial infections in IRAK-4-deficient children: IRAK-4-dependent TLRs are otherwise redundant in protective immunity. J Exp Med 2007; 204: 2407–2422.
87. Medvedev AE, Lentschat A, Kuhns DB, Blanco JC, Salkowski C, Zhang S *et al.* Distinct mutations in IRAK-4 confer hyporesponsiveness to lipopolysaccharide and interleukin-1 in a patient with recurrent bacterial infections. J Exp Med 2003; 198: 521–531.
88. Ku CL, Dupuis-Girod S, Dittrich AM, Bustamante J, Santos OF, Schulze I *et al.* NEMO mutations in 2 unrelated boys with severe infections and conical teeth. Pediatrics 2005; 115: e615–e619.
89. Koedel U, Klein M, Pfister HW. New understandings on the pathophysiology of bacterial meningitis. Curr Opin Infect Dis 2010; 23: 217–223.
90. Moens L, Verhaegen J, Pierik M, Vermeire S, De BK, Peetermans WE *et al.* Toll-like receptor 2 and Toll-like receptor 4 polymorphisms in invasive pneumococcal disease. Microbes Infect 2007; 9: 15–20.

91. Yuan FF, Marks K, Wong M, Watson S, de LE, McIntyre PB *et al.* Clinical relevance of TLR2, TLR4, CD14 and FcgammaRIIA gene polymorphisms in *Streptococcus* pneumonia infection. Immunol Cell Biol 2008; 86: 268–270.
92. Khor CC, Chapman SJ, Vannberg FO, Dunne A, Murphy C, Ling EY *et al.* A Mal functional variant is associated with protection against invasive pneumococcal disease, bacteremia, malaria and tuberculosis. Nat Genet 2007; 39: 523–528.
93. Chapman SJ, Khor CC, Vannberg FO, Frodsham A, Walley A, Maskell NA *et al.* IkappaB genetic polymorphisms and invasive pneumococcal disease. Am J Respir Crit Care Med 2007; 176: 181–187.
94. Biebl A, Muendlein A, Kazakbaeva Z, Heuberger S, Sonderegger G, Drexel H *et al.* CD14 C-159T and toll-like receptor 4 Asp299Gly polymorphisms in surviving meningococcal disease patients. PLoS One 2009; 4: e7374.
95. Smirnova I, Mann N, Dols A, Derkx HH, Hibberd ML, Levin M *et al.* Assay of locus-specific genetic load implicates rare Toll-like receptor 4 mutations in meningococcal susceptibility. Proc Natl Acad Sci USA 2003; 100: 6075–6080.
96. Emonts M, Hazelzet JA, de GR, Hermans PW. Host genetic determinants of *Neisseria meningitidis* infections. Lancet Infect Dis 2003; 3: 565–577.
97. Arbour NC, Lorenz E, Schutte BC, Zabner J, Kline JN, Jones M *et al.* TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. Nat Genet 2000; 25: 187–191.
98. Allen A, Obaro S, Bojang K, Awomoyi AA, Greenwood BM, Whittle H *et al.* Variation in Toll-like receptor 4 and susceptibility to group A meningococcal meningitis in Gambian children. Pediatr Infect Dis J 2003; 22: 1018–1019.
99. Misch EA, Hawn TR. Toll-like receptor polymorphisms and susceptibility to human disease. Clin Sci (Lond) 2008; 114: 347–360.
100. Agnese DM, Calvano JE, Hahm SJ, Coyle SM, Corbett SA, Calvano SE *et al.* Human Toll-like receptor 4 mutations but not CD14 polymorphisms are associated with an increased risk of gram-negative infections. J Infect Dis 2002; 186: 1522–1525.
101. Faber J, Meyer CU, Gemmer C, Russo A, Finn A, Murdoch C *et al.* Human toll-like receptor 4 mutations are associated with susceptibility to invasive meningococcal disease in infancy. Pediatr Infect Dis J 2006; 25: 80–81.
102. Faber J, Henninger N, Finn A, Zenz W, Zepp F, Knuf M. A toll-like receptor 4 variant is associated with fatal outcome in children with invasive meningococcal disease. Acta Paediatr 2009; 98: 548–552.
103. LeVan TD, Bloom JW, Bailey TJ, Karp CL, Halonen M, Martinez FD *et al.* A common single nucleotide polymorphism in the CD14 promoter decreases the affinity of Sp protein binding and enhances transcriptional activity. J Immunol 2001; 167: 5838–5844.
