Genetic susceptibility to *Neisseria meningitidis* infections

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ABSTRACT

The clinical presentation of infections by *Neisseria meningitidis* is highly diverse. Some patients with invasive meningococcal infections develop meningitis, while others present with sepsis or even septic shock. Three major host defence systems are activated after transepithelial passage and invasion of the bacteria into the bloodstream. These include the complement pathway, the inflammatory response by cytokines and chemokines, and the coagulation and fibrinolysis pathway. These three systems are mutually dependent. Genetic polymorphisms among components of these pathways (co)regulate the susceptibility, severity and outcome of meningococcal disease. In this paper the current knowledge of polymorphisms which are known to be associated with susceptibility to and severity of meningococcal infection is reviewed.

INTRODUCTION

Despite ongoing improvements in the treatment of infections by *Neisseria meningitidis* the mortality rates in patients with invasive disease are still very high, ranging from 4 to 40%. This is a reflection of the different clinical presentations of infection. The disease spectrum varies from meningitis to sepsis and septic shock. The first entity has a mortality rate of 4 to 6%, while in septic shock mortality rates up to 40% have been reported. The observation that septic shock can develop within several hours of onset of meningococcal invasive disease implies a major role for the innate immunity in host defence. Meningococcal lipopolysaccharide (LPS) is one of the major factors to induce the host response during bacterial invasion. This response is complex and involves the activation of three major host response systems (*figure 1*). The first is the complement pathway that apart from contribution to phagocytosis of the bacteria also functions as an inducer for the inflammatory reaction through C3a and C5a. The second system is the inflammatory reaction mediated by different chemokines and cytokines among which TNF-α and IL-1β play a central role. The third cascade is activation of the coagulation and fibrinolysis, which result in a prothrombotic stage. Activation of the host response in patients with sepsis results in a sudden onset of fever and a petechial or purpuric rash followed by hypotension. In patients with septic shock, disseminated coagulation and multiple organ failure develop. For an extensive review of the pathophysiology we refer to previous reports.[1,2]

The broad range of clinical presentations of *N. meningitidis* infections raises the question why some patients show hardly any clinical symptoms while others die within several hours of onset of symptoms. Variations in host genetic factors are known to contribute to these differences. Genetic polymorphisms are relatively stable in the human population. Single-nucleotide polymorphisms (SNPs) are distributed throughout the human genome at an estimated overall frequency of one in every 2 kb.[3] It has been shown that these polymorphisms may affect susceptibility to and severity and outcome of infectious disease. This review focuses on the current knowledge of genetic variability in the susceptibility to and severity of meningococcal infections (*table 1*).

INNATE IMMUNITY

TLR

The innate immunity plays an important role in the initial recognition of invading pathogens. This recognition was, until recently, believed to be nonspecific. However, elucidation of the function of Toll-like receptors (TLR) has shown

