SEROLOGICAL EVALUATION OF MALARIA PATIENTS IN THAILAND: ANTIBODY RESPONSE AGAINST ELECTROPHORESED ANTIGENIC POLYPEPTIDES OF *PLASMODIUM FALCIPARUM*

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Abstract. It was reported that a 47kDa antigenic polypeptide of *Plasmodium falciparum* had been strongly presented by the sera from 1) imported Japanese malaria patients with severe symptoms and 2) symptomatic and parasitemic inhabitants in endemic areas in the Sudan, Malaysia and the Philippines. In the present study, we observed the reactivity of the sera from falciparum malaria patients who had been hospitalized in the Bangkok Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, and compared the antibody response against the 47kDa antigenic polypeptide according to the severity of the patients. It was observed that antibodies to this molecule were more commonly shared in sera from severer patients, although the IFAT titers against the whole *P.falciparum* parasite antigen were lower in the group, which suggested that this antibody against the 47kDa molecule was playing a specific role at a severe stage of the infection. Determination of the immunological features of the antigenic molecules of parasites by this type of sero-epidemiological study will provide a new assay system for evaluation of immune status of individuals in different severity and suggest a way of vaccine development.

INTRODUCTION

The presence of high antibody levels against malaria parasites can be found in the sera of most adult individuals in malaria endemic areas regardless of whether these individuals have clinical malaria or not. These antibodies react specifically with various antigenic bands prepared from the parasite when analyzed using Western blotting. However, we (Kano *et al,* 1990a) reported that in a longitudinal study on the serum taken from a Japanese man with imported *P. falciparum* infection, a 47kDa band was strongly reactive only during the acute phase of the infection with progressively decreasing reactivity and was barely detectable in convalescence. We concluded that the 47kDa molecule may be useful in determining present or recent infections with clinical manifestations in serological surveys of malaria. The present study attempted to extend above findings to patients living in Thai-Myanmar border. We also discuss immunologic features of the defined antigenic molecules and their usefulness in immuno-epidemiologic studies of malaria as well as in the strategy for vaccine production.

MATERIALS AND METHODS

Subjects studied

Sera were obtained from 90 patients prior to drug treatments, who were hospitalized from July 15, 1996 to January 4, 1997, suffering from acute falciparum malaria in the Bangkok Hospital for Tropical Diseases, Mahidol University, Thailand. Most of them contracted malaria from the Thai-Myanmar border. On admission, these patients were defined by severity of the disease as follows: The first group (Group 1) consisted of those patients who were classified as severe and complicated malaria defined by WHO criteria (WHO, CTD, 1990). The second group (Group 2) was defined as moderately severe malaria, who were severe enough to require parenteral antimalaria drugs but had no complications. The third group (Group 3) was mild malaria, who were suffering from uncomplicated malaria and treated only with oral antimalaria drugs. Demographic data of patients such as age (range 15 - 66) and sex (male : female = 57 : 33) of each group of patients are described in Tables 1 to 3. These patients enrolled

gave written consent to this study, which was approved by the Ethical Committee of the Mahidol University. Immediately after drawing blood from patients (in less than 2 hours), sera from each patient were separated by aseptic technic in the Hospital laboratory and kept at -20°C, then transferred to a storage freezer (-35°C) in Japan via dry ice freezing.

Parasites used in the study

An established strain of *P. falciparum* of Gambian origin and adapted to *in vitro* culture was used in this study. This strain, SGE1, was donated by Professor Ambroise-Thomas in 1979, and was maintained in our laboratory by continual *in vitro* culture with occasional freezing . The parasite had been cultured in an ordinary CO_2 incubator (Miyagami and Waki, 1985) using RPMI 1640 with a 10% volume of human O type RBC with the addition of 10% human sera (Trager and Jensen, 1976).

Serological study

An indirect immunofluorescent antibody test (IFAT) was performed on all serum specimens using the method described by Kano *et al* (1990b). The secondary antibody employed was an FITC-conjugated rabbit immunoglobulins to human IgG (γ -chain specific; Dako, Denmark).

