HLA Class II Factors Associated with *Plasmodium falciparum* Merozoite Surface Antigen Allele Families


In *Plasmodium falciparum* malaria, certain human leukocyte antigens (HLA) and the parasite's merozoite surface antigens 1 and 2 (MSA-1, MSA-2) have been shown to influence the course of the infection. This report is on associations of distinct HLA factors with the occurrence of particular MSA families in a group of patients with either severe or mild *P. falciparum* malaria in Gabon. Different distributions of HLA-DPB1 alleles were found in the 2 groups. DR*04* alleles were observed more frequently among patients with severe malaria. Several alleles of different loci were associated with distinct MSA allele families. In addition, carriers of the amino acid methionine at position 11 of the DPA1 allele were more often infected by MSA-1 K1 parasites and less frequently by MSA-1 RO33 parasites. Furthermore, associations of HLA factors with polyclonal infections were found.

The outcome of an infection with *Plasmodium falciparum* depends essentially on both host and parasite factors. Among the host factors, distinct elements of the polymorphic HLA system have been shown to contribute to protection from *P. falciparum* malaria [1]. In regard to the parasite, particular surface antigens have been found to be variable and to be associated with the course of disease [2]. There is evidence that polymorphic parasite and host factors interact and that their polymorphism is maintained by natural selection [3, 4].

HLA class II molecules present foreign antigens to T helper cells and induce clonal proliferation of these cells. The enormous variability of the genes that encode HLA molecules and their haplotypic composition, especially in native Africans [5], has been suggested to result from requirements to fight multiple and frequently lethal infections, such as malaria.

The first study on the involvement of HLA elements in clinical malaria described protection from cerebral malaria and severe anemia that is conferred by the class I allele, HLA-Bw53, and the class II haplotype, HLA-DRB1*1302*-DQA1*0102*-DQB1*0501 [1]. This group of researchers also succeeded in molecular analysis of the HLA-Bw53 association and showed that a liver-stage specific antigen of *P. falciparum* (LSA-1) could be recognized by HLA-Bw53-positive individuals [6]. However, these findings have not been confirmed in other ethnic groups, and significant associations of parasitic molecules other than LSA-1 with HLA factors have not been revealed so far.

In studies of interactions between variable HLA molecules and polymorphic parasite factors, it has been demonstrated that the *P. falciparum* circumsporozoite protein binds to HLA-DR and -DQ molecules in vitro and correlates with in vivo immunogenicity in animal models [7].

*P. falciparum* merozoite surface antigens 1 and 2 (MSA-1, MSA-2) are expressed during the parasite stage that is infectious for erythrocytes. After the invasion of parasites into red blood cells, both antigens are also expressed on the cell surfaces. The genes coding for MSA-1 and MSA-2 are polymorphic, and balancing selection acts on this polymorphism [3]. The MSA-1 gene consists of 17 discrete blocks, with various degrees of homology within these blocks. The second block, which has been analyzed in this study, exhibits one of three allele families, namely MAD20, K1, or RO33 [8]. It must be noted, however, that other blocks of the coding genes are variable as well, giving rise to a substantial amount of conceivable parasite clones. The MSA-2 gene is organized into two highly conserved external regions flanking a variable domain, defining the allele families FC27 and 3D7 by repetitive regions [9]. MSA-1 and MSA-2 genotypes may conveniently be defined by polymerase chain reaction (PCR)-based techniques [10].

Several correlations of distinct parasite clones, reflected by one or more MSA families, with susceptibility to clinical malaria and the severity of disease have been reported [10–12]. Other reports have described the geographic differences in prevalences of these genes in asymptomatic malarial infections of children [3, 13].

It is not known whether MSA peptide fragments are presented by HLA molecules, and no associations of HLA class II variants with polymorphic parasite factors have been found.
in clinical malaria. Here we report on HLA class II associations with distinct MSA protein families and with the complexity of *P. falciparum* infection in malaria patients from Gabon.

**Material and Methods**

Two groups of patients infected with *P. falciparum* and enrolled in a case-control study on malariometric indices [10] were analyzed. HLA class II elements of 88 patients with mild malaria and of 91 patients with severe malaria were compared with respect to their individual patterns of MSA-1 and MSA-2 genotypes. The composition of the study population has been described in detail elsewhere [10]. Patients were recruited from the Albert Schweitzer Hospital in Lambaréné, Gabon.

For both MSA and HLA analyses, genomic DNA was extracted from urea-preserved blood. MSA-1 and MSA-2 alleles were assessed by determination of length polymorphisms and clone differentiation with family-specific PCR assays as described [10]. HLA class II typing was achieved with PCR-based hybridization assays with sequence-specific oligonucleotides.

