Tokai J Exp Clin Med., Vol. 23, No. 2, p.73, 1998

Single Locus Analysis of Allelic Variation in the *Plasmodium falciparum* MSP1 Gene of Field Isolates

Kazuyuki TANABE, Naoko SAKIHAMA, Osamu KANEKO*, Masatsugu KIMURA*, Marcelo U. FERREIRA**, Fumihiko KAWAMOTO** and Kenji HIRAYAMA***

Osaka Institute of Technology, Osaka, Japan
**Medical School, Osaka City University, Osaka, Japan*
***School of Medicine, Nagoya University, Aichi, Japan*
****Saitama Medical School, Saitama, Japan*

MSP1 is the major merozoite surface protein of *Plasmodium falciparum* and a malaria vaccine candidate. MSP1, however, exhibits extensive antigen polymorphism, which may compromise the development of effective vaccines based on this molecule. Since MSP1 is not only a vaccine candidate but a useful marker for the genetic diversity of *P. falciparum*, we have been studying variations in the MSP1 gene. Our previous sequence analysis revealed that variable regions of the gene are clustered into several blocks that are flanked by conserved or semi-conserved blocks and that variations are interestingly not polymorphic but dimorphic; i.e. variation is one or the other of two types, represented by MAD20 allele and K1 allele. The exception to this is block 2, which is expressed by three allelic types; MAD20-, K1- and RO33-types. Intragenic recombination has been suggested and subsequently demonstrated to generate new MSP1 alleles in the progeny after cross-fertilization in heterozygotes of parental alleles. To date, recombination is known to occur at the 5' part of the MSP1 gene (blocks 3, 4 and 5). However, only limited data are available to document allelic variation in the gene of field isolates.

To conduct a through molecular epidemiological study on allelic variation in the MSP1 gene in the natural populations of *P. falciparum*, we developed a PCR strategy that can detect 24 different MSP1 gene types and applied to filed isolates from South East Asia. Analysis of >230 isolates showed the following: (1) recombination between two representative allelic types did not exist in the central and most part of the 3' terminal part (blocks 6 to 16) of the gene, (2) 43% of isolates had multiple MSP1 gene types with a mean number of 1.7 gene types per isolate, (3) of 24 MSP1 gene types, 22 types occurred and particular types having MAD20 allelic type in blocks 6 to 16 predominated in southern Vietnam, and (4) frequency distribution of MSP1 gene types did not differ in three populations groups in the area. Frequency of MSP1 gene types observed was significantly higher-than-expected under the null hypothesis for goodness of fit by assuming random associations of allelic types in blocks 3, 4 and 5, thus rejecting the null hypothesis. After comparison of expected and observed frequencies of allelic types in pairs of blocks, non-random associations were detected between blocks 4 and 6 and random associations between other pairs of blocks. These results suggest that selection after recombination in blocks 3, 4 and 5 operates in favor of particular MSP1 gene types. We also examined sequence variation in the cysteine-rich, C-terminal 19kDa region, a strong vaccine candidate. Except one residue near the C terminus, direct sequencing of 31 field isolates with single MSP1 gene type confirmed the well recognized four dimorphic variations; E/Q and TSR/KNG. Here again, random associations were also noted in four possible combinations (ETSR, EKNG, QTSR and QKNG), indicating that recombination occurred in the 19kDa region. This result implies that recombination events should be stressed in designing effective malaria vaccines.

Kazuyuki TANABE, Osaka Institute of Technology, Ooaza 5-16-1, Asahi-ku, Osaka, Osaka 535-0000, Japan