The intensity of malaria transmission is related to the pattern of malarial disease observed in different regions, but populations may also differ in their underlying predispositions to severe malarial anemia or cerebral malaria. In western Kenya, where severe malarial anemia is much more common than cerebral malaria, the distributions of tumor necrosis factor (TNF)–α, interleukin (IL)–10, transforming growth factor (TGF)–β, IL–6, and interferon (IFN)–γ alleles were examined in a cohort of young men. The cohort displayed a marked bias toward genotypes associated with low expression of IFN–γ and IL–6, cytokines that, at high levels, have been implicated in malarial anemia and poor malaria outcomes. By contrast, the frequency of the TNF–α –238A allele, which has been associated with severe malarial anemia, was found to be similar to the frequency previously reported in comparison populations in Africa and elsewhere. IFN–γ and IL–6 genotypes may play roles in the development of severe malaria and could contribute to the relative frequency of severe malarial anemia or cerebral malaria in exposed populations.

Mortality due to the malaria parasite Plasmodium falciparum is highest among young children; death in this group may be the direct result of severe disease or the indirect result of malarial infection in the mother during pregnancy, which can lead to low birth weight and infant mortality. Total malaria morbidity is similar for a wide range of transmission intensities, although the type of syndrome caused by infection varies. Severe malarial anemia is the dominant syndrome in areas of high transmission, whereas cerebral malaria is more common in areas with lower transmission levels [1]. In addition to the effect of transmission intensity, populations may also differ in their susceptibility to these syndromes. For example, severe malarial anemia is more common in East African than in West African populations, even after accounting for the effects of transmission intensity [1].

Cytokine levels, particularly elevated levels of tumor necrosis factor (TNF–α), have been associated with both severe malarial anemia and cerebral malaria, and, thus, factors that modulate cytokine expression may be of clinical importance. Recently, polymorphisms in several cytokine genes have been described, including those of TNF–α, interleukin (IL)–10, transforming growth factor (TGF)–β, IL–6, and interferon (IFN)–γ [2, 3]. The relationship between individual cytokine polymorphisms and expression levels appears to be complex. For example, although relative levels of TNF–α production appear to be stable among individuals [4–6], relationships between specific TNF–α alleles and cytokine expression levels have varied among studies [7], in part because regulation of cytokine transcription is specific to cell type and stimulus [8].

Several studies have found associations between cytokine polymorphisms and a range of infectious, chronic, and autoimmune-related diseases [9]. Studies of the relationship between cytokine polymorphisms and malaria have focused on the −238, −308, and −376 sites in the TNF–α gene and the association of these sites with severe outcomes. Earlier work found that TNF–α −308A and −376A alleles were positively associated with cerebral malaria [10, 11] and that the TNF–α −238A allele was positively associated with severe malarial anemia [12] and negatively associated with cerebral malaria [11]. Although levels of several cytokines have been extensively examined for asso-
cations with malaria susceptibility, to our knowledge, no research has been published that examines the potential association between malaria outcomes and cytokine alleles other than those of TNF-α.

We hypothesized that cytokine allele frequencies could play an important role in the manifestation of malaria-related disease at a population level and that populations in areas of high malaria transmission might have profound biases in allele frequency resulting from selection. We therefore examined the frequency distribution of alleles for several cytokine genes, including the TNF-α, TGF-β, IFN-γ, IL-10, and IL-6 genes, in an area of East Africa where the incidence of severe malarial anemia greatly exceeds that of cerebral malaria.

Methods

Study site and cohort description. The study site in western Kenya was 10 km north of Lake Victoria, in the adjoining villages of Wangarot, Riwa Ojelo, and Waringa, Rarieda Division, Nyanza Province. The entomological inoculation rate for *P. falciparum* in this area can exceed 300 infectious bites per person per year [13]. Studies in nearby hospitals have shown that the rate of severe malarial anemia is nearly 40-fold higher than the rate of cerebral malaria among children in this population [14].

After the exclusion of individuals with abnormal results on hemograms or evidence of chronic disease on physical examination, 248 males aged 12–35 years entered the study at the beginning of the high-transmission (heavy rains) season in April 1996. These individuals were monitored for 3 rainy seasons in a treatment-reinfection study, as described elsewhere [15].

