

# Variation in the Tumor Necrosis Factor- $\alpha$ Gene Promoter Region May Be Associated with Death from Meningococcal Disease

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Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) plays a central role in the pathophysiology of sepsis. Levels of TNF- $\alpha$  are directly correlated with severity in meningococcal disease (MD). A polymorphism in the promoter region of the TNF- $\alpha$  gene is associated with differences in the secretion of TNF- $\alpha$ . The TNF2 allele is associated with higher constitutive and inducible levels of TNF- $\alpha$  secretion than is the TNF1 allele. To investigate whether possession of the TNF2 allele is associated with severity in MD, the frequency of TNF1 and TNF2 alleles in 98 children with MD was compared. There were more deaths among children who had the TNF2 allele ( $P = .03$ ; relative risk [RR], 2.5; 95% confidence interval [CI], 1.1–5.7) than in those who did not. There was also an increased risk of severe disease in children with the TNF2 allele ( $P = .02$ ; RR, 1.6; 95% CI, 1.1–2.3). Possession of the TNF2 allele predisposes to a worse outcome in children with meningococcal infection.

The cytokine tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) is thought to play a central role in the pathophysiology of sepsis. Clinical and laboratory features of sepsis can be reproduced by the infusion of TNF- $\alpha$  into animals or humans. Antibodies that block the action of TNF- $\alpha$  can prevent the lethal effects of bacterial infections in experimental animals, although their protective action in humans with sepsis and septic shock is less clear [1]. Furthermore, circulating levels of TNF- $\alpha$  have been shown to be directly correlated with disease severity and outcome in severe bacterial infections, particularly in meningococcal disease (MD) [2]. Persons dying of fulminant MD have high levels of detectable TNF- $\alpha$  in serum, whereas those with less severe MD have lower or undetectable levels.

MD has a wide clinical spectrum. The majority of infections due to *Neisseria meningitidis* are associated with uncomplicated bacterial meningitis, which has an excellent prognosis and a mortality <5%. However, 10%–15% of persons with MD develop meningococcal septicemia, with no evidence of meningitis. These patients have a mortality of 20%, rising to 60% of those who present with shock [3]. It is not known why some persons develop relatively mild meningococcal infection, as in meningitis or asymptomatic bacteremia, while others rap-

idly progress to fulminant septicemia. There is no apparent difference in the virulence of organisms that produce the different disease manifestations.

It is probable that host factors are important in determining susceptibility to fulminant MD. Increased secretion of TNF- $\alpha$  is likely to be a normal host response to bacterial invasion. As the plasma level of TNF- $\alpha$  appears to correlate closely with severity and outcome, and excessive levels are associated with fulminant disease, this raises the possibility that the development of fulminant MD may be determined by the genetic propensity of an individual to produce high levels of TNF- $\alpha$ .

Malaria is another infection in which severity has been associated with levels of circulating TNF- $\alpha$ . Children dying of cerebral malaria had 10-fold higher plasma TNF- $\alpha$  concentrations than those with uncomplicated malaria and levels 2 times higher than those who survived cerebral malaria [4].

Control of TNF- $\alpha$  secretion is believed to be regulated by variable genetic elements within the major histocompatibility complex (MHC), where the TNF- $\alpha$  gene resides [5]. A polymorphism that may directly influence the regulation of the TNF- $\alpha$  gene has been recently described in its promoter region. This biallelic polymorphism involves a single base substitution at -308 nt relative to the transcriptional start of the gene [6]. There are two allelic forms: TNF1 and TNF2. The more common TNF1 allele has guanosine in this position, whereas the TNF2 allele has adenosine. Possession of the TNF2 allele is thought to be associated with higher constitutive and inducible levels of transcription than is presence of the TNF1 allele [7].

