Genetic influence on cytokine production and fatal meningococcal disease

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Summary

Background To assess the genetic influence on cytokine production and its contribution to fatal outcome, we determined the capacity to produce tumour necrosis factor-α (TNFα) and interleukin-10 (IL-10) in families of patients who had had meningococcal disease.

Methods We studied 190 first-degree relatives of 61 patients with meningococcal disease; we also studied 26 monozygotic twins. Production of cytokines was determined during endotoxin stimulation of whole-blood samples ex-vivo. Heritability was estimated in a pedigree-based maximum-likelihood model. DNA was typed for the G to A transition polymorphisms at position −308 and −238 in the TNF gene promoter.

Findings Heritability in monozygotic twins was 0·60 for the production of TNF and 0·75 for the production of IL-10. Families with low TNF production had a tenfold increased risk for fatal outcome (OR 8·9, 95% CI 1·8–45), whereas high IL-10 production increased the risk 20-fold (19·5, 2·3–165). Families with both characteristics had the greatest risk. The transition polymorphisms in the TNF gene promoter were not associated with outcome.

Interpretation Genetic factors substantially influence production of cytokines. An innate anti-inflammatory cytokine profile may contribute to fatal meningococcal disease.

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Introduction

There is a strong genetic component to fatal infectious disease. Adoptees have a fivefold increased risk of fatal infectious disease if a biological parent has died from infection.[1] By contrast, death from infection in an adoptive parent resulted in no excess relative risks of death. Our knowledge of the factors that contribute to this genetic susceptibility is limited.

Tumour necrosis factor (TNF) is a decisive pro-inflammatory mediator in the host defence to infection. Treatment with recombinant human TNF can protect animals against lethal gram negative bacterial sepsis, whereas knock-out mice that lack the TNF-receptor type-1 are highly susceptible to infection.[2,3] Analogously, interleukin-10 (IL-10), a potent inhibitor of TNF, attenuates the clearance of various pathogens.[4,5] An anti-inflammatory cytokine profile during the initial phase of infection dampens the non-specific host response and favours growth of microorganisms (figure 1).

We have found that patients who survived fulminant meningococcal infection had low TNF production when their blood samples were incubated with endotoxin, even months to years after the acute phase of infection.[6] To examine the heritability of cytokine production in relation to outcome, we studied the production of cytokines in first-degree relatives of patients with meningococcal infection and in monozygotic twins.

Patients and methods

Between January, 1989, and February, 1994, 80 patients with meningococcal disease were admitted to the University Hospital Leiden. Diagnosis was based on the typical clinical presentation and bacterial cultures from blood and cerebrospinal fluid. Serogrouping revealed that more than 90% of isolates were *Neisseria meningitidis* group B, the remainder being group C.

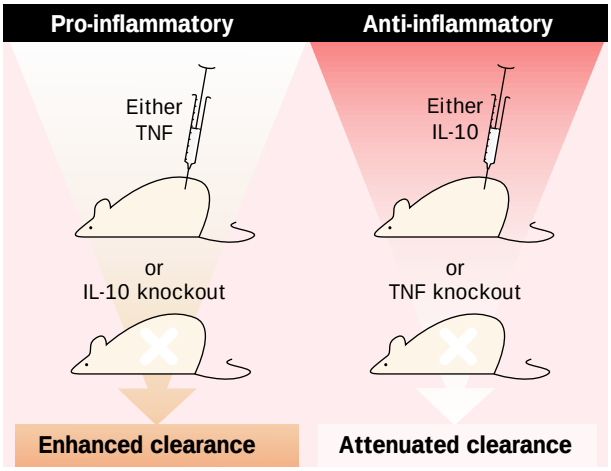

Figure 1: **Effect of TNF and IL-10 on the clearance of microorganisms in animal models**

	Relatives of:	
	Survivors (n=138)	Non-survivors (n=52)
Age range (years)	1·6–67·4	1·7–69·7
Sex (M/F)	66/72	27/25
Parents (%)	76 (55)	28 (54)
Siblings (%)	56 (41)	24 (46)
Offspring (%)	6 (4)	0

The first-degree relatives are part of 45 families of patients who survived and 16 families of patients who did not survive.

Table 1: **Characteristics of first-degree relatives of patients with meningococcal disease**

Serogroups A, X, Y, W134, and 29E were not detected. 16 patients died.