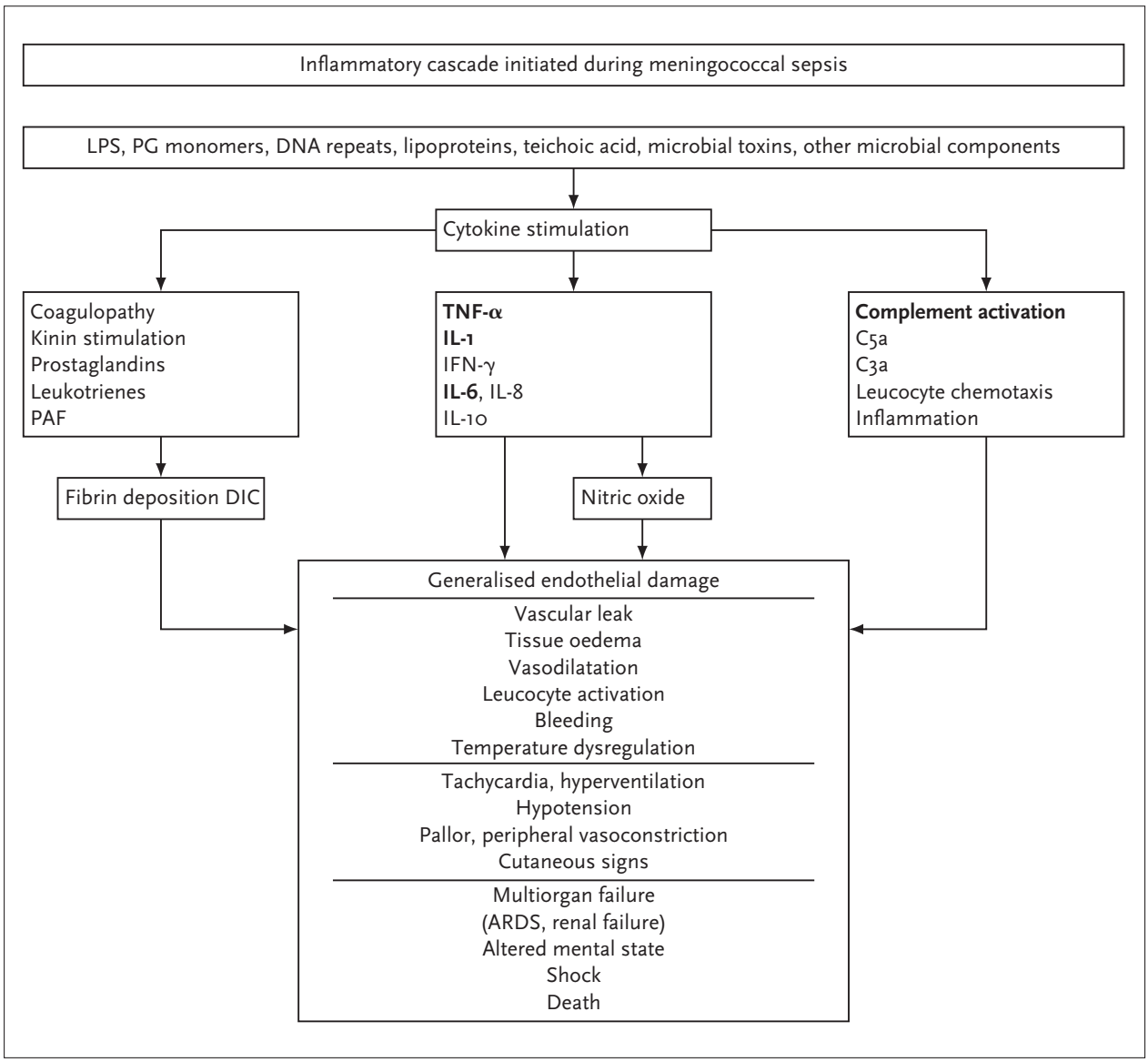

Figure 1
The three main cascades involved in the pathophysiology of meningococcal infection

The factors depicted in bold are discussed in the text. Adapted from Harcourt Publishers Ltd.

otherwise. TLRs sense different microbial molecules, covering a range of pathogens that cause infections in the host. TLR4, for example, recognises bacterial LPS. C3H/HeJ mice, having a point mutation in *tlr4* that abolishes LPS responses, are hyporesponsive to Gram-negative infections.[4,5] The common Asp299Gly polymorphism in TLR4 alters the extracellular domain of the receptor. Airway epithelial cells of patients carrying this polymorphism are hyporesponsive to LPS. However, no association has been found between the Asp299Gly polymorphism and the susceptibility to or severity of meningococcal infection.[6] Smirnova *et al.* have found an excess of rare amino acid polymorphisms in TLR4 of which the clinical relevance has not yet been studied.[7] TLR2 recognises other bacterial components, such as lipopeptides and peptidoglycan.[4] Until now the relation between polymorphisms in TLR2 and meningococcal infections has not been studied. TLRs recognise ligands in the presence of CD14 and MD-2 (TLR4) and activation of the diverse TLRs results in the activation of different signalling pathways, leading to NF-αB activation. Polymorphisms in the factors which are involved in these signalling pathways may also result in different phenotypes.

LBP and BPI

In blood LPS binds to lipopolysaccharide-binding protein (LBP) to form a complex that binds TLR4. In a recent study the Cys98Gly and Pro436Leu polymorphisms of LBP were not correlated with the susceptibility to or severity

Table 1
Genetic polymorphisms possibly associated with meningococcal infection (for details see text)

PATHWAY	GENE	POLYMORPHISM
Innate immunity	TLR4	Asp299Gly
	LBP	Cys98Gly
		Pro436Leu
	BPI	A645G
		G545C
		PstI in intron 5
	MBL	Codon 52
		Codon 54
		Codon 57
Acquired immunity	FcγRIIa	His131Arg
	FcγRIIIa	Val158Phe
	FcγRIIIb	NA1/NA2
Coagulation/ fibrinolysis	t-PA	Alu repeat insertion/deletion
	PAI-1	4G/5G insertion/deletion
	Factor V	FV^{L} G1691A
Cytokines	TNF-α	G-308A
	IL1RN	86 bp repeat in intron 2
		T2018C
	IL1B	C-511T
	IL-6	G-174C

of bacterial sepsis in all patients but a relationship was found with the outcome of bacterial sepsis in males. It has been proposed that these polymorphisms may partially explain the worse outcome of bacterial infections observed in males. In the same study the clinical effects of polymorphisms in bactericidal/permeability-increasing (BPI) protein were also evaluated. BPI is another protein binding to LPS which inhibits LPS-induced host cell responses. Neither the A645G, the G545C silent polymorphism, nor the *Pst*I in intron 5 were associated with outcome and severity of bacterial sepsis. However, in this study the limited number of patients prevented a separate analysis of patients with Gram-negative *versus* Gram-positive infections, thus introducing a bias with respect to LPS-related susceptibility. This may have limited the sensitivity in detecting an association.[8]

