LACK OF ASSOCIATION BETWEEN INTERLEUKIN-10 GENE PROMOTER POLYMORPHISM, -1082G/A, AND SEVERE MALARIA IN THAILAND

Jun Ohashi[1], Izumi Naka[1], Jintana Patarapotikul[2], Hathairad Hananantachai[2], Sornchai Looareesuwan[2] and Katsushi Tokunaga[1]

[1]Department of Human Genetics, Graduate School of Medicine, University of Tokyo, Japan;
[2]Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Abstract. Interleukin-10 (IL-10) is an important cytokine in the down - regulation of inflammatory responses, and it has been reported that a low plasma concentration of IL-10 is associated with severe anemia and cerebral malaria in *Plasmodium falciparum* infections. The *IL-10* gene is located on chromosome 1q31-32, and a promoter polymorphism (-1082G/A) is known to affect IL-10 protein production. In order to examine the possible association of the -1082G/A polymorphism with the severity of malaria, we studied 203 mild malaria, 164 non-cerebral severe malaria, and 109 cerebral malaria patients living in northwest Thailand. The genotyping was performed by a fluorescence resonance energy transfer (FRET) method. The frequencies of a major allele -1082A in mild malaria, in non-cerebral severe malaria, and in cerebral malaria patients were 92.6%, 92.1%, and 92.7% respectively. Our results showed no significant association of the -1082G/A polymorphism with the severity of malaria.

INTRODUCTION

Interleukin-10 (IL-10) plays an important role in the down-regulation of inflammatory responses. In *Plasmodium falciparum* malaria, IL-10 is considered to inhibit the production of pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α and IL-1, which are involved in the pathogenesis of malaria.

It has been shown recently that a low plasma concentration of IL-10 and a low plasma IL-10:TNF-α ratio are associated with cerebral malaria and severe anemia in *Plasmodium falciparum* malaria (Kurtzhals *et al*, 1998; Othoro *et al*, 1999; May *et al*, 2000). May *et al* (2000) reported that single nucleotide polymorphisms (SNPs) of *TNFA* promoter affect the plasma IL-10:TNF-α ratio in malaria patients; it is unclear whether genetic variants of IL-10 influence the pathogenesis of malaria.

The correlation for IL-10 production capacity was reported to be higher in monozygotic twins than in first-degree relatives (Westendorp *et al*, 1997), which suggests that the variation in the IL-10 protein level is under genetic control. It is therefore possible that there are genetic variations in the regulatory region of the *IL-10* gene. To date, several SNPs have been identified in the promoter region of the *IL-10* gene (Tounas and Cominelli, 1996; Eskdale *et al*, 1997; Turner *et al*, 1997). Of these, the -1082G/A polymorphism has been reported to be associated with IL-10 protein production (Turner *et al*, 1997; Eskdale *et al*, 1999). Turner *et al* (1997) showed that the A allele at position -1082, *ie* -1082A, was associated with lower IL-10 protein production. In contrast, Eskdale *et al* (1999) reported that the -1082A allele was associated with higher IL-10 secretion than the -1082G allele. This discrepancy may have arisen as a result of the different experimental systems that were used in these studies (Eskdale *et al,* 1999).

Although it is uncertain whether the -1082G/A polymorphism of *IL-10* affects IL-10 protein production in malaria patients, we tested the hypothesis that the -1082G/A polymorphism of IL-10 is associated with the severity of malaria.

Correspondence: Dr Jun Ohashi, Department of Human Genetics, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan.
Phone: ++81-3-5841-3693; Fax: ++81-3-5802-8619
E-mail: juno@m.u-tokyo.ac.jp

MATERIALS AND METHODS

Malaria patients

The study samples comprised 203 mild malaria controls, 164 non-cerebral severe malaria, and 109 cerebral malaria patients; all of the patients lived in northwest Thailand. Only adult patients whose age is 13 years were recruited. They were treated at the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University. This study was approved by the Ethics Committee of the Faculty of Tropical Medicine.

DNA extraction

Genomic DNA was extracted from peripheral blood leukocytes using a QIAamp blood kit (Qiagen, Hilden, Germany).

IL-10 -1082G/A typing

Genotyping for -1082G/A was performed by a fluorescence resonance energy transfer (FRET) method using LightCycler^{TM} (Roche Diagnostics, Mannheim, Germany), according to the manufacturer's instructions. The primers and probes are listed in Table 1. The final concentrations of the primers, the fluorescein probes, LCRed probes, and $MgCl_2$ were 0.5 μM, 0.2 μM, 0.4 μM, and 3 μM respectively. PCR amplification included 30 cycles of denaturation (95˚C for 0 second), annealing (56˚C for 10 seconds), and extension (72˚C for 11 seconds). Genotypes were determined by melting-curve analysis. The temperature transition rate was set at 20˚C/second for PCR and at 0.2˚C/second for melting-curve analysis.

RESULTS AND DISCUSSION

The genotype and allele frequencies in malaria patients are presented in Table 2. Neither genotype nor allele frequencies showed a significant difference among the mild malaria control, non-cerebral severe malaria, and cerebral malaria patients. Genotype frequencies did not deviate from expectations that were consistent with the Hardy-Weinberg law. The -1082A allele is at a frequency of about 50% in European populations (Koss *et al*, 2000), while this allele is frequently found in the Thai population.

