Reply

Sir—The experience reported by Myoken et al. [1] of superinfection with fungal organisms resistant to itraconazole in patients who are receiving the drug as prophylaxis is of interest. We have not seen superinfections with resistant organisms in immunocompromised HIV-infected patients receiving prophylactic itraconazole. In our experience [2] and in that of others who have reported in vitro testing results [3–5], *Penicillium marneffei* strains have been uniformly susceptible to itraconazole. However, resistance to fluconazole is not uncommon. In our double-blind trial [6], 1 (1.6%) of 63 subjects assigned to receive itraconazole developed a disseminated *P. marneffei* infection, and 11 (16.7%) of the 66 persons who received placebo developed a systemic fungal infection (7 of these patients developed cryptococcal meningitis, and 4 developed *P. marneffei* infection). We have not done in vitro testing of isolates from these patients. Because the study was blinded, we were not aware of which regimen the patients were receiving at the time that infection was diagnosed. However, the lower rates of *P. marneffei* infection seen among patients receiving itraconazole suggest that the drug may have been effective. Whether the breakthrough infection that occurred in the single patient who became infected with *P. marneffei* could have been due to dosing or pharmacokinetic issues, rather than to resistance of the organism to itraconazole, is unclear. We agree with Myoken et al. [1] that monitoring of patients receiving itraconazole who develop fungal infections and susceptibility testing of isolates from these patients could be important.

References

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Variable Number Tandem Repeat Polymorphism of the Interleukin-1 Receptor Antagonist Gene in Meningococcal Disease

Sir—We read with interest the article by Witkin et al. [1] that described the role of the variable number tandem repeat (VNTR) polymorphism of the IL-1 receptor antagonist (IL-1Ra) gene in different disease states. The role of the IL-1Ra VNTR polymorphism in meningococcal disease (MCD) has not, to our knowledge, previously been studied, and therefore we aimed to determine whether this polymorphism might influence susceptibility to disease, disease severity, or disease type.

One hundred forty-four children with a diagnosis of MCD who were admitted to a single tertiary children’s hospital were prospectively studied between December 1997 and March 1999. Informed consent was obtained from the parents of all children included in the study and from controls, and the study protocol was approved by the Local Research Ethics Committee of the Royal Liverpool Children’s National Health Service Trust. Cases were defined and investigated as described elsewhere [2]. Severity of disease was assessed using the Glasgow Meningococcal Septicaemia Prognostic Score (GMSPS) [3, 4]. Severe disease was defined by a GMSPS of ≥8. Anonymous healthy blood donors (n = 95) were used as controls; of these, 52 (53%) were male.

IL-1Ra concentrations were determined using a commercially available solid-phase enzyme-amplified sensitivity immunoassay performed on microtiter plates (Medgenix IL-1Ra EASIA Kit; Biosource). If the IL-1Ra concentrations in a blood sample were higher than the upper limit of the standard curve, additional dilutions were not performed. In the statistical analysis, nonparametric tests for ranked or ordinal data were used to account for this. A primer set was used to amplify the 86-base tandem repeat region contained in intron 2 of the IL-1Ra gene. The primer set consisted of primer 1 (5′-CTG AAG ACT CCT AT-3′) and primer 2 (5′-TCC TGG TCT GCA GGT AA-3′). PCR was performed in a Prowe Thermal cycler (Techne) programmed for 45 cycles of 50 s at 95°C, 40 s at 57.5°C, 30 s at 72°C, and 3 min at 72°C. The PCR product was electrophoresed using a 100-bp DNA ladder as a marker, at 100 V and 100 mA for 45 min on a 2% agarose gel, and the gel was

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 variable number tandem repeat polymorphism of the interleukin-1 receptor antagonist gene in meningococcal disease

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Table 1. Genotype and allele frequencies among children with meningococcal disease and controls.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients (n = 144)</th>
<th>Controls (n = 95)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype, no. (%) of subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1/A1</td>
<td>83 (58)</td>
<td>50 (53)</td>
</tr>
<tr>
<td>A2/A1</td>
<td>42 (29)</td>
<td>30 (22)</td>
</tr>
<tr>
<td>A2/A2</td>
<td>14 (10)</td>
<td>10 (11)</td>
</tr>
<tr>
<td>A1/A3</td>
<td>4 (3)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>A2/A3</td>
<td>1 (1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>A1/A4</td>
<td>0</td>
<td>1 (1)</td>
</tr>
<tr>
<td>A2 frequency, % (95% CI)</td>
<td>0.25 (0.19–0.30)</td>
<td>0.27 (0.21–0.33)</td>
</tr>
</tbody>
</table>

visualized in ultraviolet light. Four alleles were identified: A1, 410 bp; A2, 240 bp; A3, 500 bp; and A4, 325 bp.

