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Immunogenetic Control of Antibody Responsiveness in a Malaria Endemic Area

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ABSTRACT: This study builds upon the established genetic control of antimalarial immune responses and prior association studies by using a family-based approach, transmission disequilibrium testing, to identify immune response genes that influence antibody responses to *Plasmodium falciparum* infection in an endemic Tanzanian population. Candidate polymorphisms are within the interleukin-1 (IL-1) gene cluster, the IL-10 promoter, Major histocompatibility complex class II and III, the 5q31-q33 region, and the T-Cell Receptor beta variable region. There was a significant association between the IL1RN alleles and total IgE. Weak evidence for association was present between polymorphisms in the IL10 promoter region and both anti-*P falciparum* IgE and IgG4 antibodies. *Human Immunology 68, 165–169 (2007).* © American Society for Histocompatibility and Immunogenetics, 2007. Published by Elsevier Inc.

KEYWORDS: *P falciparum*; IL1; IL10; IgE; IgG; TDT; association

ABBREVIATIONS

HLA	Human leukocyte antigen
IL	Interleukin
MHC	Major histocompatibility complex
SNP	Single nucleotide polymorphism
TCRBV	T-Cell Receptor beta variable
VNTR	Variable number of tandem repeats

INTRODUCTION

Malaria is a significant cause of worldwide mortality and morbidity. Individuals living in endemic areas experience persistent subclinical malaria infection, but only a minority develops severe disease. These variations in disease pattern are attributable to a number of different factors, which include the genetic background of both host and pathogen. There is now a significant body of evidence to indicate that the genes affecting the immune response can influence the outcome of malaria infection, and the capacity to mount a humoral response [1].

In this paper, we investigate the genetic control of antimalaria antibody responsiveness to subclinical infection within a malaria-endemic area. The importance of antibody responses during malaria infection has long since been observed, with the production of specific IgG antibodies required for the acquisition of functional immunity to malaria; however, protection is subclass dependent. Malaria infection in humans has also been associated with an increase in total and malaria-specific IgE production [2]. Malaria-specific IgE has previously been associated with severity of malaria disease [2] and with parasitemia [3], although recent results from the current population demonstrate that high malaria-specific IgE levels in asymptomatic individuals are associated with reduced risk for subsequent clinical episodes [4]. The significance of the elevated total IgE in severe malaria infection is unknown [5].

There have been few candidate gene studies of immune responses in malaria. Control of parasitemia, and total IgE in nonparasitic populations, have previously been linked to chromosome 5q31-q33, which contains gene coding for the cytokines IL-4 and IL-13 [6, 7].

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Polymorphisms in the IL-1 gene cluster have been implicated in the control of both malaria and IgE levels [8–10], and the down regulatory cytokine IL-10 is also involved in the differential control of IgE and IgG4 levels [11]. Human leukocyte antigen (HLA) class II restriction of the cellular response to specific erythrocytic stage antigens have been demonstrated to exist and reveal associations with a number of different HLA class II alleles [12]. Although the allied T-cell receptor has yet to be closely investigated with respect to malaria, it is an attractive candidate due to its variant nature and essential role in the immune response to malaria.

In the current study, we have investigated the role of polymorphisms in a number of candidate genes for control of antibody responses to malaria in a well-studied Tanzanian population by using family-based association methods. Candidate genes (IL1, IL10, MHC class II and III, IL4, IL13, and TCRBV) were chosen for their potential role in the control of both antibody responses and malaria infection.

MATERIALS AND METHODS

Patients

The fishing village of Nyamisati, 150 km south of Dar-es-Salaam, Tanzania is a holoendemic area for malaria, with transmission increasing during the two rainy seasons (April to June and November to December), the predominant malaria species being *Plasmodium falciparum*. There is no cerebral malaria observed in the village. The study population is of Bantu origin and has previously been described [4].

To enable family-based analysis, parental information was collected and 167 extended pedigrees compiled, composed of two or more generations and containing 303 nuclear families and 1469 individuals. Venous blood was collected in 1999—1050 samples, of which 860 samples were from related individuals and 190 from unrelated individuals—with informed consent and approval of the local ethics committees. Human genomic DNA was extracted from the frozen venous blood by using standard procedures of salting out, followed by phenol-chloroform extraction.

Genotyping

SNP genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) by using previously published methods on the following polymorphisms. The observed heterozygosity is also presented for each locus as a measure of information provided by the polymorphism: $IL1B_{-511}$ (heterozygosity: 0.417); $IL10_{-592}$ (heterozygosity: 0.49) and $IL10_{-1082}$(heterozygosity: 0.495); TNF_{-308} (heterozygosity: 0.152) and LTA_{-368} (heterozygosity: 0.499); IL13 (heterozygosity: 0.375); and BV8S3 (heterozygosity; 0.22), BV24S1 (heterozygosity: 0.478), BV2S1 (heterozygosity: 0.22), BV15S1 (heterozygosity: 0.167), and BV3S1 (heterozygosity: 0.376). VNTRs were typed by polymerase chain reaction (PCR) and agarose gel electrophoresis: $IL1RN_{VNTR}$ (heterozygosity: 0.238) and $IL4_{VNTR}$ (heterozygosity: 0.43). HLA class II loci were typed using sequence-specific oligonucleotide probes to detect polymorphisms in HLA-DQB1 (heterozygosity: 0.839), HLA-DQA1 (heterozygosity: 0.838), and HLA-DRB1 (heterozygosity: 0.87). Genotype frequencies at all SNP and VNTR loci did not differ significantly from those expected under Hardy-Weinberg equilibrium, with the exception of BV2S1. The genotyping at this locus was repeated and genotypes confirmed. Known haplotypes were determined from family data for IL10 polymorphisms.

Serology

The levels of total IgE antibodies and of antimalarial IgE and IgG antibodies were determined by ELISA as described previously [4]. *P falciparum* specific assays used lysates of infected erythrocytes (*P falciparum* laboratory line F32) [2, 4]. Antimalarial IgG4 antibodies were determined using lysates of infected erythrocytes (*P falciparum* laboratory line NF54 3D7). Thirty-three individuals manifested a clinical episode at the time of sampling; antibody levels did not differ significantly between individuals with and without clinical disease.

Frequency distributions of antibody levels were positively skewed, so data were log transformed before analysis. Antibody levels were adjusted for age and sex effects by multiple regression. Heritability of the adjusted values was estimated using SOLAR [13].

Statistical Analysis

Associations between antibody levels and alleles at candidate loci, together with IL-10 haplotypes, were tested by a quantitative transmission disequilibrium test [14] with the program QTDT version 2.1.3 [14]. To control for any population stratification, we tested the within-family association in a model including environmental, polygenic heritability, and additive major locus variance components. A multiallelic test was performed for multiallelic loci, thereby reducing multiple testing and generating a single p value per locus. To control for any nonnormality, empirical p values were calculated by permutation. Uncorrected p values are presented. However, since eight independent loci were tested (*i.e.,* not in linkage disequilibrium with another typed locus), only results with $p < 0.00625$ (equivalent to 0.05 divided by 8) were considered significant. Linkage disequilibrium between adjacent loci was calculated on