Blood sample collection and processing. In the second and third seasons, volunteers donated 10 mL of blood, collected in heparinized tubes, 2 weeks after receiving treatment with quinine and doxycycline. Blood was spotted onto filter paper and stored at room temperature for subsequent DNA extraction. Peripheral blood mononuclear cells (PBMC) were separated by ficoll-hypaque (Sigma) density centrifugation, resuspended in 10% dimethyl sulfoxide in fetal bovine serum, cryopreserved using temperature-controlled freezing boxes, and then placed in long-term storage in liquid nitrogen. Sufficient PBMC for cytokine analyses were obtained from 141 of 173 volunteers in the second season and 120 of 143 volunteers in the third season. Production of TNF-α, IFN-γ, and IL-10 by PBMC after stimulation of the cells with phytohemagglutinin or malaria antigen was measured as described elsewhere [15].

DNA extraction and genotyping. DNA was extracted from blood spots dried on filter paper using a commercial kit (Gentra Systems) according to the manufacturer’s instructions. Genotyping for the TNF-α −308G/A; TGF-β +869C/T and +915G/C; IFN-γ +874A/T; IL-10 −1082G/A, −819C/T, and −592C/A; and IL-6 −174G/C polymorphisms was carried out with a commercially available kit (OneLambda) that uses a sequence-specific priming polymerase chain reaction (PCR).

TNF-α −238 and −376 sites were genotyped using a nested PCR approach. A 650-bp fragment was amplified with universal primers N1F (5′-CTCAAGGAGCTCGTCTTTC-3′) and N1/2R (5′-CCGGATCATGCTTTCAGTG3′) [12], and then allele-specific upstream primers were used in conjunction with the downstream N1/2R primer to selectively amplify the −238A or −238G [10] and −376A or −376G (modified from [16]) alleles to yield 459- and 599-bp fragments, respectively. Allele-specific primers included N2 238G F (5′-AGACCCCCCTCGGAATCG-3′), N2 238A F (5′-AGACCCCCCTCGGAATCG-3′), N2 376G F (5′-TCTGTGATCCTGTCTGGGAAG-3′), and N2 376A F (5′-TCTGTGATCCTGTCTGGAAA-3′).

A duplex reaction was performed during the nested amplification that included primers that amplified 1 of 2 allele pairs: 238G and 376A or 238A and 376G. First-reaction conditions were 1 cycle of 95°C for 5 min; 45 cycles of 95°C for 30 s, 48°C for 45 s, and 72°C for 60 s; and then 1 cycle of 72°C for 4 min. Nested-reaction conditions were 1 cycle of 95°C for 5 min; 32 cycles of 95°C for 30 s, 54°C for 45 s, and 72°C for 60 s; and 1 cycle of 72°C for 4 min. Amplified products were sized by separation through 2% agarose gel (Sigma).

Validation of the results from the cytokine gene analysis was undertaken using 2 separate techniques: molecular beacon probes, for the −238 site, and restriction fragment–length polymorphism (RFLP) analysis, for the −376 site. For molecular beacon analysis, a nested reaction amplified an 84-bp fragment centering on the −238 position, using primers 238F (5′-CAGTCACTTTGGCCCAAGAG-3′) and 238R (5′-GGACACACAAACATCAAG-3′), and product was detected with probes for allele 238G (5′-AGCGCAGGAGTGCT-3′) and 238A (5′-AGACTCGGAAAGTCT-3′). Reaction conditions were 1 cycle of 95°C for 120 s and 35 cycles of 95°C for 10 s, 52°C for 30 s, and 72°C for 15 s; a Smart Cycler (Cepheid) was used. For RFLP analysis, a 627-bp segment amplified with primers N2F (5′-GCCCTCCGATTTCTGCTATC-3′) and N1/2R was subjected to digestion with the restriction enzyme Taq1 (New England Biolabs). The −376G allele was identified as a 627-bp uncut fragment, and the −376A allele as 581-bp and 46-bp digested fragments. One-fourth of the samples were validated using the molecular beacon and RFLP techniques, and the results previously obtained with the nested, allele-specific PCR assays were confirmed.

Statistical analysis. The distribution of cytokine allele frequencies in the present cohort was compared with the distribution in other populations reported in the literature by contingency table analyses and weighted analyses. We examined the relationships between cytokine genotypes and measures of parasitemia, including time to reappearance of parasitemia, mean parasitemia, and frequency of detectable parasitemia. The time to reappearance of parasitemia was examined with Kaplan-Meier models (group differences were evaluated with the log-rank test). Mean parasitemia and frequency of parasitemia required to be significant. Evidence for disequilibrium in genotype frequencies was examined with the Hardy-Weinberg equation. Cytokine responses, mean parasitemia, and frequency of parasitemia required log transformation (ln[ ]) to obtain normal distributions. Analyses were performed with StatView (version 5.0.1 for Macintosh; SAS Institute) and SPSS software (version 10.0 for Windows).