104. van Furth AM, Roord JJ, van Furth R. Roles of pro-inflammatory and anti-inflammatory cytokines in pathophysiology of bacterial meningitis and effect of adjunctive therapy. Infect Immun 1996; 64: 4883–4890.
105. Kornelisse RF, Hack CE, Savelkoul HF, van der Pouw Kraan TC, Hop WC, van Mierlo G *et al.* Intrathecal production of interleukin-12 and gamma interferon in patients with bacterial meningitis. Infect Immun 1997; 65: 877–881.
106. Carrol ED, Guiver M, Nkhoma S, Mankhambo LA, Marsh J, Balmer P *et al.* High pneumococcal DNA loads are associated with mortality in Malawian children with invasive pneumococcal disease. Pediatr Infect Dis J 2007; 26: 416–422.

107. Freyer D, Weih M, Weber JR, Burger W, Scholz P, Manz R *et al.* Pneumococcal cell wall components induce nitric oxide synthase and TNF-alpha in astroglial-enriched cultures. Glia 1996; 16: 1–6.
108. Prinz M, Kann O, Draheim HJ, Schumann RR, Kettenmann H, Weber JR *et al.* Microglial activation by components of gram-positive and -negative bacteria: distinct and common routes to the induction of ion channels and cytokines. J Neuropathol Exp Neurol 1999; 58: 1078–1089.
109. Angstwurm K, Freyer D, Dirnagl U, Hanisch UK, Schumann RR, Einhaupl KM *et al.* Tumour necrosis factor alpha induces only minor inflammatory changes in the central nervous system, but augments experimental meningitis. Neuroscience 1998; 86: 627–634.
110. Tsao N, Chang WW, Liu CC, Lei HY. Development of hematogenous pneumococcal meningitis in adult mice: the role of TNF-alpha. FEMS Immunol Med Microbiol 2002; 32: 133–140.
111. Zwijnenburg PJ, van der Poll T, Florquin S, Roord JJ, van Furth AM. IL-1 receptor type 1 gene-deficient mice demonstrate an impaired host defense against pneumococcal meningitis. J Immunol 2003; 170: 4724–4730.
112. Klein M, Paul R, Angele B, Popp B, Pfister HW, Koedel U. Protein expression pattern in experimental pneumococcal meningitis. Microbes Infect 2006; 8: 974–983.
113. Koedel U, Bernatowicz A, Frei K, Fontana A, Pfister HW. Systemically (but not intrathecally) administered IL-10 attenuates pathophysiologic alterations in experimental pneumococcal meningitis. J Immunol 1996; 157: 5185–5191.
114. Paul R, Koedel U, Winkler F, Kieseier BC, Fontana A, Kopf M *et al.* Lack of IL-6 augments inflammatory response but decreases vascular permeability in bacterial meningitis. Brain 2003; 126 (Part 8): 1873–1882.
115. Ostergaard C, Yieng-Kow RV, Larsen CG, Mukaida N, Matsushima K, Benfield T *et al.* Treatment with a monocolonal antibody to IL-8 attenuates the pleocytosis in experimental pneumococcal meningitis in rabbits when given intravenously, but not intracisternally. Clin Exp Immunol 2000; 122: 207–211.
116. Zwijnenburg PJ, van der Poll T, Florquin S, Akira S, Takeda K, Roord JJ *et al.* Interleukin-18 gene-deficient mice show enhanced defense and reduced inflammation during pneumococcal meningitis. J Neuroimmunol 2003; 138: 31–37.
117. Malipiero U, Koedel U, Pfister W, Fontana A. Bacterial meningitis: the role of transforming growth factor-Beta in innate immunity and secondary brain damage. Neurodegener Dis 2007; 4: 43–50.
118. Hausler KG, Prinz M, Nolte C, Weber JR, Schumann RR, Kettenmann H *et al.* Interferon-gamma differentially modulates the release of cytokines and chemokines in lipopolysaccharide- and pneumococcal cell wall-stimulated mouse microglia and macrophages. Eur J Neurosci 2002; 16: 2113–2122.
