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HLA-B*4601 Increased in the Adult Patients with Severe Malaria at Mae Sod Hospital in Thailand

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To investigate the host genetic factors affecting the clinical course of falciparum malaria, HLA-B gene polymorphism was analyzed in the outpatients and hospital patients with different clinical severity of malaria.

Materials and Methods

Subjects. Two hundred and twenty outpatients with positive blood smear of *P. falciparum* at Mae Sod Malaria Clinic were examined for their complete blood cell counts (CBC), hemoglobin concentration (Hb), hematocrit (Ht), and the percentage of the infected RBC in the blood smear. At the same time, physical examination including palpebral anemic change, bulbar icterus, and hepatosplenic palpitation was done by the same physician. After the diagnosis, all the subjects were immediately treated by anti-malarial drugs. We have also collected blood samples from 13 severe malaria patients at Mae Sod National Hospital. The nationality of the patients was Myammars (115 from Karen, 105 from other Burmese). The average ages of the outpatients and the hospitalized patients with severe malaria were 27.6 ± 9.2 and 23.3 ± 7.15, and the sex ratios were 187 : 33 and 9 : 4 respectively.

HLA-B gene polymorphism. From 0.5-1ml of EDTA blood, whole genomic DNA was extracted. 100ng of the DNA were applied to PCR solution containing primers specifically designed for the amplification of Exon 2 of the HLA:A-B gene. The amplified HLA-B gene fragments were analyzed for their polymorphic sites by using 20 different sequence specific oligoprobes (SSOs) and dot blot hybridization.

Results

The first criteria we used for the evaluation of the severity of malaria was parasitemia as shown in Table 1. We tentatively set the cut off point, 1.4%, to divide a high parasiteamia and a low. There was no difference in the allele distributions of HLA-B between high and low parasiteamia groups. Then we used the hemoglobin concentration for another criteria for the definition of the clinical severity. As shown in Table 2, mild anemic patients group which showed less than 10g/dl of hemoglobin had more allele frequency of HLA-B*1301 than that of high-

Table 1.

ECB type	all patients n=220		low P.f < 1.4% n=153		high P.f > 1.4% n=51	
B*1301	20	9.1%	13	8.5%	6	11.8%
B*1521	45	20.5%	30	19.6%	11	21.6%
B*3902	12	5.5%	7	4.6%	3	5.9%
B*40 complex	15	6.8%	9	5.9%	5	9.8%
B*4601	18	8.2%	12	7.8%	4	7.8%
B*5201	29	13.2%	20	13.1%	8	15.7%
B*62 complex	62	28.2%	41	26.8%	14	27.5%

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Table 2.

ECB type	all patients n=220		mild Hb > 10g/dl n=179		anemic Hb < 10g/dl n=31	
B*1301	20	9.1%	11	6.1%	8	25.8% Corrected p value P < 0.05
B*1521	45	20.5%	37	20.7%	5	16.1%
B*3902	12	5.5%	6	3.4%	4	12.9%
B*4601	18	8.2%	16	8.9%	2	6.5%
B*5201	29	13.2%	23	12.8%	5	16.1%
B*62 complex	62	28.2%	47	26.3%	8	25.8%

Table 3.

ECB type	mild all patients n=220		severe cerebral malaria n=13	
B*4601	18	8.2%	5	38.5% Corrected p value P < 0.02
B*1521	45	20.5%	3	23.1%
B*1508	1	0.5%	3	23.1%

er Hb group (Corrected P value < 0.05).

Thirteen cerebral malaria patients were also examined for their HLA-B alleles. Table 3 shows that the frequency of HLA-B*4601 significantly increased in the patients compared with the outpatients at Malaria Clinic with mild symptoms (Corrected P value < 0.02).

Discussion

Human major histocompatibility complex (MHC) is known as HLA system consisted of 3 major gene clusters, namely class I, class II and class III regions. Gambian study using younger children clearly showed the decreased frequency of HLA-B53 allele in the severe malaria patients who showed severe anemia (less than 5g/dl of Hb) and/or neurological symptoms. Subsequently, they showed that cytotoxic T cells were activated in the HLA-B53 positive patients by the stimulation of Liver Stage Specific Antigen-1 (LSA-1). They discussed the beneficial role of HLA-B53 to induce infected hepatocyte specific CD8 T cells to inhibit the proliferation of the parasites.

We searched for the same kind of HLA-B in South East Asia. In Malaysia, we analyzed the natural resistant population of Aborigines called Orang Asli for their HLA-B polymorphism and found that HLA-B* 1513 which was dominant allele in the population that showed significantly lower titer of anti-schizont IgG antibody in the serum. The antigen binding pocket of the HLA-B* 1513 showed similarity with HLA-B53 suggesting that the same kind of immunological mechanisms worked here in South East Asia.

In the present study, we focused on the adult patients who would be expected to be resistant against malaria by the acquired immunity. Therefore, the clinical symptoms were relatively milder than small children but still it was possible to categorize their severity by using several criteria. Within the criteria, especially CNS symptoms and hemoglobin concentration could successfully identify susceptible HLA-B alleles, HLA-B*4601 and 1301 respectively as shown in Tables 2 and 3. Pathogenesis of cerebral malaria and anemia must be different from each other according to the previous studies. Further immunological analysis of those susceptible alleles should be done to explain the mechanisms involved in the pathogenesis.

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