Results

Cytokine genotype and phenotype frequencies. Genotyping data from 148–150 volunteers were compared with data from
Table 1. Frequency of tumor necrosis factor (TNF–α), interferon (IFN–γ), and interleukin (IL–6) genotypes and alleles in the present study and in comparison populations.

<table>
<thead>
<tr>
<th>Population, reference(s)</th>
<th>TNF–α – 308 genotype and allele frequencies, %</th>
<th>TNF–α – 238 genotype and allele frequencies, %</th>
<th>IFN–γ + 874 genotype and allele frequencies, %</th>
<th>IL–6 – 174 genotype and allele frequencies, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G/G G/A A/A A</td>
<td>G/G G/A A/A A</td>
<td>T/T A/T A/A A</td>
<td>G/G C/G C/C C</td>
</tr>
<tr>
<td>Present study</td>
<td>85.3 14 0.7 7.7</td>
<td>88.7 10.6 0.7 6</td>
<td>20.6-20.7 53.9–54.3 25.0–25.5 47.6–47.9</td>
<td>98.7 1.3 0 0.7</td>
</tr>
<tr>
<td>Black, United States [17]</td>
<td>85.9 12.5 1.6 7.8</td>
<td>90.8–97.3 3.7–9.2 0–0.3 1.5–4.6</td>
<td>81.3–91.1 8.9–18.6 0 4.5–9.3</td>
<td>81.3–91.1 8.9–18.6 0 4.5–9.3</td>
</tr>
<tr>
<td>African, regions of perennial malaria transmission [18, 19]</td>
<td>82.8–82.9 15.3–16.2 1.0–1.8 9.1–9.5</td>
<td>91.0–99.0 1.8–3.0 0.0–0.5 0.5–4.7</td>
<td>83.3–45.1 39.2–44.1 15.7–24.2 35.3–45.5</td>
<td>83.3–45.1 39.2–44.1 15.7–24.2 35.3–45.5</td>
</tr>
<tr>
<td>African, regions of seasonal malaria transmission [10, 16, 24]</td>
<td>69.0–85.3 14.7–29.8 0–4.2 7.4–16.3</td>
<td>84.3–91.8 8.2–14.5 0.0–1.2 4.1–8.5</td>
<td>83.3–45.1 39.2–44.1 15.7–24.2 35.3–45.5</td>
<td>83.3–45.1 39.2–44.1 15.7–24.2 35.3–45.5</td>
</tr>
<tr>
<td>White [20–23] b</td>
<td>56.6–75.6 23.1–38.6 1.2–7.4 12.3–23.8</td>
<td>90.0–99.0 1.8–3.0 0.0–0.5 0.5–4.7</td>
<td>83.3–45.1 39.2–44.1 15.7–24.2 35.3–45.5</td>
<td>83.3–45.1 39.2–44.1 15.7–24.2 35.3–45.5</td>
</tr>
<tr>
<td></td>
<td>G/G G/A A/A A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present study</td>
<td>88.7 10.6 0.7 6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African, regions of seasonal malaria transmission [11, 16, 25]</td>
<td>90.8–97.3 3.7–9.2 0–0.3 1.5–4.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White [21, 23, 26] d</td>
<td>91.0–99.0 1.8–3.0 0.0–0.5 0.5–4.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present study</td>
<td>88.5 10.8 0.7 6</td>
<td>87.2–88.5 10.9–11.5 0.0–0.8 6.0–7.4</td>
<td>20.6–20.7 53.9–54.3 25.0–25.5 47.6–47.9</td>
<td>98.7 1.3 0 0.7</td>
</tr>
<tr>
<td>African, regions of seasonal malaria transmission [11, 12, 16]</td>
<td>84.3–91.8 8.2–14.5 0.0–1.2 4.1–8.5</td>
<td>83.3–45.1 39.2–44.1 15.7–24.2 35.3–45.5</td>
<td>83.3–45.1 39.2–44.1 15.7–24.2 35.3–45.5</td>
<td>83.3–45.1 39.2–44.1 15.7–24.2 35.3–45.5</td>
</tr>
<tr>
<td>White [21, 23, 26] d</td>
<td>91.0–99.0 1.8–3.0 0.0–0.5 0.5–4.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present study</td>
<td>2 20.8 77.2 12.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black, United States [27] d</td>
<td>7 55.8 37.2 34.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White [3, 27] d</td>
<td>20.6–20.7 53.9–54.3 25.0–25.5 47.6–47.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present study</td>
<td>98.7 1.3 0 0.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black, United States and United Kingdom [2, 27] e</td>
<td>81.3–91.1 8.9–18.6 0 4.5–9.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White [2, 27, 28] e</td>
<td>33.3–45.1 39.2–44.1 15.7–24.2 35.3–45.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE. P values were calculated by the χ² test; individual allele frequencies for the cohort of the present study were compared to the weighted averages for previously reported populations.

