Jpn. J. Infect. Dis., 54, 114-116, 2001

Short Communication

Absence of Association between the Allele Coding Methionine at Position 29 in the N-terminal Domain of ICAM-1 (ICAM-1^{Kilifi}) and Severe Malaria in the Northwest of Thailand

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

Department of Human Genetics, Graduate School of Medicine, University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo 113-0033, Japan and
[1]Faculty of Tropical Medicine, Mahidol University, 420/6 Rajvithi Road, Bangkok 10400, Thailand

(Received June 6, 2001. Accepted July 27, 2001)

SUMMARY: Intercellular adhesion molecule 1 (ICAM-1) is known to be the endothelial receptor for *Plasmodium falciparum*-infected erythrocytes. Associations of the variant allele coding methionine at position 29 in the N-terminal domain of ICAM-1, ICAM-1^{Kilifi}, with severe malaria have been investigated in African populations, and the results of these investigations have varied widely. In this study, we investigated a possible association between the ICAM-1^{Kilifi} and severe malaria in adult malaria patients living in northwest Thailand. The frequencies of the ICAM-1^{Kilifi} among patients with mild malaria, with non-cerebral severe malaria, and with cerebral malaria were 1.7%, 2.7%, and 2.3%, respectively. This variant showed neither positive nor negative association with severe malaria in Thailand.

Intercellular adhesion molecule 1 (ICAM-1) is the essential endothelial ligand for binding of *Plasmodium falciparum*-infected erythrocytes (1). The interaction between the infected erythrocytes and host endothelium is considered to play a crucial role in the pathogenesis of severe malaria. The sequestration of mature parasitized erythrocytes from the peripheral circulation in *Plasmodium falciparum* infections by adhesion to the postcapillary venular endothelium is one of the remarkable features of cerebral malaria. The binding site is known to locate in the first N-terminal domain of ICAM-1 (2,3). Recently, a mutation causing an amino acid substitution from lysine to methionine at position 29 in the N-terminal domain of ICAM-1 was found in Kilifi, Kenya. The allele coding methionine, known as ICAM-1^{Kilifi} or 29M, was first reported to be significantly associated with susceptibility to cerebral malaria in Kenya (4). More recently, however, the 29M allele was found to be protective against severe malaria in Gabon (5). Also, a lack of association between the 29M allele and severe malaria was reported in West Africa (6). Surprisingly, the results are quite different among these three previous studies. Thus, the association between 29M and severe malaria has not been established yet, and to the best of our knowledge, association studies on the K29M ICAM-1 polymorphism have never been carried out in Asian populations. For this reason, we here investigated the possible association between the 29M allele and severe malaria in adult malaria patients living in northwest Thailand.

In order to examine the association between 29M and severe malaria, malaria patients living in northwest Thailand were analyzed. Informed consent was obtained from all individuals. Two-hundred three patients with mild malaria, 165 with non-cerebral severe falciparum malaria, and 109 with cerebral malaria who were treated at the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University from 1998 to 2000 were recruited for participation in this study. All patients were 13 years of age or older, and the mean ages for mild malaria, non-cerebral severe falciparum malaria, and cerebral malaria were 25.5, 23.7, and 28.6, respectively. Genomic DNA was extracted from peripheral blood leukocytes using a QIAamp blood kit (Qiagen, Hilden, Germany). A 263 bp fragment in exon 2 encompassing the mutation causing the K29M polymorphism was amplified by PCR using a 5′ primer ICAM-1F (5′-GACCTGGCAATG CCCAGACATCTGTGTCC-3′) and a 3′ primer ICAM-1R (5′-GTACACGGTGAGGAAGGTTTAGCTGTTG-3′). PCR was performed using GeneAmp reagents and AmpliTaq Gold DNA polymerase (Perkin-Elmer Applied Biosystems, Foster City, Calif., USA). The conditions of the amplification consisted of initial denaturation at 96°C for 10 min, followed by 38 cycles of denaturation at 96°C for 1 min, annealing at 63°C for 1 min, and extension at 74°C for 1 min, using a thermal cycler (GeneAmp PCR system 9700; Perkin-Elmer Applied Biosystems). The PCR products of 10 randomly chosen samples were used for the direct sequencing with an ABI PRISM™ 310 Genetic Analyzer (Perkin-Elmer Applied Biosystems). The samples with and without the 29M allele were used as references in the PCR-single strand conformation polymorphism (PCR-SSCP) analysis. Two microliters of solution containing the PCR product was mixed with 6 μl of denaturing solution (95% formamide, 20 mM EDTA, 0.05% bromphenol blue, 0.05% xylene cyanol FF). The mixtures were denatured at 96°C for 5 min and immediately cooled on ice. One microliter of the mixtures was applied to 10% polyacrylamide gel (acrylamide: bisacrylamide = 49:1). Electrophoresis was carried out in 0.5 × TBE (45 mM Tris-borate [pH 8.0], 1 mM EDTA) under constant current of 20 mA/gel, using a minigel electrophoresis apparatus with a constant temperature control system (90 × 80 × 1 mm, AE 6410 and AE 6370; ATTO, Tokyo). Single-strand DNA

