An Interleukin-1 Genotype Is Associated with Fatal Outcome of Meningococcal Disease


To determine whether known variants of the interleukin-1 (IL-1) and tumor necrosis factor (TNF) gene families are associated with severe manifestations of meningococcal disease, 276 white patients 4–70 years of age (median, 17 years) were genotyped. All patients had microbiologically proven Neisseria meningitidis infection; 39 died and 237 survived. A significant association \((P < .001)\) was found between fatal outcome and genotype at \(IL1B\) (nucleotide position \(-511\)). Homozygous individuals, both for the common \(1/1\) and the rare \(2/2\) alleles, had increased odds ratios (ORs) for death, compared with heterozygous individuals \(1/2\): ORs (95\% confidence intervals [CIs]) were 3.39 (1.39–8.29) and 7.35 (2.51–21.45), respectively. The mortality rates according to genotype at \(IL1B\) (\(-511\)) were 18.0\% (\(1/1\)), 6.1\% (\(1/2\)), and 32.3\% (\(2/2\)), compared with 14.2\% overall. The composite genotype, consisting of heterozygosity of \(IL1B\) (\(-511\)) together with homozygosity of the common allele of the IL-1 receptor antagonist gene (\(IL1RN\)) at \(+2018\), was significantly associated with survival \((P = .018; \text{OR}, 7.78 [95\% \text{CI}, 1.05–59.05])\). There was no association between TNF genotype and fatal outcome. These data suggest that IL-1 genotype influences the severity of meningococcal disease.

The death rate from meningococcal disease is 5\%–20\%, despite antimicrobial therapy and intensive care treatment. The host’s cytokine response, which is genetically determined, reflects the severity of the disease [1]. The cytokines tumor necrosis factor (TNF)–\(\alpha\) and interleukin (IL)–1 are key mediators of the inflammatory response. Two genes, \(IL1A\) and \(IL1B\), located on human chromosome 2q13 encode the proinflammatory cytokines IL-1\(\alpha\) and IL-1\(\beta\), respectively, and a third gene, \(IL1RN\), encodes the IL-1 receptor antagonist (IL-1ra) [2].

Single-nucleotide polymorphisms (SNPs) are substitutions of a single base at a particular site. These are stably carried by a proportion of the population and have the potential to affect the protein product of the gene to which they are related. For example, \(TNFA\) has a biallelic polymorphism at position \(-308\) (i.e., 308 nt upstream of the transcriptional start), which is associated with a higher rate of transcription of TNF [3]. Likewise, there are SNPs within \(IL1A\) at position \(+4845\) [4] (i.e., 4845 nt downstream of the transcriptional start) and within \(IL1B\) at positions \(+3954\) [5] and \(-511\) [6]. The polymorphism at position \(-511\) is in 99.5\% linkage disequilibrium with another at position \(-31\) that has been shown to affect the transcription-initiation complex of \(IL1B\) and is associated with an increased risk of hypochlorhydria and gastric cancer in patients infected with Helicobacter pylori [7]. In addition, there is a variable-number tandem repeat repeat in \(IL1RN\), of which the less common allele 2 (\(IL1RN*2\)) is also associated with an adverse outcome of Helicobacter infection [7]. This allele is in linkage disequilibrium with several polymorphisms in \(IL1RN\), including one at position \(+2018\) within exon 2, which was previously denoted “(+8006)” [8].

To test whether known polymorphisms within the IL-1 and TNF genes are related to the outcome of meningococcal disease, we compared their distributions among DNA samples derived from survivors and fatal cases drawn from the populations of England and Wales.

Methods

Patients. Since October 1996, the Meningococcal Reference Unit (MRU) for England and Wales has offered a nationwide service for the detection of Neisseria meningitidis by polymerase
chain reaction (PCR) in blood or cerebrospinal fluid (CSF) samples, in addition to strain characterization in culture-proven cases and meningococcal serology. From January 1997 to June 1998, we archived consecutively received whole-blood samples from white patients 4–70 years of age who had had meningococcal disease. Samples were included in the study if meningococcal disease was verified by culture or PCR detection of N. meningitidis in blood or CSF. Once clinical information had been collated, samples were coded so that individuals could no longer be identified.

**Laboratory methods.** DNA was extracted by standard methods. We probed for 5 SNPs: IL1A (+4845), IL1B (+3954), IL1B (−511), IL1RN (+2018), and TNFA (−308) using a validated 5’ nucleic assay (TaqMan allelic discrimination test; Perkin Elmer Biosystems, Foster City, CA) [7]. Probes, primer sequences, and cycling conditions are available in an Appendix published only in the electronic edition of the Journal (http://www.journals.uchicago.edu/JID/). We indicate the frequent allele of each gene variant as “1” and the rarer allele as “2,” as in the standard nomenclature. Homozygosity is indicated as “1/1” or “2/2,” and heterozygosity is indicated as “1/2.” Carriage of one particular allele is indicated, for example, as “2+” for the rarer allele, meaning individuals who are either 2/2 or 1/2.