A recent study of the frequency of the TNF2 allele in Gambian children showed an increased prevalence of homozygotes for the TNF2 allele in children with cerebral malaria, especially those who died or suffered severe neurologic sequelae, compared with nonmalaria controls or children with mild malaria [8]. Cases of severe malarial anemia without cerebral complications had a frequency of TNF2 homozygotes similar to that in the control groups.

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Approval for this study was obtained from the Ethics Committee of St. Mary's Hospital. Informed consent was obtained from the patients or their parents or guardians. Human experimentation guidelines of St. Mary's Hospital Medical School were followed in the conduct of this clinical research.

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We postulated that possession of the TNF2 allele might predispose to fulminant MD and therefore studied the distribution of TNF1 and TNF2 alleles in children with MD.

### Patients, Materials, and Methods

MD was diagnosed in children with suspected MD following microbiologic confirmation, including positive Gram's stain or culture for *N. meningitidis* or latex agglutination of capsular polysaccharide antigen from blood or cerebrospinal fluid or following a rise in specific antibody to meningococcal antigens in convalescent serum. A diagnosis of MD was made in patients presenting with a purpuric rash and fever and features of sepsis if no other pathogen was isolated despite thorough bacteriologic and virologic investigation.

Severity of MD was classified using the pediatric risk of mortality (PRISM) score [9], which has been validated as an accurate indicator of severity in MD [10]. This was assessed on hospital admission and was converted to a percent risk of mortality ( $p$ ) for each patient by using the described equation. Children with MD were stratified for severity using the PRISM score. They were divided into 2 groups, mild ( $p \leq 10\%$ ) and severe ( $p > 10\%$ ) disease. The figure of  $p \leq 10\%$  was prospectively chosen as an arbitrary cutoff between mild and severe MD. It obtains support from a study by Algren et al. [10], in which there was no mortality in a group with  $p < 15\%$ . Outcome was assessed at 28 days after admission and was classified as either survival or death.

DNA was prepared by standard methods [11] from citrated whole blood collected on admission from 98 children with acute MD of varying severity. A 107-bp fragment of the TNF- $\alpha$  gene promoter region containing the G-to-A substitution was amplified using the polymerase chain reaction (PCR). Primers used to amplify this region were designed to incorporate this polymorphism into an *NcoI* restriction site, as described by Wilson et al. [12]. A single base change at the 3' end of the 5' primer was required for the formation of the *NcoI* recognition sequence. The amplified DNA product was incubated with *NcoI* (GIBCO, Paisley, UK) according to the manufacturer's instructions. The presence of the TNF1 allele is associated with the *NcoI* restriction site, which produces 2 DNA fragments (87 and 20 bp), while the TNF2 allele does not have the *NcoI* restriction site.

After digestion, the PCR product was resolved by electrophoresis on a 9% polyacrylamide gel, stained with ethidium bromide, and visualized under UV light. Each run included a negative control and samples that were known to be positive and negative for the *NcoI* restriction site.

### Results

All children with a diagnosis of MD admitted to the Paediatric Unit at St. Mary's Hospital over a 24-month period were included in the study. The mean and median predicted risk of mortality calculated from the PRISM score for the group overall were 23% and 11%, respectively, so the number of deaths predicted for the 98 children in the study was 23. The observed mortality was 18 deaths. All deaths occurred in the group with severe MD ( $p > 10\%$ ).

Possession of the TNF2 allele was associated with a significantly increased risk of severe disease and death ( $P = .03$ ,  $\chi^2$  test). In children with the TNF2 allele, there was an increased proportion who had severe MD (22/33, 67%) compared with those who did not have the TNF2 allele (27/65, 41%;  $P = .02$ ,  $\chi^2$  test; relative risk [RR], 1.6; 95% confidence interval [CI], 1.1–2.3). The proportion of children who had the TNF2 allele and died (10/33, 30%) was significantly larger than the proportion of children who did not have the TNF2 allele and died (8/65, 12%;  $P = .03$ ,  $\chi^2$  test; RR, 2.5; 95% CI, 1.1–5.7). All deaths occurred within 14 days of admission (median, 24 h following illness onset).