*Corresponding author: Tel: +81-3-5841-3693, Fax: +81-3-5802-8619, E-mail: juno@m.u-tokyo.ac.jp

Table 1. Genotype and allele frequencies of the 29M allele in the Thai malaria patients

	Mild malaria controls (n = 203)[1]	Non-cerebral severe malaria (n = 165)	Cerebral malaria (n = 109)
Genotype frequency			
29M / 29M	0 (0.0)[2]	0 (0.0)	0 (0.0)
29M / 29K	7 (3.4)	9 (5.5)	5 (4.6)
29K / 29K	196 (96.6)	156 (94.5)	104 (95.4)
Allele frequency[3]			
29M	7 (1.7)	9 (2.7)	5 (2.3)
29K	399 (98.3)	321 (97.3)	213 (97.7)

[1] no. of patients.
[2] percentage.
[3] no. of genes.

Table 2. Interaction of ICAM-1 29M, TNFR2 196R, and severe malaria in Thailand

	Mild malaria controls (n = 201)[1]	Non-cerebral severe malaria (n = 163)	Cerebral malaria (n = 108)
Combination of allele positivity			
29M + / 196R +	1 (0.5)[2]	3 (1.8)	4 (3.7)
29M + / 196R −	6 (3.0)	6 (3.7)	1 (0.9)
29M − / 196R +	49 (24.4)	45 (27.6)	23 (21.3)
29M − / 196R −	145 (72.1)	109 (66.9)	80 (74.1)

[1] no. of patients.
[2] percentage.

fragments in the gel were visualized by silver staining (Daiichi Pure Chemicals, Tokyo).

The genotype and allele frequencies of the 29M allele in malaria patients are shown in Table 1. 29M was rare in Thailand, and showed neither positive nor negative association with severe malaria in adult Thai patients (Chi-square test based on a 2×2 contingency table).

Tumor necrosis factor receptor 2 (TNFR2) is known to mediate upregulation of ICAM-1 (7). Recently, an amino acid substitution from methionine to arginine at position 196 in exon 6 of the TNFR2 was found, and the allele coding arginine (196R) was reported to be significantly associated with systemic lupus erythematosus in Japanese (8,9). Therefore, we investigated a possible association of the 196R allele with severe malaria in the same cohort, but detected no significant association (10). In order to analyze the interaction between 29M and 196R in regard to the severity of malaria, the present case-control data were stratified by the allele positivities of the 29M and 196R alleles (Table 2). Although no significant difference was observed among the three malaria groups, the number of individuals possessing both alleles was marginally higher in the group of patients with cerebral malaria than in those with mild malaria (Fisher's exact test; $P = 0.052$). Because we cannot exclude the possibility that the small P value occurred due to chance after the stratification, this problem should be investigated in the future. Furthermore, the data were also stratified by the allele positivities of the 29M and tumor necrosis factor α-308A (TNFA-308A) allele investigated in our previous study (10), and no statistically significant difference was detected (data not shown).

Our study revealed no association of the 29M allele with severe malaria, while the frequency of this allele was rare in the Thai malaria patients. We therefore cannot exclude the possibility that the sample size was too small to detect a difference in frequency of the 29M allele among the groups, since a large sample size is statistically required to detect a susceptibility allele with a low frequency (11). However, considering the low frequency of the 29M allele, we may say that this variant is unlikely to be selectively advantageous against malaria in Thailand.