For statistical analyses, $\chi^2$ tests were calculated to estimate $P$ values for allele associations. In the contingency tables for comparisons of the overall allele distributions in the mild and severe malaria groups (two columns), the number of rows corresponded to the number of alleles of the respective HLA class II locus (DRB1, DQA1, DQB1, DPA1, DPB1) observed $\geq 5$ times plus 1 for a group of alleles observed $<5$ times. This is in accordance with common strategies and leads to an enhancement of the power of analysis. For small groups, Fisher’s exact test was applied.

**Results**

Analyses were performed to assess associations of HLA class II factors with the severity of malaria, the occurrence of distinct MSA families, and the complexity of *P. falciparum* infection as reflected by the numbers of MSA families. The distribution of DPB1 alleles in the groups with mild and severe malaria was significantly heterogeneous ($\chi^2 = 21.2, df = 11, P = .03$). DPA1, DQA1, DQB1, and DRB1 alleles did not exhibit differences of their distributions. Two HLA class II alleles were associated with severe malaria. The HLA DRB1*04 allele group and the DPB1 allele DPB1*1701 were observed more frequently among patients with severe than mild malaria (17% vs. 4%, $P < .005$, and 7% vs. 0%, $P < .05$, respectively).

In mild malaria, several HLA associations with the occurrence of distinct MSA families could be defined (table 1). DPB1*0201-positive individuals were more frequently MSA-1 RO33-positive than individuals not carrying that allele (80% vs. 31%). Individuals positive for DPB1*0101 were more often MSA-1 MAD20-positive than DPB1*0101-negative individuals (66% vs. 31%). Inversely, the presence of DQB1*0604 was negatively associated with MSA-2 3D7 (17% vs. 76%). Of interest, DQB1*0605 never occurred together with K1 in mild malaria (0% vs. 47%). In severe malaria, the strongest positive association was that of DQA1*0102 with the MSA-2 FC27 family (60% vs. 28%).

We looked next at the multiplicity of individual infections (table 1). As previously described, more than one MSA-1 or MSA-2 family was found in the minority of the individuals [10]. Therefore, calculations were restricted to these few individuals. However, several associations were seen. DQA1*0201- and DQA1*0401-positive individuals with mild infections had more than one MSA-1 or MSA-2 family more frequently than did individuals negative for these alleles (67% vs. 19% and 67% vs. 15%, respectively). In severe malaria, polyclonal infections were found mainly in DPB1*0301- (36% vs. 4%) and DRB1*12-positive individuals (42% vs. 10%).

It has been hypothesized that position 11 of the DPα chain, an epitope shown to play an important role in the outcome of *Onchocerca volvulus* and *Schistosoma haematobium* infection, might also be relevant in *P. falciparum* malaria [14, 15]. In mild malaria, Met-11-positive individuals were more rarely infected by RO33-positive *P. falciparum* clones than were Met-11-negative individuals (26% vs. 59%) (figure 1). This association was also seen in severe malaria (14% vs. 33%). Inversely, in severe malaria, Met-11-negative individuals were more frequently infected by RO33-positive *P. falciparum* clones than were Met-11-positive individuals (42% vs. 10%).

**Table 1.** Associations of HLA alleles with distinct or multiple MSA factors.

<table>
<thead>
<tr>
<th>HLA allele</th>
<th>MSA factor(s)</th>
<th>+ +</th>
<th>+ -</th>
<th>- -</th>
<th>OR</th>
<th>CI</th>
<th>$P$</th>
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<tbody>
<tr>
<td>Mild malaria:</td>
<td></td>
<td></td>
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<tr>
<td>DPB1*0101</td>
<td>MAD20</td>
<td>23</td>
<td>12</td>
<td>16</td>
<td>35</td>
<td>4.2</td>
<td>(1.7–10.2)</td>
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<tr>
<td>DPB1*0201</td>
<td>RO33</td>
<td>8</td>
<td>2</td>
<td>23</td>
<td>52</td>
<td>9.0</td>
<td>(2.2–37.3)</td>
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<tr>
<td>DQB1*0604</td>
<td>3D7</td>
<td>1</td>
<td>5</td>
<td>62</td>
<td>20</td>
<td>0.06</td>
<td>(0.01–0.4)</td>
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<tr>
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<td>Multiple</td>
<td>6</td>
<td>3</td>
<td>15</td>
<td>63</td>
<td>8.4</td>
<td>(2.2–31.6)</td>
</tr>
<tr>
<td>DQA1*0401</td>
<td>Multiple</td>
<td>4</td>
<td>2</td>
<td>12</td>
<td>70</td>
<td>11.7</td>
<td>(2.6–52.8)</td>
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<td>Severe malaria:</td>
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<tr>
<td>DQA1*0102</td>
<td>FC27</td>
<td>37</td>
<td>25</td>
<td>8</td>
<td>21</td>
<td>3.9</td>
<td>(1.5–9.9)</td>
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<tr>
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<td>7</td>
<td>3</td>
<td>77</td>
<td>14.7</td>
<td>(3.7–58.5)</td>
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<tr>
<td>DRB1*12</td>
<td>Multiple</td>
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<td>7</td>
<td>8</td>
<td>70</td>
<td>6.3</td>
<td>(1.8–21.7)</td>
</tr>
</tbody>
</table>