Although the *TNFA*-308A homozygote has been reported to increase the risk of cerebral malaria (McGuire *et al*, 1994), we previously found no association of the *TNFA*-308G/A polymorphism with cerebral malaria in Thailand (Hananantachai *et al*, 2001). Since SNPs of the *TNFA* promoter affect the plasma level of IL-10 over TNF-α in malaria patients (May *et al*, 2000) and the *IL-10* -1082G/A polymorphism is associated with the IL-10 protein production (Turner *et al*, 1997; Eskdale *et al*, 1999), there could be an interaction or association between *TNFA* promoter and *IL-10* polymorphisms in malaria patients. In order to examine this possibility, malaria patients were stratified by the positivities of *IL-10* -1082A and *TNFA* -308A alleles (Table 3). Although

Table 1
Primers and hybridization probes used in this study.

Name	Sequence
Primer	
IL10-1082F	5′-CAACTGGCTCCCCTTACCTT-3′
IL10-1082R	5'-AAGCTTCTGTGGCTGGAGTC-3′
Probe	
IL10F2	5′-GCTTCTTTGGGAGGGGGAAGTAGG-3′ (3′ FITC)
IL10LC2	5′-ATAGGTAAGAGGAAAGTAAGGGACCTCCTATCCAGCCTCCATGGAA-3′ (5′ LCRed640)

Table 2
Genotype and allele frequencies in malaria patients.

IL-10-1082G/A	Mild malaria controls (2n = 406)	Non-cerebral severe malaria (2n = 328)	Cerebral malaria (2n = 218)
Genotype			
AA	175 (86.2)	139 (84.8)	93 (85.3)
AG	26 (12.8)	24 (14.6)	16 (14.7)
GG	2 (1.0)	1 (0.6)	0 (0.0)
Allele			
A	376 (92.6)	302 (92.1)	202 (92.7)
G	30 (7.4)	26 (7.9)	16 (7.3)

Data are expressed as number and (percentage).

Table 3
Interaction of IL-10-1082G/A, TNFA-308G/A, and the severity of malaria in Thailand.

	Mild malaria controls (n = 203)	Non-cerebral severe malaria (n = 163)	Cerebral malaria (n = 109)
Combination of allele positivity			
-1082A + / -308A +	16 (7.9)	20 (12.3)	14 (12.8)
-1082A + / -308A -	185 (91.1)	142 (87.1)	95 (87.2)
-1082G - / -308G +	1 (0.5)	0 (0)	0 (0.0)
-1082G - / -308G -	1 (0.5)	1 (0.6)	0 (0.0)

Data are expressed as number and (percentage).

the positivity of the *TNFA* -308A exhibited a tendency to increase in severe malaria patients compared to mild malaria controls in Table 3, no significant difference was detected. In Thai malaria patients, the disease severity would not be influenced by *IL-10* -1082G/A and *TNFA* -308G/A polymorphisms.

ACKNOWLEDGEMENTS

This study was supported by the Core University System Exchange Program of the Japan Society for the Promotion of Science, which is coordinated by the University of Tokyo and Mahidol University, further support was funded by the National Research Council of Thailand and a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Science, Sports and Culture.

REFERENCES

Eskdale J, Keijsers V, Huizinga T, Gallagher G. Microsatellite alleles and single nucleotide polymorphisms (SNPs) combine to form four major haplotype families at the human interleukin-10 (IL-10) locus. *Genes Immun* 1999;1:151-5.

Hananantachai H, Patarapotikul J, Looareesuwan S, Ohashi J, Naka I, Tokunaga K. Lack of association of -308A/G TNFA promoter and 196R/M TNFR2 polymorphisms with disease severity in Thai adult malaria patients. *Am J Med Genet* 2001;102:391-2.

Koss K, Fanning GC, Welsh KI, Jewell DP. Interleukin-10 gene promoter polymorphism in English and Polish healthy controls. Polymerase chain reaction haplotyping using 3´ mismatches in forward and reverse primers. *Genes Immun* 2000;1:321-4.

Kurtzhals JAL, Adabayeri V, Goka BQ, *et al*. Low plasma concentrations of interleukin 10 in severe malarial anaemia compared with cerebral and uncomplicated malaria. *Lancet* 1998;351:1768-72.

May J, Lell B, Luty AJF, Meyer CG, Kremsner PG. Plasma interleukin-10:tumor necrosis factor (TNF)-α ratio is associated with TNF promoter variants and predicts malarial complications. *J Infect Dis* 2000;182:1570-3.

McGuire W, Hill AV, Allsopp CE, Greenwood BM, Kwiatkowski D. Variation in the TNF-α promoter region associated with susceptibility to cerebral malaria. *Nature* 1994;317:508-10.

Othoro C, Lal AA, Nahlen B, Koech D, Orago ASS, Udhayakumar V. A low interleukin-10 tumor necrosis factor - a ratio is associated with malaria anemia in children residing in a holoendemic malaria region in western Kenya. *J Infect Dis* 1999;179:279-82.

Tounas NA, Cominelli F. Identification and initial characterisation of two polymorphisms in the human IL-10 promoter [abstract]. *Eur Cytokine Network* 1996;7:578.

Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV. An investigation of polymorphism in the interleukin-10 gene promoter. *Eur J Immunogenet* 1997;24:1-8.

Westendorp RGJ, Langermans JAM, Huizinga TWJ, *et al*. Genetic influence on cytokine production and fatal meningococcal disease. *Lancet* 1997;349:170-3.