COMPLEMENT

MBL

Activation of the complement system forms a significant part of the innate immunity. Besides beneficial, also deleterious effects are associated with complement activation. The severity of hypotension is in part regulated by complement activation. At present, three activation pathways are considered (*figure 2*).[9] Firstly, the classical pathway which is activated by antibody-antigen interactions, and secondly, the two pathways of the innate immunity, which do not require antibody.

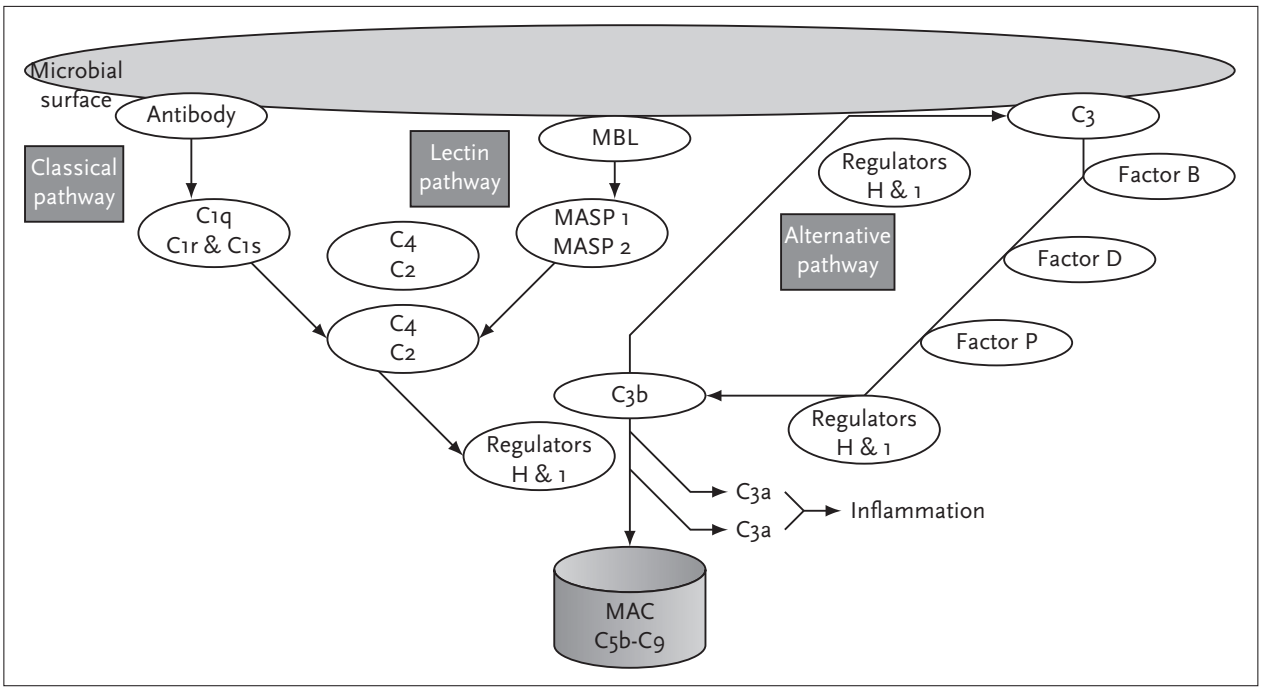

Figure 2
The three pathways of complement activation, connecting into a common late pathway generating the membrane attack complex (MAC) and inflammatory mediators C3a and C5a

Adapted from Hibberd et al.[9]

The alternative pathway of innate immunity is activated through interaction of C3 with factor b, factor d and properdin to generate C3b. The additional innate activation pathway is activated through mannose-binding lectin (MBL). All three pathways converge in a common final pathway. MBL first forms trimers, which in turn further assemble to form multimers. The MBL multimers bind to sugars and LPS on the bacterial surface. This complex then activates the mannose-associated serine proteases MASP1 and MASP2. MASPs activate C4 of the classical pathway.
Three allelic variants of MBL have been described in codons 52, 54 and 57 of exon 1. A mutation in codons 54 and 57 causes the disruption of the axial glycine repeats (Gly-Xaa-Yaa) resulting in aberrant trimers. These trimers cannot form correct multimers and are unstable. Through an unknown mechanism, the codon 52 mutation also results in unstable trimers. Heterozygotes for the variant alleles show a decrease in MBL serum levels to 10% of normal, while in people homozygous for any of the three mutations levels drop to 1% of normal.[9,10] In children the variant alleles were associated with an increased susceptibility to meningococcal infections. Patients homozygous for the variant alleles showed a trend towards slightly less severe disease than heterozygous and wild-type individuals. This observation, however, did not reach significance.[11]