**NOTE.** Associations meeting $P < .005$ are given. + + is positive for both HLA allele and MSA factor(s), + - is positive for HLA allele but negative for MSA factor(s), - - is negative for HLA allele but positive for MSA factor(s), and - - is negative for both HLA allele and MSA factor(s). Multiple = $\geq 2$ MSA families, indicating polyclonal *P. falciparum* infections. OR = odds ratio, CI = 95% confidence interval.
malaria. K1 was more frequently associated with the occurrence of DPA1 Met-11 (73% vs. 49%).

Discussion

Two associations with severe disease after infection with P. falciparum, namely those of the group of DRB1*04 alleles and of DPB1*1701, were observed. However, no associations with mild courses of malaria could be defined, thus not confirming previous findings [1].

Distinct MSA families have previously been shown to play a role in severe malaria. In particular, RO33 variants were more frequent in clinical malaria in Brazil and Senegal [11, 12]. Inversely, RO33 was associated with asymptomatic malaria in Gabon [13]. This is consistent with the recent observation that RO33 is found in mild malaria rather than in severe cases [10]. In that report, MAD20 was also shown to be associated with mild malaria and K1 with severe malaria. If it is true that MSA families influence the outcome of P. falciparum malaria, associated HLA factors should also be involved in the course of disease. Given the fact that such interactive effects of host and parasite factors exist, this would explain the clusters of distinct HLA elements and the regionally different prevalences of MSA genotypes.

In the present study, particular MSA families were associated with HLA class II alleles and the DPA1 Met-11 epitope. The relevance of such associations on the course of malaria as well as on susceptibility or protection from the infection itself remains, at present, unclear. The concomitant occurrence of a given HLA allele with a specific MSA genotype might reflect tolerance of the corresponding parasite factor. Different associations of MSA genotypes with HLA elements have been found in mild and in severe malaria. It is likely that immune responses, elicited by HLA peptide binding with subsequent presentation to T cells, differ in mild and severe courses of malaria. The manifestations of malaria correlate with the production capacity of interferon-γ by leukocytes (Kremsner PG, unpublished data); on the other hand, interferon-γ up-regulates the expression of HLA genes by acting on regulatory promoter regions.

With respect to the different distributions of HLA alleles and haplotypes in different geographic regions and among various ethnic groups, associations with infectious diseases have to be considered and compared critically. Different distributions of associated HLA alleles do not necessarily exclude equal distributions of isolated amino acid residues that are shared by several alleles and that are functionally relevant (i.e., directly involved in the shaping of the antigen-binding cleft). This could be the case for the association of distinct MSA genotypes with DPA1 Met-11, a residue common to DPA1*02021, DPA1*02022, and DPA1*0301, which, as alleles, were not associated with any particular MSA genotype.

The occurrence of distinct HLA and polymorphic parasite factors in different geographic regions depends, most likely, on selective pressure. There is evidence for selection in favor of particular MSA-1 types [16], because nonrandom linkage of blocks 4 and 6 of the MSA-1 variants has been observed. It has been demonstrated that this particular selection does not occur during the parasite’s sexual cycle within the transmitting mosquitoes or during the act of transmission [17], suggesting rather that host factors are involved. The situation is still more complex, because cohabiting parasite strains can facilitate each other’s survival by down-regulating cellular immune responses [4].

Despite the small study group, associations of MSA-1 and MSA-2 families with HLA alleles and the DPA1 Met-11 epitope were significant (P<.005). The typing protocol used in our study allows assessment of the number of different MSA allele families that are representative for selected genomic regions of the P. falciparum gene but, certainly, leads to an underestimation of the true number of different parasite clones. With regard to the limited sample size, however, this conservative technique of MSA-typing appears appropriate.

Notably, several alleles of DPB1, the only locus with a heterogeneous overall distribution in both mild and severe malaria, were also associated with the RO33 and MAD20 families and with multiple infections. RO33 and MAD20 have also been shown to be associated with mild disease manifestation in the same study group [10]. These observations and the DPA1 Met-11 associations with the K1 family point toward a specific role of DP molecules in susceptibility or resistance to P. falciparum malaria.
References