Previously, we studied 50 survivors of infection.[6] For the present study all first-degree relatives of the survivors (n=50) and the non-survivors (n=16) were invited to participate. From 222 first-degree relatives aged over 18 months, 190 (86%) were enrolled (table 1). Five families of survivors refused to take part, whereas all the families of dead patients participated, leaving a total of 61 families for analysis. Additionally, we studied 26 monozygotic twins (six men and 20 women, age range 31 to 71 years). None of the twins had a family history of meningococcal disease. The study was approved by the local hospital ethics committee and informed consent was obtained from all patients or their parents.

All participants visited the outpatient clinic between 0800 h and 1200 h to limit circadian variation in cytokine production.[7] All were free from clinical disease and did not use medication that might have affected cytokine production. The production of cytokines was determined in whole-blood samples ex vivo.[8] The concentration of cytokines was measured by ELISA. Unlike our earlier study, samples were collected from heparin-anticoagulated blood instead of edetic acid-anticoagulated blood. Consequently, the concentrations of cytokines in the present study are a magnitude higher. In two individuals endotoxin stimulation failed for technical reasons. In six families (24 samples) overnight stimulation was not done and IL-10 data are not available.

DNA was isolated from sodium dodecyl sulphate-lysed and proteinase-K-treated peripheral blood cells by phenol-chloroform extraction. Fragments of the TNF gene promoter were amplified by PCR to detect the G-to-A transition polymorphism at positions –238 and –308.[9,10]

Heritability of cytokine production—the proportion of the population variation attributable to genetic variation—was determined with a pedigree-based maximum likelihood method.[11] For a given pedigree of individuals a vector of observations and a vector of expected values is defined. These vectors depend on fixed variables such as group membership—ie, family members of surviving or dead patients, or monozygotic twins. The covariances between the family members for the residual part of the observations—ie, the part that is not accounted for by the fixed variables—depend on the relationships between the pedigree members and on the genetic model assumed for the observations. We modelled (FISHER package) the variance not accounted for by the fixed variables as consisting of additive genetic and random environmental variance. We also tested to see if familial resemblance could be accounted for by shared environment instead of shared genes. The likelihoods obtained for the different models were compared with χ^2 difference tests.[12]

Between groups, cytokine production was compared with non-parametric tests that avoided any distributional assumptions. Relative risks for fatal meningococcal disease, and the corresponding 95% CIs, were estimated with the exposure odds ratio (OR) by simple cross tabulation. Allele frequencies were compared with χ^2 tests.

Results

First, the correlations for cytokine production capacity between spouses were constrained at zero (table 2). The likelihood of the model did not significantly decrease indicating that the cytokine production capacity of spouses is not correlated (model 2,3). In a similar analysis, the correlations between siblings were similar and equal to the parent-offspring correlations (model 4,5,6). When the correlations between siblings were constrained equal to the correlations between monozygotic twins, the likelihood of the model decreased (model 7). This decrease was, however, not significant for TNF. The likelihood of the model also decreased significantly when the correlations between siblings or monozygotic twins were constrained at zero (model 8,9). Hence, a model with positive but unequal correlations for siblings and monozygotic twins appears to be the best model (model 6). In this model, the mean (SE) correlation for TNF in monozygotic twins was 0·60 (0·17) and higher than the correlation in siblings (0·22 [0·07], not significantly different). In monozygotic twins, the correlation for IL-10 was 0·75 (0·12) and also higher than the correlation in siblings (0·33 [0·06], p=0·03).

To further explore the hypothesis that shared environment contributes little to the production of cytokines we studied the differences in 47 parents. Four pairs were excluded because they were divorced. For all other parents it was assumed that they had shared environment since the age of 20 years. Differences between spouses in the production of TNF were not associated with the time they lived together (r=0·008, p=0·95). Similar results were obtained for the production of IL-10 (r=0·02, p=0·88).

Under basal conditions, the production of TNF and IL-10 was low and not different between the relatives of survivors or non-survivors (table 3). During 6 h incubation with 10 and 1000 ng/mL endotoxin from *Escherichia coli* O111:B4, TNF production in the relatives of dead patients was only half the production in the relatives of patients who survived (p<0·001, table 3). During 24 h stimulation with 1000 ng/mL endotoxin, IL-10 production was twofold higher in the relatives of