LCCD and factor D

Patients with late complement component deficiency (C5-C9) (LCCD) are known to suffer from recurrent Gram-negative bacterial infections. Failure to form a membrane attack complex (MAC) might underlie this increased susceptibility. Interestingly, these infections result in milder disease severity than observed for the total patient population, suggesting that there are also adverse effects of complement activation during meningococcal infections. Complement D deficiency was found in members of a family suffering from severe *N. meningitidis* infections. The alternative pathway for complement activation was impaired. This pathway, in contrast to the classical pathway, appears to be prominent in meningococcal infection.[12,13]

ACQUIRED IMMUNITY

Fcγ receptors

Fcγ receptors (FcγRs) belong to a heterogeneous family of receptors and are grouped in three classes (FcγRI, II and III). Three subtypes of receptors responsible for IgG-mediated signalling, FcγRIIa, FcγRIIIa and FcγRIIIb, are thought to be important in host defence against meningococci. FcγRIIa is located on leucocytes and mononuclear macrophages and is sensitive to IgG2 and IgG3. Two alleles are known to differ at amino acid position 131 because of single-nucleotide polymorphism (SNP) in exon 4. The 131Arg allotype confers lower interaction efficiency on IgG2 and IgG3 than the 131His haplotype. Two allotypes known for FcγRIIIa, 158Phe and 158Val show different binding of IgG1, IgG3 and IgG4, as interaction with FcγRIIIa-158Val is stronger. FcγRIIIb is expressed on neutrophils and has the neutrophil antigen (NA) polymorphism representing four amino acid substitutions in the membrane-distal loop of the receptor. FcγRIIIb-NA2 binds IgG1 and IgG3 less efficiently than FcγRIIIb-NA.[14] One can imagine that differences in efficiency in binding IgGs result in different host response effectiveness and thereby variable susceptibility to and/or severity of disease.
A study in survivors of meningococcal disease and first-degree relatives of survivors and nonsurvivors revealed no differences in FcγR distribution. The FcγRIIa-131Arg allele was more often found in meningitis patients compared with sepsis patients. The FcγRIIIa-158Val allelic frequency was markedly increased in relatives of meningitis patients compared with relatives of patients presenting with haemodynamic instability. The Arg/Arg-Phe/Phe-NA2/2 frequency that represents the least efficient FcγR combination, and is responsible for diminished phagocytosis, was tripled in first-degree relatives compared with healthy nonrelated controls. These data suggest an association between FcγR haplotype and susceptibility to and severity of meningococcal disease.[14]

COAGULATION

Activation of coagulation and fibrinolysis is the result of the acute inflammatory response in patients with invasive meningococcal disease. The prothrombotic endothelium surface results from activation by cytokines.
Subsequently, tissue factor production results in activation of the extrinsic pathway of coagulation and the production of platelet-activating factors. The fibrinolytic system is initially activated but is subsequently inhibited. This results in a marked imbalance in coagulation and fibrinolysis resulting in a net procoagulant state and ultimately disseminated intravascular coagulation (DIC). This leads to deposition of fibrin, the formation of microthrombi and bleeding. Multiorgan failure and death are the most severe clinical findings regarding this imbalance.

t-PA

Tissue-type plasminogen activator (t-PA) is a serine protease that converts plasminogen into its active form, plasmin, which in turn leads to fibrinolysis. Impaired t-PA function leads to insufficient lysis of thrombi. Differences in t-PA production or impaired function can therefore affect the severity of meningococcal disease. An insertion/deletion polymorphism of an Alu element in t-PA considered to affect the basal levels of t-PA has been investigated in

relation to meningococcal disease. No association could be observed between the different alleles and the severity or outcome of meningococcal disease.[15] The authors have regarded the use of an intensive care unit (ICU) as a criterion for severe meningococcal disease. In our opinion this only holds partially true, since not all patients admitted to the ICU for observation because they are at risk of septic shock actually develop septic shock. This might have prevented them from finding significant differences between the study populations. Further research regarding polymorphisms in t-PA with respect to meningococcal disease is therefore indicated.