a. P < 0.046
b. P = 0.001
c. P < 0.077
d. P < 0.001
e. P < 0.01

other populations for single-nucleotide polymorphisms in TNF–α [10–12, 16–26], IFN–γ [3, 27], and IL–6 [2, 27, 28] genes (table 1) and haplotypes of TGF–β (table 2) and IL–10 (table 3) genes [3, 27]. The distribution of genotypes or putative cytokine expression phenotypes (i.e., expression levels predicted by genotypes) in this Kenyan cohort differed significantly from the distributions reported in other populations for all cytokine genes except TNF–α/H11002 238.

Differences in frequency were greatest in comparison with those in white populations. Differences were also significant in comparison with those in populations of West African ancestry, such as black US and Caribbean populations, for IFN–γ, IL–6, and TGF–β but not for TNF–α – 308 and IL–10. Black populations in urban areas on the eastern seaboard of the United States are commonly of West African ancestry with an ~30% white admixture [17]. Frequencies of TNF–α alleles but not IFN–γ, IL–6, IL–10, and TGF–β alleles have been reported for West Africa.

TNF–α – 308A and –376A allele frequencies in this cohort differed significantly from those in some but not all European and West African populations. By contrast, the TNF–α – 238A allele frequency was similar to that in comparison populations in Africa, Asia, and Europe. The bias in IL–6 alleles was most dramatic. The –174C allele was present in just 0.7% of study subjects (all heterozygotes), compared with an allelic frequency of 35.3%–45.5% among white individuals, 13.9% in Gujarati Indians, and 4.5%–9.3% in populations of West African ancestry. The frequency of IFN–γ genotypes was also profoundly biased. For example, the +874A/A genotype associated with low IFN–γ expression occurred more than twice as often in this Kenyan population (77.2%) than in white and black US populations (25%–37.2%). Hardy-Weinberg analysis found the distribution of all genetic polymorphisms to be in equilibrium.

Linkage between alleles. In pairwise analysis of linkage between alleles, only TNF–α/H11002 238A and –376A were significantly associated (P < 0.001). This finding was reported elsewhere as a complete linkage, with –376A occurring only in a subset of individuals who possess –238A [11]. The most common geno-
type, found in 94 (63%) of 149 subjects, was TNF-α −308GG/IFN-γ +874AA/IL-6 −174GG. Individuals with this genotype were significantly more likely to have an IL-10 genotype that was associated with high expression than were individuals without this genotype (15% vs. 4%; P = .03).

Relationship between cytokine genotype and cytokine production. Details of antigen- and mitogen-specific cytokine production and its relationship to parasitemia in this cohort have been described elsewhere [15]. No association was found between cytokine production by PBMC and putative expression phenotypes of TNF-α, IL-10, or IFN-γ.

Cytokine genotypes and measures of parasitemia and anemia. In the third transmission season, individuals with the TNF-α −308A allele (n = 17) had a 40% frequency of parasitemia, compared with 29% in individuals without the allele (n = 99) (P = .02), and became reinfected more rapidly after treatment (P = .014). This association was not observed in the other 2 transmission seasons. Individuals with the TNF-α −308GG/IFN-γ −874AA/IL-6 −174GG genotype were found to be infected less frequently but had higher mean parasite densities, a trend that did not achieve significance in all seasons (data not shown). No relationship between other cytokine alleles and measures of parasitemia or anemia (hemoglobin and ferritin levels) was detected.

Discussion

Efforts to understand the pathogenesis of severe disease due to malaria may be crucial for the development of novel therapies and control strategies. In the present study, we examined the frequency of cytokine genotypes in a cohort of young Kenyan men from an area where severe malarial anemia is the major sequela of childhood malaria. We compared the frequency of specific alleles, or frequency of cytokine-expression phenotypes predicted by the genotyping results, with the frequencies reported for other populations.

