Concise Communication

HLA-DQB1*0501–Restricted Th1 Type Immune Responses to Plasmodium falciparum Liver Stage Antigen 1 Protect against Malaria Anemia and Reinfections

Jürgen May,1,2 Bertrand Lell,1,4 Adrian J. F. Luty,1 Christian G. Meyer,1 and Peter G. Kremsner1,4

Protective immunity against Plasmodium falciparum requires constant exposure to the pathogen. T cell–mediated immune responses are induced by T cell epitopes of pre-erythrocytic stage antigens of P. falciparum and involve HLA-restricted CD4 and CD8 cells. Cytotoxic T cell responses to a conserved epitope of P. falciparum liver stage antigen (LSA) type 1 are restricted by the HLA class I allele Bw53. The role of HLA class II alleles in mediating cellular responses against P. falciparum LSA-I has not yet been demonstrated. In a longitudinal study performed for >4 years, associations were found between the HLA class II allele DQB1*0501 and protection from malaria anemia and malarial reinfections in Gabonese children. Children carrying DQB1*0501 had a higher frequency of interferon-γ responses to LSA-1 T cell epitopes, compared with noncarriers.

Immunity to Plasmodium falciparum requires continuous reinfection and T cell immune responses. Radiation-attenuated sporozoites confer immunity, which has focused attention on parasite antigens expressed by pre-erythrocytic stages and on mechanisms that inhibit intrahepatic parasite maturation [1]. Considerable efforts have been made to design vaccines that stimulate protective parasite-specific cytotoxic T lymphocyte (CTL) responses [2]. A potential target, the P. falciparum liver stage antigen (LSA) type 1, expresses immunogenic T and B cell epitopes, and a conserved LSA-1 epitope is recognized by HLA class I Bw53–restricted CTL [3]. HLA-Bw53 and the HLA class II haplotype DRBI*1302-DQA1*0102-DQB1*0501 are independently associated with protection from severe malaria [4].

In this study, we examined long-term clinical courses after mild or severe malaria and P. falciparum reinfections in relation to the HLA class II candidate alleles DRBI*1302-DQA1*0102-DQB1*0501. We further analyzed interferon (IFN)–γ responses triggered by T cell epitopes of LSAs and their relationship to these HLA class II alleles.

Patients and Methods

Study group. We studied 100 children with severe malaria (median age, 39 months; range, 13–133 months) and 100 with mild malaria (mean age, 41 months; range, 8–140 months) in a prospective matched-pair study. The children were enrolled at the Albert Schweitzer Hospital, Lambaréné, Gabon. Reinfections were identified through case finding and parasitologic follow-up of subjects every 2 weeks. Mothers were asked to bring their children to the clinic for any illness. Clinical and parasitologic observations in the study group were described elsewhere [5]. Monocyte pigmentation assessed by modified Giemsa-stained thick blood smears was used to estimate antecedent and long-lasting high parasite burden and anemia [6]. All patients with clinical disease were treated. Children with sickle cell anemia were excluded from the study, and those with homozygous α-thalassemia were excluded from analysis because of inherent anemia associated with this condition.

HLA typing. HLA typing was done according to protocols of the 11th International HLA Workshop. Subtyping was done for the DRw52 group. In brief, the second exons of the HLA class II loci were amplified from genomic DNA by polymerase chain reaction. Amplicons were linked onto nylon membranes and hybridized with HLA-specific probes.

Received 1 May 2000; revised 7 August 2000; electronically published 10 November 2000.

Presented in part: 5th Deutscher Kongreß für Infektions und Tropenmedizin, Munich, November 1999 (abstract Sy 275).

Informed consent was obtained from parents or guardians of patients after ethics committee approval (International Foundation of the Albert Schweitzer Hospital, University of Tübingen ethics committee). The human experimentation guidelines of the Albert Schweitzer Foundation were followed in the conduct of the clinical research.

Financial support: European Community (EU-INCO DC), Deutsche Forschungsgemeinschaft, World Health Organization Multilateral Initiative on Malaria, and fortune Program, University Tübingen.

Reprints or correspondence: Dr. Jürgen May, Institut für Tropenmedizin Berlin und Medizinische Fakultät Charité, Humboldt-Universität zu Berlin, Spandauer Damm 130, D-14050 Berlin, Germany (juergen.may@gmx.de).

The Journal of Infectious Diseases 2001; 183:168–72

© 2001 by the Infectious Diseases Society of America. All rights reserved.
bridized with digoxigenin-labeled sequence-specific oligonucleotides. After membranes were washed with tetramethyl ammonium chloride, hybrids were visualized with an anti-digoxigenin-alkaline phosphatase conjugate.