Model	TNF production capacity			IL-10 production capacity		
	Log-likelihood	χ^2	p	Log-likelihood	χ^2	p
1 General	−192·66	..	..	−129·62	..	..
2 Spouse correlation dead=0	−192·76	<1	p>0·05	−130·43	1·62	p>0·05
3 Spouse correlation survived=0	−194·57	3·62	p>0·05	−131·49	2·12	p>0·05
4 Sibling correlation dead=survived	−194·64	<1	p>0·05	−132·91	2·84	p>0·05
5 Parent offspring correlation dead=survived	−196·99	4·7	0·03	−133·04	<1	p>0·05
6 Sibling=parent offspring correlation	−197·46	<1	p>0·05	−133·32	<1	p>0·05
7 Sibling=parent offspring=MZ correlation	−198·81	2·69*	p>0·05	−135·76	4·88*	0·03
8 Sibling=parent offspring correlation=0	−202·09	9·26*	0·002	−144·98	23·32*	<0·001
9 MZ correlation=0	−200·41	5·90*	0·02	−138·81	30·98*	<0·001

The general model estimates mean effects, variances, and familial correlations for the three groups separately. MZ=monozygotic twins. *Compared with model 6. In model 6 the mean (SE) correlations for TNF were estimated as 0·60 (0·17) in MZ and 0·22 (0·07) in first-degree relatives. Correlations for IL-10 were estimated as 0·75 (0·12) in MZ and 0·33 (0·06) in first-degree relatives.

Table 2: **Model fit for the production of cytokines from a pedigree-based maximum-likelihood method**

Cytokine production	Relatives of survivors	Relatives of non-survivors	p
	Mean (median [IQR])	Mean (median [IQR])	
TNF production (pg/mL)*			
Basal conditions	28 (10 [0–30])	46 (17 [11–29])	>0·05
+10 ng/mL endotoxin	3653 (3236 [2011–4343])	1615 (1388 [967–2007])	<0·0001
+1000 ng/mL endotoxin	5881 (5276 [4124–7225])	3929 (3584 [2761–4588])	<0·0001
IL-10 production (pg/mL)†			
Basal conditions	29 (18 [6–34])	47 (8 [4–20])	>0·05
+1000 ng/mL endotoxin	2298 (2028 [1237–2964])	4524 (3949 [2598–6418])	<0·0001

IQR=interquartile range. *Data available from 136 relatives of survivors and 52 of non-survivors. †Data available from 121 relatives of survivors and 43 of non-survivors. Production of TNF was determined in a whole blood stimulation assay after 6 h incubation and the production of IL-10 after 24 h incubation. Similar significant differences are obtained when parents only were included in the analysis.

Table 3: **Production of cytokines in relatives of patients who survived or did not survive meningococcal disease**

patients who died (p<0·001, table 3). Similar associations were obtained when the relatives were arranged according to the severity of the disease during initial hospital admission (p<0·05). The production of TNF after 6 h incubation, and IL-10 after 24 h incubation, were not associated (r=0·11, p=0·19).

Using a dichotomy around the median, we classified cytokine production within families as low or high (figure 2). Families with low TNF production had a tenfold increased risk for fatal meningococcal disease (OR 8·9, 95% CI 1·8–45). Families with high IL-10 production had a 20-fold increased risk for fatal disease (19·5, 2·3–165). Families with both characteristics had the highest risk (OR indeterminately high).

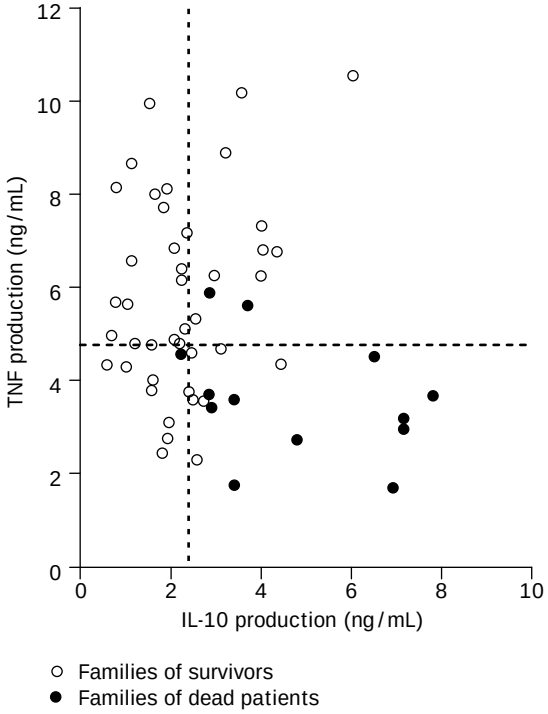

Figure 2: **Production of TNF and IL-10 in whole blood samples incubated with 1000 ng/mL endotoxin**