Only 3 children in our study population were homozygous for the TNF2 allele. Two had severe and 1 had mild MD. All of these children survived.

There was no apparent difference between the prevalence of the TNF2 allele in our population of children with MD (0.17) and the known gene frequency of the TNF2 allele in both Caucasians and Africans (0.16) [8, 12]. There was no obvious association of racial origin or socioeconomic status with outcome in our study. We did not formally address socioeconomic factors. However, in the United Kingdom, emergency medical care is provided free of charge, and all inhabitants have immediate access to emergency medical care from both family practitioners and emergency physicians.

### Discussion

In children with MD, possession of the TNF2 allele appears to be associated with an increased risk of more fulminant infection, resulting in death. The RR of death in TNF1/TNF2 heterozygotes was 2.5 compared with that of children homozygous for the TNF1 allele. There was also a significant association between possession of the TNF2 allele and increased severity of meningococcal disease. This suggests that factors that may control secretion of TNF- $\alpha$  may be important in the determination of severity of meningococcal infection.

We found no difference in the frequency of the TNF2 allele in children with MD compared with that in other populations previously studied. Our results suggest that while the presence of the TNF2 allele does not increase the risk of acquiring invasive meningococcal infection, once an individual is infected with *N. meningitidis*, possession of the TNF2 allele appears to increase the risk of fulminant infection.

In MD, the level of circulating TNF- $\alpha$  has been shown to correlate directly with severity and outcome [2]. It follows that children who have a genetic propensity to secrete higher levels of TNF- $\alpha$  following invasion by meningococci would have a worse prognosis. In our present study of 98 children with MD of varying severity, the presence of this polymorphism in the promoter region of the TNF- $\alpha$  gene, which is thought to be associated with secretion of higher levels of TNF- $\alpha$  [7], was associated with more fulminant MD.

Our results differ from those described for Gambian children with malaria, a disease in which severity also correlates with level of TNF- $\alpha$ . In malaria, heterozygosity alone with the TNF2 allele is not associated with an increased risk of cerebral malaria or death. Only children homozygous for the TNF2 allele are at increased risk of severe cerebral malaria [8]. It is perhaps not surprising that our results differ from those for children with malaria. Apart from the association between level of TNF- $\alpha$  and disease severity, the pathophysiology of malaria differs markedly from that of MD.

The small number of children in our study who were homozygous for the TNF2 allele did not allow sufficient power to address adequately whether there was any association between fulminant MD and homozygosity for this allele.

It is likely that presence of the TNF2 allele is only one of several risk factors for fulminant MD. The TNF- $\alpha$  gene is in linkage disequilibrium with several HLA alleles that may also be involved with the control of TNF- $\alpha$  secretion or that may be independent risk factors for the development of MD or other forms of sepsis [6]. However, the Gambian study of children with malaria found that this polymorphism at -308 nt in the TNF- $\alpha$  gene promoter region is an independent risk factor for the severity of cerebral malaria and was not linked to MHC HLA class I or II variation [8]. There are no clear data regarding HLA linkage in children with MD or other forms of sepsis, although there is a suggestion that particular complement deficiencies, which are known to predispose to MD, are linked to certain HLA haplotypes, particularly HLA-B27 [13].

Apart from other, as yet unknown genetic and environmental controls of TNF- $\alpha$  secretion, the level of circulating TNF- $\alpha$  is not the only factor associated with fulminant disease. Other important factors involved in the outcome from MD include the rapidity of receiving appropriate medical care (including parenteral antibiotics and fluid resuscitation), level of plasma endotoxin, and derangements of coagulation and complement factors, which have all been shown to have an influence on outcome [3].

Our study suggests that polymorphisms that result in altered function of genes that control the immune response to infection

and the inflammatory response may be important in determining the clinical manifestations and outcome of severe infections such as meningococcal disease.

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