**Statistical analysis.** Heterozygous and homozygous odds ratios (ORs) for survival and death were calculated for each locus as estimates of the genotypic relative risks [9]. Subsequently, data were either analyzed by χ² analysis in a 2 × 3 genotype table or a 2 × 2 table for carriage of a specific allele. Composite genotype analyses over multiple loci were also done where appropriate. Monte Carlo techniques were used to calculate empirical P values [10] (Monte Carlo composite genotype method [MCCG]) when the standard χ² test on the relevant contingency table was invalid because of small expected values. A study-wide significance level of .01 was considered a reasonable threshold and is probably conservative, since we have previously shown a high degree of linkage disequilibrium in this region [11] and, therefore, individual tests are not strictly independent.

**Results**

**Patients and microbiologic data.** During the study period, 1120 microbiologically proven cases of meningococcal disease in England and Wales were verified, among patients 4–70 years of age, by the MRU. Over the same period, whole-blood samples for PCR detection of N. meningitidis were received from 5075 patients in this age group with suspected meningococcal infection. Of these, we identified 276 with culture- or PCR-proven meningococcal disease. Of the DNA samples, 39 were from patients who had died, and 237 were from patients who had survived. The mean age of patients who had died (26.4 ± 19.7 years) was not significantly greater than that for those who had survived (20.6 ± 14.9 years; P = .2, Mann-Whitney U test). The median age in both cases was 17 years. The serogroups of N. meningitidis detected by culture or PCR of samples from patients who had died were B (91 patients [39%]), C (104 patients [44%]), W135 (2 patients [1%]), and ungrouped (40 patients [17%]).

**Genotype distributions.** The distribution of gene polymorphisms among subjects with meningococcal disease are shown in table 1. Allelic distributions for IL1A (+4845), IL1B (+3954), and TNFA (−308) were similar. However, IL1B (−511) genotypes were significantly different between the groups (P < .001). Individuals homozygous (1/1 or 2/2) at IL1B (−511) were more likely to die from meningococcal disease, and heterozygotes were more likely to survive. The percentage of deaths in genotype groups 1/1, 1/2, and 2/2 were 18.0%, 6.1%, and 32.3%, respectively, and the ORs for death of individuals homozygous for allele 1 or 2 (1/1 and 2/2), compared with the ORs for heterozygous individuals, were 3.39 (95% confidence interval [CI], 1.39–8.29) and 7.35 (95% CI, 2.51–21.48), respectively.

We also observed an increased, but not significant, risk of death for individuals carrying the rare allele (2+) of IL1RN (+2018): Mortality rates were 11.3% and 17.3% for 1/1 and 2+., respectively. Composite genotype analysis was therefore done over IL1B (−511) and IL1RN (+2018). Table 2 illustrates the 2 × 6 contingency table that was generated. A χ² analysis (5 df) illustrated significant evidence for an association between the composite genotype over these loci and risk of death or survival (P < .001). For each genotype combination across these two loci, the mortality rate was higher when the individual was carrying the rare allele at IL1RN (+2018) (table 2). The lowest mortality rate (2.4%) was for the composite genotype 1/2 at IL1B (−511) and 1/1 at IL1RN (+2018), and the highest mortality rate (42.1%) was for the composite 2/2 and 2+. The OR for mortality, when compared between these two genotype combinations only, was extremely strong (OR, 29.09 [95% CI, 3.28–258.21]; P < .001). This increased risk of mortality was also observed when the “protective” composite (1/2 and 1/1) was compared against all other groups of genotype combinations together (OR, 7.78 [95% CI, 1.05–59.05]; MCCG P = .0183); only 1 (2.4%) of 41 patients with this composite genotype had died versus 38 (16.8%) of 226 who did not have this genotype. We found no
effect of age, sex, or year of presentation on genotype distribution, at any of the loci tested, among those who had survived and those who had died.

For technical reasons, not all samples were successfully genotyped. Of the 276 samples analyzed, 31 could not be genotyped for TNFA (−308), 17 for IL1A (+4845), 23 for IL1B (+3954), 8 for IL1B (−511), and 2 for IL1RN (+2018). Of the 276 blood samples analyzed, 28 (26 survivors, 2 deaths) were collected from a single infectious disease facility. To investigate the possibility of false-positive results due to population stratification, from a single infectious disease facility. To investigate the possibility of false-positive results due to population stratification, we reanalyzed the data after exclusion of this cohort. All significant associations remained.