Symbols represent the family means of TNF production and IL-10 production. Open circles represent cytokine production in 42 families of patients who survived (121 first-degree relatives), and closed circles represent production of cytokines in 13 families of dead patients (43 first-degree relatives). Dotted lines indicate medians of the family estimates for both cytokines

Polymorphism	Relatives of:		Controls (%)
	Survivors (%)	Non-survivors (%)	
−308 GG	79 (59)	29 (57)	50 (57)
−308 GA	50 (38)	20 (39)	34 (34)
−308 AA	4 (3)	2 (4)	4 (2)
−238 GG	121 (91)	42 (82)	78 (89)
−238 GA	12 (9)	9 (18)	10 (11)

Controls are healthy blood donors. In a few samples DNA isolation failed. Distributions are similar when parents only are included in the analysis.

Table 4: **Gene frequencies in first-degree relatives of patients with meningococcal disease**

Distributions of bi-allelic polymorphisms at the –308 and the –238 nucleotide are presented in table 4. No differences were observed between the –308 genotype frequencies in relatives of survivors and non-survivors (χ^2 0·2, p>0·8). A small difference was observed in the distribution of the –238 genotypes (χ^2 2·7, p=0·09). Cytokine production capacity was not materially different for the various genotypes (data not shown).

Discussion

Our study identifies a strong genetic influence on the production of cytokines upon ex-vivo stimulation of whole blood with endotoxin. Approximately 60% of the variation in TNF production and 75% of the variation in IL-10 production appears genetically determined. Families characterised by low TNF production had a tenfold increased risk for fatal outcome, whereas high IL-10 production increased the risk 20-fold. Families with both characteristics had the highest risk. Taken together the data suggest that innate capacity to produce cytokines contributes to familial susceptibility for fatal meningococcal disease.

It is tempting to speculate which mechanisms contribute to the regulation of TNF production upon stimulation with endotoxin. The two G-to-A transition polymorphisms at position –308 and –238 in the TNF gene promoter were not associated with outcome and could not explain the marked interindividual variation in TNF production capacity. This finding is in line with our in-vitro experiments showing that production of TNF is not affected by the –308 polymorphism.[9] These observations call into question the significance of the –308 transition polymorphism in the outcome of infectious disease.[13]

The complement system plays a pivotal part in the host response against meningococci. The genes for the complement system are located within the major histocompatibility complex class III region, in the vicinity of the TNF gene. It is, however, unlikely that deficiencies in the complement system have influenced our results. Complement deficiencies are associated with the serogroups X, Y, Z, W135, 29E, and, to a lesser extent, serogroup C.[14] Apart from three patients with group C, these serogroups were absent in our patient series.

The high correlation between cytokine production capacity in monozygotic twins shows that cytokine production can reliably be assessed in a whole-blood stimulation assay ex-vivo. Other investigators have shown less than 15% variation when individuals are sampled over time.[7] Low doses of endotoxin have been administered to healthy volunteers and their circulatory cytokine concentrations measured.[15] Subsequently the volunteers' cytokine production capacity was determined in a whole-blood stimulation assay.[16] The observed

interindividual differences in whole-blood stimulation assay were similar to the differences in cytokine concentrations after intravenous administration of endotoxin.

The anti-inflammatory cytokine profile in relatives of dead patients contrasts with the high circulating concentrations of TNF in patients with meningococcal infection during hospital admission.[17] However, in meningococcal sepsis the circulating concentrations of endotoxin are grossly elevated too.[18] Although production of endotoxin may differ between bacterial strains, high concentrations of lipopolysaccharide indicate a large bacterial load. Consequently, the high TNF concentrations may be interpreted as the expression of advanced infection, notwithstanding the fact that the disease may only be present for 12 to 24 h.

The low production of TNF in the first-degree relatives of dead patients is in accordance with the low TNF inducibility found in the survivors of fulminant meningococcal disease.[6] These data refute our earlier interpretation that low TNF production among the survivors of a fulminant course is the result of selective mortality. Families who combine low TNF and high IL-10 production have the highest risk for an adverse outcome. Apparently, an innate anti-inflammatory cytokine profile increases susceptibility for fatal meningococcal disease, an observation that is in line with current experimental evidence.[19] It is not yet clear, however, if these findings are generalisable to other infectious diseases.

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