Statistical analysis was performed using SPSS 10.0 for Windows and Epi Info software, version 6 (Centers for Disease Control and Prevention). The difference in mean values was determined using the Mann-Whitney test for 2 groups and the Kruskal-Wallis test for 3 groups. Associations were determined using the χ² test (and Fisher's exact test, where appropriate), and relative risk analysis was used to determine risk.

The age range of the patients was 0.1–15.7 years (median age, 2.8 years [interquartile range, 1.0–7.0 years]). A total of 78 (54%) of the 144 children were male. A total of 63 (44%) had a GMSPS of ≥8 at admission; 10 (7%) of the 144 had meningitis alone, 73 (51%) had both meningitis and septicaemia, and 61 (42%) had septicemia alone. The diagnosis was confirmed microbiologically in 101 children (70%) by culture of blood or CSF samples, blood antigen testing, or PCR analysis of blood or CSF for meningococcal DNA. IL-1Ra concentrations ranged from 100 to >1750 pg/mL; of the 125 children for whom IL-1Ra was found in samples, 116 (93%) had concentrations >1750 pg/mL.

The frequencies of the different genotypes are shown in table 1. There was no difference in the frequency of A2 between patients and controls. There was no significant difference in IL-1Ra concentrations between subjects with A2 and subjects without that allele. Among patients with A2, the relative risk of severe disease was 1.0 (95% CI, 0.69–1.47), and the OR was 1.01 (95% CI, 0.49–2.09). The relative risk of death due to MCD among patients with A2 was 2.54 (95% CI, 0.63–10.23), and the OR was 2.69 (95% CI, 0.53–14.94).

Fewer children who had septic shock or severe disease or who required ventilation had A2 than did not (11 [41%] vs. 16 [59%] of 27 children, 25 [40%] vs. 38 [60%] of 63 children, and 23 [38%] vs. 38 [62%] of 61 children, respectively). However, this difference did not reach statistical significance. No significant associations were seen between genotype and disease type.

This polymorphism has been shown to be functional—A2 is associated with higher levels of IL-1Ra [5, 6]. Persons homozygous for allele 2 of the IL-1Ra gene have a more prolonged and more severe proinflammatory immune response than do persons with other IL-1Ra genotypes. Being A2 homozygous may therefore be beneficial in combating infectious agents. The reason that this was not demonstrated in our study is unclear. Most of the patients had very high concentrations of IL-1Ra. It may be that because additional dilutions were not performed in the determination of the IL-1Ra concentrations, it was not possible to discriminate between subjects who had extremely high levels and subjects whose levels were slightly higher than the upper limit.

Our study did not find any significant associations between the presence of A2 and disease severity, type of disease, or risk of dying. However, there was a trend suggesting that A2 was associated with less-severe disease. There was no significant difference in A2 frequency between patients and controls; therefore, this polymorphism does not appear to influence susceptibility to disease. The polymorphism itself may regulate IL-1Ra production, or it may be in linkage disequilibrium with another polymorphism regulating IL-1Ra production or another genetic determinant of susceptibility to and severity of MCD.

A larger study examining the role of the IL-1Ra VNTR polymorphism in MCD and in IL-1Ra production and expression is needed to confirm this finding and is being planned. The robustness of any results can be confirmed using the transmission disequilibrium test [7, 8], which analyzes whether the transmission of an allele from parents to the index case patient is significantly higher than expected and removes the confounding effect of ethnicity.

A common functional polymorphism of the IL-1Ra gene does not appear to influence disease susceptibility or risk of death from MCD, but it may influence disease severity. This suggests that IL-1Ra function may not be a critical determinant of the host response to meningococcal infection.

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