119. Lapinet JA, Scapini P, Calzetti F, Perez O, Cassatella MA. Gene expression and production of tumor necrosis factor alpha, interleukin-1beta (IL-1beta), IL-8, macrophage inflammatory protein 1alpha (MIP-1alpha), MIP-1beta, and gamma interferon-inducible protein 10 by human neutrophils stimulated with group B meningococcal outer membrane vesicles. Infect Immun 2000; 68: 6917–6923.
120. van Deuren M, van der Ven-Jongekrijg J, Bartelink AK, van Dalen R, Sauerwein RW, van der Meer JW. Correlation between pro-inflammatory cytokines and anti-inflammatory mediators and the severity of disease in meningococcal infections. J Infect Dis 1995; 172: 433–439.
121. van Deuren M, van der Ven-Jongekrijg J, Vannier E, van Dalen R, Pesman G, Bartelink AK *et al.* The pattern of interleukin-1beta (IL-1beta) and its modulating agents IL-1 receptor antago-

nist and IL-1 soluble receptor type II in acute meningococcal infections. Blood 1997; 90: 1101–1108.

122. Moller AS, Bjerre A, Brusletto B, Joo GB, Brandtzaeg P, Kierulf P. Chemokine patterns in meningococcal disease. J Infect Dis 2005; 191: 768–775.
123. Schaaf B, Rupp J, Muller-Steinhardt M, Kruse J, Boehmke F, Maass M *et al*. The interleukin-6 -174 promoter polymorphism is associated with extrapulmonary bacterial dissemination in *Streptococcus pneumoniae* infection. Cytokine 2005; 31: 324–328.
124. Schaaf BM, Boehmke F, Esnaashari H, Seitzer U, Kothe H, Maass M *et al*. Pneumococcal septic shock is associated with the interleukin-10-1082 gene promoter polymorphism. Am J Respir Crit Care Med 2003; 168: 476–480.
125. Doernberg S, Schaaf B, Dalhoff K, Leng L, Beitin A, Quagliarello V *et al*. Association of macrophage migration inhibitory factor (MIF) polymorphisms with risk of meningitis from *Streptococcus pneumoniae*. Cytokine 2011; 53: 292–294.
126. Balding J, Healy CM, Livingstone WJ, White B, Mynett-Johnson L, Cafferkey M *et al*. Genomic polymorphic profiles in an Irish population with meningococcaemia: is it possible to predict severity and outcome of disease? Genes Immun 2003; 4: 533–540.
127. Carrol ED, Mobbs KJ, Thomson AP, Hart CA. Variable number tandem repeat polymorphism of the interleukin-1 receptor antagonist gene in meningococcal disease. Clin Infect Dis 2002; 35: 495–497.
128. Read RC, Cannings C, Naylor SC, Timms JM,Maheswaran R, Borrow R *et al*. Variation within genes encoding interleukin-1 and the interleukin-1 receptor antagonist influence the severity of meningococcal disease. Ann Intern Med 2003; 138: 534–541.
129. Endler G, Marculescu R, Starkl P, Binder A, Geishofer G, Muller M *et al*. Polymorphisms in the interleukin-1 gene cluster in children and young adults with systemic meningococcemia. Clin Chem 2006; 52: 511–514.
130. Koedel U, Angele B, Rupprecht T, Wagner H, Roggenkamp A, Pfister HW *et al*. Toll-like receptor 2 participates in mediation of immune response in experimental pneumococcal meningitis. J Immunol 2003; 170: 438 .
131. Echchannaoui H, Frei K, Schnell C, Leib SL, Zimmerli W, Landmann R. Toll-like receptor 2-deficient mice are highly susceptible to *Streptococcus pneumoniae* meningitis because of reduced bacterial clearing and enhanced inflammation. J Infect Dis 2002; 186: 798–806.
132. Klein M, Obermaier B, Angele B, Pfister HW, Wagner H, Koedel U *et al*. Innate immunity to pneumococcal infection of the central nervous system depends on toll-like receptor (TLR) 2 and TLR4. J Infect Dis 2008; 198: 1028–1036.
133. Cook DN, Pisetsky DS, Schwartz DA. Toll-like receptors in the pathogenesis of human disease. Nat Immunol 2004; 5: 975–979.
134. Cundell DR, Gerard NP, Gerard C, Idanpaan-Heikkila I, Tuomanen EI. *Streptococcus pneumoniae* anchor to activated human cells by the receptor for platelet-activating factor. Nature 1995; 377: 435–438.