PAI-1

Plasminogen activator inhibitor type 1 (PAI-1) is responsible for the inhibition of fibrinolysis both directly and indirectly through inhibition of t-PA (*figure 3*). In turn PAI-1 is inhibited by activated protein C. In septic shock, laboratory findings show decreased levels of all coagulation factors including protein C.[16] The gene encoding PAI-1 has several polymorphic loci, including a 3'-HindIII site, a CA(n) repeat in intron 3 and a 4G/5G insertion/deletion polymorphism at -675 in the promoter. PAI-1 activity has been shown to be significantly higher in control subjects homozygous for the 4G allele than in subjects homozygous for the 5G allele.[17] The production of PAI-1 mRNA after IL-1 stimulation appeared to be higher in HepG2 cells bearing the 4G allele.[18] In patients with meningococcal sepsis the levels of PAI-1 are positively related to severity of disease, outcome, cytokine levels, acute-phase proteins and coagulation parameters.[16] In nonsurvivors the PAI-1 levels were shown to be 1.9 times higher for the same TNF-α levels than in survivors. In children suffering from meningococcal disease a relationship has been observed between genotype, PAI-1 levels and outcome of disease. The subjects with the 4G homozygous genotype had higher PAI-1 levels and had an increased risk of death. No association was observed with severity of disease.[19]

A similar study performed by Westendorp *et al.* showed a higher incidence of the 5G/5G genotype among relatives of patients with meningitis. Patients whose relatives were carriers of the 4G/4G genotype had a sixfold higher risk of developing septic shock than meningitis.[20]

FV^L

Factor V Leiden (FV^L) is associated with thrombotic events, and is therefore an interesting candidate for involvement in the development of meningococcal purpura fulminans. A study comparing children with meningococcal disease, healthy controls and parents of children with meningococcal disease did not reveal an association between the FV^L mutation and susceptibility. Patients heterozygous for the mutation showed increased complications, as assessed by requirement for skin grafting, referral to plastic surgeon and/or amputation. A significant effect on mortality was not observed.[21]