The frequencies of all cytokine alleles in the study population were significantly different from those in white populations, except for the frequencies of TGF-β and TNF-α −238. Surprisingly, the TNF-α −238A allele, which has been associated with severe malarial anemia, was not more common in this population, in which severe malarial anemia is a major cause of childhood mortality, than it was in others. On the one hand, an increased frequency might have implicated TNF-α −238A as a factor in the extremely high rates of severe malarial anemia observed among young children in the study area. On the other hand, the mortality resulting from severe malarial anemia in this area of high malaria transmission would be expected to select against an allele contributing to susceptibility. All cytokine gene polymorphisms, including TNF-α −238 alleles, were in Hardy-Weinberg equilibrium, and, therefore, those results did not provide evidence for selective mortality in the present cohort, although small deviations might not be detected in this sample of 150 male subjects.

Frequencies of TNF-α −308 and −376 alleles in this cohort differed significantly from those in some but not all West African and European comparison populations. For example, the frequency in Kenyan populations of the −308A allele, which has been found to be associated with cerebral malaria, was similar to the frequency in healthy Gambian adults and Gambone children with mild malaria but significantly lower than the frequency in European populations and in Gambian populations with malaria or other illnesses. Conversely, the frequency of the TNF-α −376A allele, which has been found to

Table 2. Frequency of transforming growth factor−β +869/+915 haplotypes and putative expression phenotypes in the present study and in comparison populations.

<table>
<thead>
<tr>
<th>Population, reference(s)</th>
<th>High-expression haplotype/phenotype frequencies, %</th>
<th>Intermediate-expression haplotype/phenotype frequencies, %</th>
<th>Low-expression haplotype/phenotype frequencies, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Haplotype</td>
<td>Haplotype</td>
<td>Haplotype</td>
</tr>
<tr>
<td></td>
<td>GCC/GCC Phenotype</td>
<td>GCC/GCC Phenotype</td>
<td>GCC/GCC Phenotype</td>
</tr>
<tr>
<td>Present study</td>
<td>TG/TG 32.0, 39.3, 71.3</td>
<td>TG/CC 4.7, 18.7, 0.0</td>
<td>CG/CC 4.7, 0.0, 0.0</td>
</tr>
<tr>
<td>Black, United States [27]</td>
<td>31.1, 33.3, 64.4</td>
<td>6.7, 13.3, 0.0</td>
<td>11.1, 0.0, 4.4</td>
</tr>
<tr>
<td>White [3, 27]</td>
<td>26.5–41.1, 35.5–49, 75.5–76.6</td>
<td>8.8–12.1, 4.7–9.8, 0.0</td>
<td>4.9–5.6, 0.9–1.0, 0.0</td>
</tr>
</tbody>
</table>

* P < .004, vs. the present study.

Table 3. Frequency of interleukin-10 −1082/−819/−592 haplotypes and putative expression phenotypes in the present study and in comparison populations.

<table>
<thead>
<tr>
<th>Population, reference(s)</th>
<th>High-expression haplotype/phenotype frequencies, %</th>
<th>Intermediate-expression haplotype/phenotype frequencies, %</th>
<th>Low-expression haplotype/phenotype frequencies, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Haplotype</td>
<td>Haplotype</td>
<td>Haplotype</td>
</tr>
<tr>
<td></td>
<td>GCC/GCC Phenotype</td>
<td>GCC/GCC Phenotype</td>
<td>GCC/GCC Phenotype</td>
</tr>
<tr>
<td>Present study</td>
<td>10.7, 10.7</td>
<td>20.0, 33.3, 53.3</td>
<td>6.0, 15.3, 36.0</td>
</tr>
<tr>
<td>Black, United States [27]</td>
<td>2.4, 2.4</td>
<td>17.0, 43.9, 61.0</td>
<td>7.3, 19.5, 36.6</td>
</tr>
<tr>
<td>White [3, 27]</td>
<td>16.8–30.0, 16.8–30.0</td>
<td>20.6–29.7, 21.2–24.8, 41.8–54.5</td>
<td>5.9–8.2, 12.4–16.8, 5.9–7.6, 28.2–28.6</td>
</tr>
</tbody>
</table>

* P < .001, vs. the present study.
be associated with cerebral malaria, was higher in this population than in all other African and European populations previously examined, but the differences were not consistently significant in pairwise comparisons. Furthermore, TNF-α −376A allele frequencies did not vary between East African and West African populations.