Over the last few years, the association of the 29M allele with severe malaria has been the subject of controversy (4-6). Different parasite strains show different avidity for the ICAM-1 ligand (12), suggesting that ICAM-1 alleles predisposing to severe malaria vary among different populations, since malaria strains themselves are considered to be different from population to population. Thus, the discordant results among the present and three previous association studies may be due to the difference in strains of *P. falciparum*.

ACKNOWLEDGMENTS

This study was supported by the Core University System Exchange Programme under the auspices of the Japan Society for the Promotion of Science, coordinated by the University of Tokyo and Mahidol University, by the National Research Council of Thailand, and by a grant-in-aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

REFERENCES

1. Berendt, A. R., Simmons, D. L., Tansey, J., Newbold, C. I. and Marsh, K. (1989): Intercellular adhesion molecule-1 is an endothelial cell adhesion receptor for *Plasmodium falciparum*. Nature, 341, 57-59.
2. Ockenhouse, C. F., Betageri, F. R., Springer, T. A. and Staunton, D. E. (1992): *Plasmodium falciparum*-infected erythrocytes bind ICAM-1 at a site distinct from LFA-1, Mac-1, and human rhinovirus. Cell, 68, 63-69.

3. Berendt, A. R., McDowall, A., Craig, A. G., Bates, P. A., Sternberg, M. J., Marsh, K., Newbold, C. I. and Hogg, N. (1992): The binding site on ICAM-1 for *Plasmodium falciparum*-infected erythrocytes overlaps, but is distinct from, the LFA-1-binding site. Cell, 68, 71-81.
4. Fernandez-Reyes, D., Craig, A. G., Kyes, S. A., Peshu, N., Snow, R. W., Berendt, A. R., Marsh, K. and Newbold, C. I. (1997): A high frequency African coding polymorphism in the N-terminal domain of ICAM-1 predisposing to cerebral malaria in Kenya. Hum. Mol. Genet., 6, 1357-1360.
5. Kun, J. F. J., Klabunde, J., Lell, B., Luckner, D., Alpers, M., May, J., Meyer, C. and Kremsner, P. G. (1999): Association of the ICAM-1^{Kilifi} mutation with protection against severe malaria in Lambarene, Gabon. Am. J. Trop. Med. Hyg., 61, 776-779.
6. Bellamy, R., Kwiatkowski, D. and Hill, A. V. S. (1998): Absence of an association between intercellular adhesion molecule 1, complement receptor 1 and interleukin 1 receptor antagonist gene polymorphisms and severe malaria in a West African population. Trans. R. Soc. Trop. Med. Hyg., 92, 312-316.
7. Lucas, R., Lou, J., Morel, D. R., Ricou, B., Suter, P. M. and Grau, G. E. (1997) TNF receptors in the microvascular pathology of acute respiratory distress syndrome and cerebral malaria. J. Leukoc. Biol., 61, 551-558.
8. Komata, T., Tsuchiya, N., Matsushita, M., Hagiwara, K. and Tokunaga, K. (1999): Association of tumor necrosis factor receptor 2 (TNFR2) polymorphism with susceptibility to systemic lupus erythematosus. Tissue Antigens, 53, 527-533.
9. Tsuchiya, N., Komata, T., Matsushita, M., Ohashi, J. and Tokunaga, K. (2000): New single nucleotide polymorphisms in the coding region of human *TNFR2*. Genes. Immun., 1, 501-503.
10. Hananantachai, H., Patarapotikul, J., Ohashi, J., Naka, I., Looareesuwan, S. and Tokunaga, K. (2001): 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., 102, 391-392.
11. Ohashi, J., Yamamoto, S., Tsuchiya, N., Hatta, Y., Komata, T., Matsushita, M. and Tokunaga, K. (2001): Comparison of statistical power between 2×2 allele frequency and allele positivity tables in case-control studies of complex disease genes. Ann. Hum. Genet., 65, 197-206.
12. Adams, S., Turner, D. D. H., Nash, G. B., Micklem, K., Newbold, C. I., Craig, A. G. (2000): Differential binding of clonal variants of *Plasmodium falciparum* to allelic forms of intercellular adhesion molecule 1 determined by flow adhesion assay. Infect. Immun., 68, 264-269.