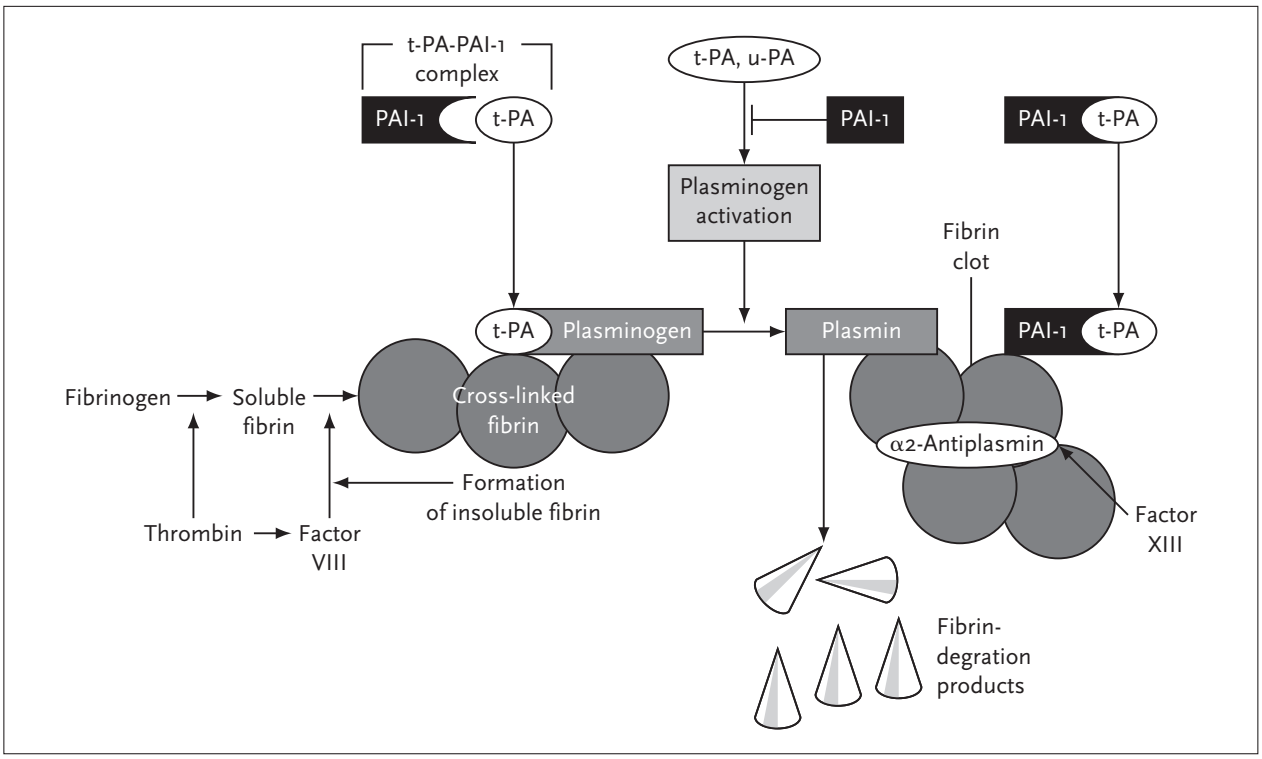

Figure 3
Involvement of PAI-1 and t-PA in coagulation and fibrinolysis

CYTOKINES

TNF-α

Tumour necrosis factor α (TNF-α) plays a central role in the activation of the inflammatory response, and serum levels are raised in all patients with meningococcal disease. TNF-α activates adhesion-promoting receptors and ligands enabling leucocytes to cross the endothelium and reach the site of inflammation. Other inflammatory mediators such as IFN-γ, IL-6 and IL-8 are activated by TNF-α. A procoagulant state is promoted by the induction of tissue factor release. TNF-α blood levels are positively associated with disease severity, coagulopathies and outcome.[22] Westendorp *et al*. found a higher TNF-α response in *ex vivo* blood samples after stimulation with endotoxin in patients who had experienced a moderately severe disease course compared with patients with a mild course. The TNF-α response was low again in survivors of fulminant disease. On admission for meningococcal disease, patients who did not survive had initial TNF-α levels three times higher than those in survivors with a clinical disease presentation of similar severity.[23,24]

The G-308A polymorphism in the TNF-α promoter region has been shown to be associated with outcome of meningococcal disease. The TNF2 allele (-308A) is associated with an increased risk of death. Controversial opinions exist about the role of this polymorphism and the severity of disease. The diverse results of the various studies may reflect the differences in patient and control populations. Some researchers study meningococcal sepsis only, while others look into the relevance of the G-308A polymorphism in patients suffering from sepsis on an ICU after surgery.[25-27] No association has been observed for the G-308A polymorphism and susceptibility to meningococcal disease.[25,26] It is clear that TNF-α production is influenced by genetic factors in meningococcal sepsis, but the relative importance of the known polymorphisms is still under discussion.

IL-1 family

The interleukin-1 (IL-1) family consists of both proinflammatory and counterinflammatory members. The genes IL1A and IL1B encode the proinflammatory proteins IL-1α and IL-1β, respectively. The IL-1 receptor antagonist (IL-1RA) represents the anti-inflammatory component. IL-1RA can bind the IL-1 receptor without inducing signal transduction. Several polymorphisms are known in the IL-1 family. Five alleles of IL1RN, the gene encoding IL-1RA, are known to differ by a variable number repeat of 86 bp in intron 2.[28] This repeat contains transcription factor binding sites. The IL1RNA2 allele has two repeats and is in linkage disequilibrium with SNP T8006, also known as T2018C.[29,30] The A2 allele has been shown to be associated with susceptibility to severe sepsis in patients in a surgical intensive care unit.[31] Read *et al*. have investigated whether variants of the IL-1 and TNF gene families are associated with severe manifestations of meningococcal infection. All patients included had microbiologically proven infection by *N. meningitidis*. A significant association has been found between the outcome and the IL1B C-511T polymorphism. Patients homozygous for either the common or the rare allele had increased odds ratios for death, compared with heterozygous individuals. The combination of heterozygosity of IL1B (-511) together with homozygosity of the common allele of IL1RN at +2018 was significantly associated with survival. In this study no association between the TNF -308 genotype and fatal outcome was demonstrated.[30] These data suggest that IL-1 genotype influences the outcome of meningococcal disease.

IL-6

Interleukin 6 (IL-6) is a major pyrogen and is responsible for the induction of hepatic acute-phase proteins and antibody production by B cells. IL-6 blood levels are increased in meningococcal infection.[32] The G-174C polymorphism in the promoter region of IL-6 is associated with outcome in meningococcal infection, since the -174G/G genotype is associated with an increased mortality risk.[33] However, this polymorphism is part of a complex haplotype associated with differences in IL-6 production. The -579G, -572G, -373A9/T11, -174G haplotype shows higher transcription of IL-6 in an ECV304 cell line after IL-1 induction than the other polymorphism combinations. This study clearly shows that different polymorphisms can have an influence on transcription, but they are not functioning independently.[34] When assessing the contribution of the G-174C polymorphism in meningococcal disease, the complete haplotype should be considered.