Discussion

In patients with meningococcal disease, we observed that those heterozygous at IL1B (−511) were more likely than homozygotes to survive (OR, 4.08 [95% CI, 1.73–9.62]; P < .001). The mortality rate for individuals with genotype 1/2 was 6.1%, compared with 20.9% for those who were homozygous. The polymorphism at position −511 is a substitution of cytosine by thymidine; 43.5% of individuals in a northern England population are heterozygous at this position. We also observed an association between fatal outcome and composite genotype over IL1B (−511) and IL1RN (+2018) (P < .001). The composite genotype consisting of heterozygosity at IL1B (−511) plus homozygosity for the common allele at IL1RN (+2018) appeared to be protective against death in patients with meningococcal disease (OR, 7.78 [95% CI, 1.05–59.95]; MCCG P = .0183). This composite was present in 16.8% of the survivors but in only 2.4% of the patients who had died.

The potential importance of IL1B and IL1RN polymorphisms in other infectious diseases, notably periodontitis [12] and infection by H. pylori [7], has been demonstrated. The same (or linked) proinflammatory polymorphisms at IL1B (−511) and IL1RN have been shown to be involved in the interaction between hypochlorhydra, gastric cancer, and infection by H. pylori [7]. IL-1β and IL-1ra are proinflammatory and anti-inflammatory, respectively, in vivo, and the IL-1β:IL-1ra molar ratio is the most important determinant of IL-1 activity. Although this work demonstrates a significant association of IL1B (−511) with death from meningococcal disease, it is intriguing that persons homozygous for both the common and the rare alleles appear to be at increased risk. This is a surprising observation and should be confirmed in a larger study. It is possible that the advantage for persons heterozygous at IL1B (−511) is based on a gene dose effect on the early IL-1 response to N. meningitidis infection. The IL-1β:IL-1ra molar ratio in heterozygotes may be neither too great nor too small to overcome meningococcal infection without contributing to dangerous metabolic disruption. The involvement of homozygosity at IL1RN (+2018) in a “protective” composite genotype may reflect the fact that variation within IL1RN has been shown to enhance IL-1β release in vitro [13].

Other cytokine gene polymorphisms may also contribute to the severity or outcome of meningococcal disease. Nadel et al. [14] studied 98 infants and children with meningococcal disease, 18 of whom died. Among the children who carried the rarer allele of TNFA (−308), there were more deaths (OR, 2.5, P = .03) and a higher incidence of severe disease (OR, 1.6, P = .02). Our study of a different and older population did not confirm this association and included sufficient numbers of patients to detect a 1.2 increase in allelic frequencies (of this polymorphism), with a power of 80%. Severity of meningococcal disease is likely to be under polygenic influence. Variations in the gene encoding plasminogen-activator–inhibitor–1 are also associated with severity of meningococcal sepsis [15].

Some caution should be applied to the interpretation of data derived from specimens referred to a reference laboratory rather than from a population-based sample. We were careful to include only DNA from patients with microbiologically proven disease, but this may have introduced bias caused by an effect of cytokine genotype on diagnostic yield (unlikely) or some other unidentified effect related to referral of the sample. The only outcome variables we sought to identify were death and survival, as these are easily defined and verifiable, but we acknowledge that sociomedical factors, such as access to medical care, inevitably influence individual clinical outcomes. We genotyped patients between the ages of 4 and 70 years, to exclude

### Table 2. Composite genotype analysis of the interleukin (IL)–1 gene polymorphisms IL1B (−511) and IL1RN (+2018).

<table>
<thead>
<tr>
<th>Patient group</th>
<th>1/1 1/2 2/2</th>
<th>Total no. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1/1 1/2 2/2</td>
<td></td>
</tr>
<tr>
<td>Deceased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survivors</td>
<td>85 36 24</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>85 36 24</td>
<td></td>
</tr>
<tr>
<td>Mortality rate</td>
<td></td>
<td>15.3 25.0 2.4 8.1</td>
</tr>
</tbody>
</table>

NOTE. P = .0005, χ², 5 df; 1. Upper line (1/1, 1/2, and 2/2) is IL1B (−511), and lower line (1/1 and 2+) is IL1RN (+2018). 2. Carriage of (frequent allele; 2, carriage of rare allele; 2+, carriage of rare allele of IL1RN (+2018) (either homozygous or heterozygous).
patients at the extremes of age who may have had features that distort the influence of genetic factors on outcome. In the United Kingdom during 1995–1998, 3.2% of identified cases were >70 years of age, but this group represented 10% of deaths from meningococcal disease, a discrepancy that increases with age. Patients <4 years of age represented 43% of cases but only ~32% of deaths (E.B.K., unpublished observations derived from data from the Public Health Laboratory Service).

In summary, our data suggest that IL-1 allelic variants may be important disease modifiers of meningococcal disease, and polymorphisms of the IL-1 genes may be among a number of genetic markers of poor prognosis in *N. meningitidis* infection.

**Acknowledgments**

We thank Michael McKendrick, Stephen Green, John Anson, Simone Gregory, and Pauline Whitaker for their clinical, laboratory, or administrative expertise.

**References**

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