Immunologic assays. For peripheral blood mononuclear cell (PBMC) stimulations, cells were separated from blood. After the cells were washed, cultures were established with medium alone, with recall peptide (purified protein derivative), and with synthetic \( P. falciparum \) LSA-1 peptides LSA-J (ERRAKEKLEQEQRD-LEQRKADT)K and ls6 (KPIVQYDNF; concentration, 10 \( \mu \)g/mL). The peptides represent epitopes of the T6/96 \( P. falciparum \) clone and have distinct T cell stimulatory capacities [3]. We measured the IFN-\( \gamma \) concentration in the culture supernatants after 6 days. Monoclonal antibodies (Mabtech) were used in a capture sandwich ELISA. The detection limit was 2 pg/mL; all lower values were assigned a value of 0. Nonresponders were defined as subjects in whom the difference between cytokine concentrations in unstimulated cultures and peptide-stimulated cultures was 0.

Statistics. Contingency analyses were performed to test for heterogeneity of HLA class II alleles among IFN-\( \gamma \) responders and nonresponders after stimulation with L6 or LSA-J. DQ associations with monocyte pigmentation, number of reinfections, and LSA responses were estimated by \( \chi^2 \) tests (\( P < .05 \) was considered significant). We used the nonparametric Mann-Whitney \( U \) test to assess differences in minimal hematocrit values during reinfections in DQB1*0501-positive and -negative subjects. Kaplan-Meier analyses allowed us to determine differences between DQB1*0501 carriers and noncarriers in the longitudinal analysis of reinfections. The log-rank test enabled us to describe the significance of differences between groups. In analyses of reinfections, subjects with <12 months of follow-up were excluded. Linkage disequilibria were defined by \( \Delta \) values for nonrandom assortment of alleles as \( \Delta = dln - (b + d)(c + d)/n^2 \), where \( b, c, \) and \( d \) represent frequencies of +/-, -/+ and --, respectively, from 2 \( \times \) 2 tables (\( n \) individuals).

Results

The study design involved follow-up of subjects every 2 weeks for 52 months. Analyses included findings for 88 children who presented initially with mild malaria and 91 with severe malaria from an original cohort of 200 children. Hematocrit levels at admission and subsequent reinfections differed significantly between the groups (median hematocrit: mild malaria, 37% vs. 32%, \( P = .034 \); severe malaria: median 31% vs. 28%, \( P = .012 \); figure 1A). In both groups, DQB1*0501-homozygous subjects had the highest median hematocrit (37% in mild malaria and 32% in severe malaria). In DQB1*0501 carriers with severe malaria, the median hematocrit was also higher at admission (29% vs. 26%, not significant [NS]). We found no associations of lowered hematocrit with sickle cell trait, heterozygous \( \alpha \)-thalassemia, or glucose-6-phosphate dehydrogenase gene variants.

Children with DQB1*0501 have a lower reinfection rate. In the severe malaria group, the absence of DQB1*0501 was linked to an increased risk of \( >1 \) reinfection per year (odds ratio [OR], 0.3; 95% confidence interval, [CI], 0.1–0.8; \( P = .026 \); table 1). A similar trend was observed in children with mild malaria (<1 reinfection, 39% DQB1*0501 positive; \( >1 \) reinfection, 23% DQB1*0501 positive; OR, 0.4; 95% CI, 0.1–1.3; NS). Reinfection profiles were further analyzed by considering each reinfection as a single outcome variable. In total, 688 reinfections were detected. Kaplan-Meier analyses of the group with severe malaria revealed that intervals between reinfections were longer in DQB1*0501 carriers than in individuals negative for that allele (\( P = .043 \); figure 1C). A similar trend was observed in the mild malaria group (figure 1B).

Pigmentation of monocytes. We used the number of pigmented monocytes per microliter of blood to estimate \( P. falciparum \) infections and malaria-related anemia before the study [6]. The third quartile was set as the cutoff point, to detect the highest infection rates. In subjects with severe malaria, DQB1*0501 carriers had a 70% lower risk of preceding malaria-related anemia, as assessed by the number of pigmented monocytes (OR, 0.1; 95% CI, 0.02–0.6; \( P < .001 \); table 1).

IFN-\( \gamma \) response to LSA-1 epitopes. To determine whether protection conferred by DQB1*0501 is mediated by cytokines, IFN-\( \gamma \) responses to the LSA-1-derived peptides LSA-J and ls6 were analyzed. Ls6 is a protective CTL epitope restricted by HLA-Bw53 [3], and LSA-J is a peptide of 24 amino acids that matches with the peptide size required for class II–restricted presentation. IFN-\( \gamma \) responses to LSA-J and ls6 were more frequent in PBMC from DQB1*0501 carriers than in those from noncarriers (LSA-J: OR, 5.5; 95% CI, 1.3–23.4; \( P = .023 \); ls6: OR, 1.5; 95% CI, 0.4–6.6; NS; table 1). Of note, DQB1*0501 was not associated with IFN-\( \gamma \) responses against merozoite surface antigens.