DISCUSSION

Meningococcal disease comprises a complex pathophysiology resulting in a spectrum of disease presentation in affected individuals. Assessing the contribution of host factors in infection therefore requires a strict definition of the patient population. Patients suffering from meningococcal meningitis might have different 'susceptibility genotypes' to septic shock patients, as different pathophysiological pathways are activated. Combining these study populations is therefore not advisable. The same problems occur when comparing patients with sepsis of unknown microbiological origin. Host response to Gram-positive microbes might differ from the response towards Gram-negative bacteria.[4] It must be noted, however, that in approximately 10% meningococcal infection cannot be proven with microbiological techniques, while the clinical presentation is typical.
In addition, it might be difficult to obtain patient groups of sufficient numbers to show an association between disease

susceptibility, severity and outcome when investigating genotypes with extremely low allele frequencies. Ethnic differences in the study population might also interfere with the supposed associations. Studies including relatives of patients as controls might overcome this problem. These studies compare the observed and expected inheritance of the different alleles. One must, however, consider the possibility that disease is not linked to the studied gene, but to another gene in linkage with the first.
Another interesting focus of investigation is the interaction of the polymorphisms and the cumulative effect on the course of meningococcal disease. These interactions might be either synergistic or counteracting. In the IL-1 family such interactions have been found for IL-1B(-511) and IL1RN (2018). The development of high-throughput molecular genetic techniques is of great importance in our efforts to unravel the immense complexity of genetic interactions.

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Discussion following lecture by P.W.M. Hermans

Netea: I would like to ask you two questions; one is regarding the tumour necrosis factor (TNF) levels in meningococcal sepsis. You told us that they are high and that this increases the severity and the risk of death. Is this really an increased production capacity leading to death and leading to increased circulating levels, or is there actually a reduced production capacity leading to multiplication of bacteria, which leads to a lot of stimulus, which then leads to high amounts of cytokines? I am thinking of first-degree relatives of patients with meningococcal sepsis. If I remember rightly, they have a reduced production capacity of TNF. My second question is a more conceptual one, which is more or less for everybody. As we are now looking at Toll-like receptor (TLR) polymorphisms in various diseases, what are our chances of finding something important with a genetic approach? Because at the moment we find a very significant polymorphism or a very significant mutated gene, the host is already dead. So the closer we are to the truth in looking for a determinant gene, the smaller the chance we will ever find it. Only less significant polymorphisms are found which are compatible with survival, whereas the really significant ones are not detected since the hosts are already dead. I am thinking of TLRs, because if it is really a polymorphism which leads to a TLR-2 defect, I do not think this is compatible with survival. It may be the same with some important polymorphisms for TNF. For example, I do not know anyone who is really deficient in TNF.

Hermans: Quite complex comments, but starting with the last part, I think it is absolutely true that quite a few interesting polymorphisms have been studied now, and as soon as new polymorphisms pop up, I immediately get the feeling, OK, we have to increase the number of patients again to see any relevance in interaction. You are in fact driving towards a complete human-specific genetic passport for that particular human being. If I have got your point right, I think that these extremely complex and difficult studies might not give us the appropriate answers we were hoping for. Coming back to TNF, TNF-α expression actually, for many cytokines, when patients come in, as far as I understand from the clinicians, the cytokine responses and the cytokine levels are extremely high. Immediately after admission they tend to decrease, do you agree, Dr van Deuren?

Van Deuren: Yes.

Hermans: The turnover is extremely high, which means that the half-life is quite short.

Netea: I am just thinking that the circulating levels that you are measuring are in fact a result of the interaction between our capacity to make it and how many bugs are multiplying in our system. It is very difficult to discern and to say, a sick person has a lot of TNF, so that is a bad thing. It might very well be possible he was unable to make enough in the beginning to suppress the multiplication of the bacteria.

Hermans: I agree with that.

Van Deuren: I would like to ask a few questions and make a few remarks on meningococcal disease. Of course, it is a blood-borne infection with an impressive clinical picture and blood is the most easily obtained tissue. So a lot of measurements have been done in blood. But it is really the question whether that is the most important site of the body to look at. The gate for the meningococcal bacterium is the oronasal mucosa. At that site there are several interactions between the bacterium and our immune system: the meningococcal pylus is attached to CD46 for instance, the meningococcal pore A outer membrane protein is attached to the carcinoembryogenic antigen CEA, CD66a, b and c. And there are a lot of polymorphisms in these types of receptors or ligands too, for instance relating to the intramucosal survival of the meningococcus, because it is able to survive passing through the cells. There are

some studies showing that they are influencing bactericidal proteins, enabling them to survive and to pass through the bloodstream. Are you aware of any studies looking at the polymorphisms in this site of the body? Because that will really determine our susceptibility, because all the rest in the blood really is a matter of severity.

Hermans: As far as I know, such studies have not been undertaken, but the initial point you made that has not been studied either, in detail at least, is the genetic differences among these bugs, and it is definitely not only man C *versus* man B, but there are many more molecules showing a huge variety within this particular species.

Van Deuren: That is the second important property of the bug, of course, its switching off and switching on molecules so rapidly enabling it to enter the bloodstream. A second remark about the levels of TNF: the higher the concentration of endotoxin in your blood, the more TNF you produce. And really the best predictor of mortality is not the TNF level but the endotoxin level, as already mentioned by Brandtzaeg in his series of 150 patients. There is a good correlation between the level of TNF and endotoxin. So for me it is not the genetic trait or the capacity of the person to produce TNF, but it is the amount of endotoxin that he tolerates, and that will determine the amount of TNF produced and, thus, the amount of procoagulant and anticoagulant activities.