Like previous investigators [18, 19], we were unable to demonstrate any consistent relationship between polymorphisms and measures of parasite burden. Because the population of young male subjects included in the present study would have acquired substantial antiparasite immunity, the effects of polymorphisms on parasitemia could be masked, and future studies in children may be more revealing. Furthermore, if cytokine promoter polymorphisms modulate disease, rather than parasitemia, as has been observed for nonimmunologic resistance genes such as hemoglobin S [29], this would not be revealed by measures of parasitemia.

We observed dramatic differences in frequency between the present study and comparison populations for IL-6 and IFN-γ alleles. Polymorphisms in these cytokine genes have not, to our knowledge, previously been examined for associations with malaria outcomes. The striking bias favoring the IL-6 −174G allele over −174C made it difficult to conduct any statistical testing of relationships with malaria outcomes. High plasma IL-6 levels have been associated with severe disease and death due to malaria [30, 31], and the IL-6 −174C allele has been associated with increased in vivo expression of IL-6 in neonates [32] and adults [33] during acute-phase reactions. Selection against the IL-6 −174C allele might result from malaria-related mortality early in life, although disequilibrium suggesting selection was not detected by Hardy-Weinberg analysis in the present study.

Our study population was also profoundly skewed, compared with white and black US populations, toward an IFN-γ genotype associated with low cytokine expression [34]. High plasma levels of IFN-γ have been associated with severe malaria [31] and with low birth weight in infants born to women who had malaria during pregnancy [35]. Thus, an allele that decreases IFN-γ expression might be favorably selected as a consequence of either malaria during pregnancy or childhood malaria.

In particular, IL-6 and IFN-γ may play roles in the development of severe malarial anemia in malaria-exposed populations. In a study of in vitro cytokine expression by immunocytes collected from individuals in an area of Malawi in which malaria is endemic, chronic iron deficiency was most strongly associated with elevated production of IL-6 and IFN-γ [36]. IL-6 causes transferrin-receptor density on hepatocytes to increase [37, 38], ferritin synthesis to increase, and transferrin synthesis to decrease [37]. In patients with anemia resulting from multiple organ dysfunction syndrome, the reticulocyte response to erythropoietin is inversely correlated with IL-6 levels [39]. IFN-γ increases ferritin heavy-chain gene transcription [40] and also inhibits the growth of erythroid precursors in vitro [41, 42]. Together, IL-6 and interferon-γ may inhibit erythropoiesis, either directly or by diversion of iron from erythroblasts to other cellular stores.

One limitation of this study is the exclusion of other genetic information in the examination of polymorphisms. Additional cytokine gene polymorphisms (e.g., IL-6 −597, −572, and −373) could not be genotyped. Another constraint was the absence of data on IL-6 and IFN-γ allele frequencies from a wider range of exposed populations. We therefore used data reported for black populations in the Caribbean and the United States, because they share West African ancestry. The 30% white admixture in black populations on the eastern seaboard of the United States is an important confounding variable, but that does not explain the bias in IFN-γ allele frequencies observed in the present study. We found no association between any cytokine genotypes and cytokine production in response to stimulation with malaria antigens or with mitogen. The lack of concordance between putative expression phenotypes and in vitro production may reflect difficulty in determining cytokine levels at only 2 sampling times, variable intercurrent illness in the host, or strong relationships between cytokine levels and differential exposure to P. falciparum. Alternatively, the cytokine genotypes studied may not be related to in vivo cytokine production in this population.

In summary, this study examined cytokine genotype frequencies in a young, male, Kenyan cohort from an area where severe malarial anemia is frequent and cerebral malaria is uncommon during childhood. The TNF-α −238A allele, which is associated with severe malarial anemia, occurred at a frequency in this population that is similar to the frequency in West African and white populations. Preferred biases exist in the distribution of IL-6 and IFN-γ genotypes, in comparison with the distribution in populations of European and West African ancestry, and these genotypes have been found to be associated with low levels of expression of both cytokines. Further work in different geographic areas is needed to confirm whether these biases in IL-6 or IFN-γ allele frequencies contribute to the relative frequency of malarial anemia or cerebral malaria in different populations.

Acknowledgments

We are grateful to Raphael Onyango, Samuel Oduor Wangowe, and Frederick Onyango, for supervising the field studies, and to the study participants from western Kenya. Stephen McGarvey critically reviewed the manuscript.

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