Linkage of DQB1*0501 with other HLA class II loci. DQB1*0501 was found in strong linkage disequilibria with DQA1*0101 (\( \Delta = 0.054; P < 10^{-7} \); haplotype frequency, 0.168). The strongest linkage of DRB1 alleles with DQB1*0501 was moderate (DRB1*12, \( \Delta = 0.023; P < .01 \)); the combination DRB1*12-DQB1*0501 was found in only 8.5% of the study population.
Discussion

Our data show that HLA class II DQB1*0501 confers protection against malaria anemia in consecutive infections over long periods of exposure, which is consistent with a lower rate of reinfections in persons with that allele. Associations were stronger in children who presented initially with severe malaria. Reinfection rates in that group were significantly higher than in children with mild malaria [5]. This cannot be explained by the presence of DQB1*0501, because this allele occurred at similar frequencies in both groups. Other factors that mask the effect of the DQB1 allele might contribute to protection in the mild malaria group.

HLA class II alleles were previously found to be involved in protection from or susceptibility to severe malaria, but conflicting results were obtained for mild malaria [7]. Longitudinal studies on the relationship between HLA alleles, cytokine patterns, and the outcome of P. falciparum infection are scarce. One study could not confirm the association of HLA-Bw53...
with protection from malaria [8], and correlations between incidences of clinical attacks and HLA class II, but not class I alleles, have been reported [7].

An HLA-dependent cytokine profile could explain associations of HLA factors with distinct malaria conditions. Pro-inflammatory molecules such as IFN-γ are key regulators of antiparasite cellular immunity [5], and class II molecules, in particular DQ molecules, are associated with IFN-γ production of T cells [9]. We reported elsewhere that IFN-γ responses confer resistance to reinfection with *P. falciparum* [5]. IFN-γ responses to LSA-1 peptides are predominantly produced by CD8 cells [10]. However, the mechanisms that generate appropriate IFN-γ levels are not clearly defined. CD8 T cells, activated by interaction with HLA-peptide complexes on the surface of infected hepatocytes, might secrete IFN-γ, which leads to parasite destruction [11].

HLA-dependent differences of IFN-γ production could reflect linkage with genes other than HLA genes on human chromosome 6. Polymorphisms in the tumor necrosis factor (TNF)–α region, for example, are associated with particular HLA class II alleles. In our study, we found no associations of DQB1*0501 with TNF promoter polymorphisms, TNF plasma levels, or TNF production capacity.

The pre-erythrocytic parasite stages could be targets of protective responses, and both CD8 and CD4 T cells appear to be involved in an HLA-restricted manner [12]. CD8 T cell-dependent cytotoxicity is mediated by HLA-Bw53 restriction [3]. However, CTL specific for *P. falciparum* antigens have not been identified consistently, and some researchers conclude that induction of CTL against plasmodial antigens does not necessarily reflect protection in vivo.

Th1 responses to pre-erythrocytic antigens are protective in human malaria [13]. CD4 T cell–mediated protective immunity against sporozoites involving IFN-γ can be achieved by immunization with synthetic linear peptides derived from pre-erythrocytic stages. The underlying mechanism could be a stimulation of cytokines by antigen-presenting cells (e.g., Kupffer or endothelial cells), leading to expression of class II molecules in the microenvironment of infected liver cells. On the other hand, hepatocytes express class I and II molecules in the course of various infections [14]. The pathways of transport and processing of *P. falciparum* LSA, eventually leading to class II restricted CD4 T cell responses, are not understood.

The HLA-DQB1*0501–associated protection from anemia can be confirmed as occurring in persons of different ethnicities with defined phenotypes of malaria. Our findings underline the significance of HLA class II–restricted recognition of LSA-1 epitopes by CD4 T cells and subsequent IFN-γ production during the parasite’s early stages in single and repeated infections. This is compatible with a Th1 phenotype of immune response and with the notion that HLA molecules can direct the immune response toward a Th1 or Th2 phenotype [15]. Consequences include a reduction of the parasite burden and the clonal complexity of the infection. The fact that clonal complexity depends on particular HLA factors and that selection in favor of distinct parasite genotypes occurs [16] corresponds well with the idea that host genes are intimately involved in prevention of growth and maturation of parasite variants [17].

Acknowledgments

We thank the children of the study group and their families for study participation. We are also grateful to B. Bojowald, O. Danglmaier, B. Greve, K. Herbach, H. Knoop, L. Lehman, D. Luckner, P. Matousek, A. Missinou, D. Schmid, R. Schmidt-Ott, and M. Sovric (Sektion Humanparasitologie, Institut für Tropenmedizin der Universität Tübingen, Tübingen, Germany).

References