Appelmelk: You showed that you get an impressive increase in relative risk when you add the various polymorphisms. Where will this end? From a scientific point of view you can go on, but do we need to know all this? Which knowledge regarding polymorphisms will lead to clinical actions? You read in the papers about DNA passports and you see companies eager for dollars, but how important is it for the actual patient management?

Hermans: Your question actually was the main reason for me to show this study on tissue-type plasminogen activator (t-PA) therapy, which is in part guided by the existing polymorphisms and genetic variability. Indeed, when you have these details, there is a possibility of performing patient-specific therapy in the long run. At the moment that is not feasible. But I think such data are of definite value for the patient. It is not so that you are just born with a 'genetic passport' and that nobody can change anything about that. I think when you have these data in advance and you know where to interact, you can intervene in these processes.

Appelmelk: Do you think that it is realistic when we have a patient and are at the bedside to take his blood, for us to put it on a chip and then decide what to do?

Hermans: By that time it is too late!

Kusters: Can I give one brief comment or ask a question? You are now looking at only one type of disease and one type of bug trying to find the ideal 'genetic passport', but obviously evolution has gone a long way and the so-called wrong passport may in fact be an ideal passport for survival. Maybe an increased risk of a certain type of disease is the flip side of a better survival of other types of disease.

Hermans: When a patient comes in with a meningococcal sepsis, having an excellent overall survival passport, still at that time his survival clock has started ticking towards zero. When you know the disadvantages of that particular passport, if you can alter it, that would be a major advantage. Of course, such an intervention might be disadvantageous with another infection.

Kuijpers: Whether lipopolysaccharide (LPS) and the levels of TNF strictly correlate, depends on which test system you use, which ELISA, or especially which firm you have addressed to purchase this ELISA system from. Just comparing TNF ELISA test systems as such would already give you two or three papers probably. It is very difficult. With genes you cannot lie – that is an advantage, but on the other hand it is of course a disadvantage that if you then start to shift from genes to concepts, as for instance TNF-induced responses, and one observes the staggering lack of effect of blocking TNF for instance in meningococcal disease or sepsis, I very much doubt whether this approach will have any impact in the short run on how to treat patients.

Kimman: It is nice to comment on this issue of course, whether we should do this work in order to improve the treatment of individual patients. I do not see a future in which patients will be treated only after their genotype has been assessed, but on the other hand, you nicely showed that this kind of work can lead the way to pathways which can be influenced whatever the genotype of the patient is. For instance, you identify a PAI/t-PA pathway and then you have a lead for treatment irrespective of the genotype of the patient. Unfortunately it did not work, but you identified something which you can tackle.

De Groot: I would like to support Dr Hermans in relation to the question from Dr Appelmelk and the point that is raised here. I will give you a practical example why I think this genetic approach will have additional value and how it could already have in the current situation. Let's consider a polytrauma patient and suppose you knew the genetic profile in advance when he came in, thus being able to predict whether he was in a high-risk group or in a low-risk one. That would be helpful in the choice of therapy. Now Dr Hermans mentioned one therapy, t-PA, but another one

is known to all clinicians from a publication in the New England Journal of Medicine last year, namely activated protein C.[1] Both therapies have serious potential side effects in terms of cerebral haemorrhage. The selection of patients who would benefit from these very invasive therapies would therefore be balanced depending on their risk. So this is the way of thinking I believe we should pursue as clinicians in terms of making a choice actually for these drugs where we know that in these desperately ill patients there certainly will be a very narrow kind of safety margin.

Van Deuren: Just to address a limitation of this approach. In meningococcal disease I have looked at the moment – I call it the kinetics of dying – the patient dies from that disease. From literature and from my own experience I have collected approximately 300 deaths, not cases, but deaths, and from that it can be seen that one-third of the patients who will die have already died within six hours of admission, half have died within 12 hours, 66% within 24 hours and 80% within 48 hours. So already one-third of your patients have been lost within six hours. I do not know how long it takes to run a chip array.

Verbrugh: It is quite interesting that we are here discussing the value of our core business as a science, the creation of new knowledge. We would rather like to pose the question beforehand or we would like to know which knowledge we can use and what knowledge we cannot. This is a poor question to ask. Sometimes we do have to ask, because there are financial constraints, but primarily the business is to get new insights and new knowledge.

REFERENCE

1. Bernard GR, Vincent JL, Laterre PF, et al. Efficacy and safety of recombinant human activated protein C for severe sepsis. N Engl J Med 2001;344:699-709.