Western blotting study

Asynchronous cultured parasites, in which were included no gametocytes, were harvested when parasitemia reached >20%, and the infected erythrocytes were hemolyzed with 0.05% saponin. The lysate was centrifuged with a refrigerating centrifuge at 3,600 rpm for 15 minutes, and then washed 3 times with phosphate buffered saline (PBS). After the final wash, the sediment was dissolved in the antigen solution (2.3% SDS, 5% 2-ME, 1 mM PMSF, 62.5 mM Tris-Cl, pH6.8). The resulting material was stored at -35OC until use, and was adjusted to a concentration of 30 µg/20 µl when applied to one-dimensional electrophoresis in 10% acrylamide gels, following the method of Laemmli (1970). The reference markers used were SDS-PAGE Standards (BioRad, CA). Western blotting was performed by electrotransfer to polyvinylidene difluoride filter (PVDF) (Clear Blot-P, ATTO, Tokyo) using Horizeblot (ATTO, Tokyo) with absorbent papers soaked in blotting buffer (0.1M Tris-Cl, 0.192M Glycine, 20% methanol). Non-specific reactions to the PVDF were blocked by soaking the membrane in 10% skimmed milk in PBS at 4°C overnight and then washed three times with PBS. The blotted PVDF strips were incubated with each serum sample at 37°C for 2 hours. After washing, the strips were then incubated with peroxidase-labelled goat anti-human IgG (Dako, Denmark) at 37°C for 1 hour. The substrate for the enzyme reaction was from a Konica immunostain kit (Konica, Tokyo, Japan) and the molecular weights (MW) of fractionated antigen bands were determined using the curves obtained with the standard markers.

RESULTS

Results obtained by electrophoresis and the IFAT using the serum samples from Group 1 are shown in Table 1. Many bands of lower molecular masses were commonly shared by the group of patients of which 47kDa molecule was shown by 78.3 % of them. The IFAT titers ranged from 1:4 to 1:4096 with geometric mean reciprocal titer (GMRT) at 1:226. The profiles of moderately severe malaria patients (Group 2) are shown in Table 2. The GMRT of IFAT was 1:329 which was higher than that of Group 1. However, no more than 45.5% of them showed positive reactivity against the 47kDa molecule. The third profile in Table 3 is of mild malaria patients (Group 3). Bands of higher molecular weights (200kDa, 150kDa, 115kDa and 110kDa) were relatively strongly presented by the sera. Positivity rate of the 47kDa became only 33.3%, although the GMRT was the highest of the three groups.

The pattern of the other bands present varied considerably by serum specimen, and thus consistent findings concerning the immunological features of the corresponding molecules could not be properly speculated. However, one of the strongest bands commonly shared among three groups was the 95kDa molecule, whose positivity rates were 56.5% (Group 1), 63.6% (Group 2) and 83.3% (Group 3).

DISCUSSION

We previously reported that the 47kDa molecule was strongly exhibited by sera of non-immune Japa-

nese patients who were at the acute stage of falciparum malaria (Kano *et al,* 1990a). This was also true of Sudanese children who lived in an endemic locality and developed fever and parasitemia. It therefore appeared that presentation of the 47kDa molecule indicated a current or recent symptomatic episode of malaria developed in susceptible individuals. This results was supported by the report from the Philippines which indicated that the 47kDa molecule was likely to be manifested by sera from people who were less resistant to malaria, but not readily by those from immune individuals who even showed parasitemia (Rivera *et al,* 1997). Similar findings were also reported by Norazmi *et al* (1996) using *P.falciparum* antigen of Malaysian origin. In the present study employing sera from patients in Thailand, we concluded that severer patients were likely to show strong reactivity against the 47kDa. In other words, the molecule showed strong antigenicity against those individuals who were symptomatically severe, playing immunologically a certain role for those patients to overcome those severe symptoms.

In mild cases (Group 3), the results of the IFAT revealed higher titers. This finding suggests that humoral immunity is important for the modulating the patients' severity. Thus, distribution of the positive bands in the blotted map of Table 3 gives us some hints on the role of antibodies against respective antigenic molecules. Relatively larger molecules, such as 200kDa, 150kDa, 115kDa, and 110kDa, are expected to be immunologically very important for the host to produce effective antibodies against *P.falciparum* parasites for the protection of the disease. The 68kDa molecule was also highly shared by many mild malaria isolates, which also requires further investigation to determine its immunological significance.

The 95kDa molecule was recognized most commonly by patients in all Groups. The potential of this molecule in diagnosis of *P.falciparum* infection will be envisaged. One of the objectives of the present study is to provide a new malariometric index using defined molecules. Determination of the characteristics of each molecule is, of course, indispensable for the seroepiemiological studies before these antigenic molecules are to be used.

In conclusion, we have conducted serological studies of malaria patients in Thailand, referring to antibody response against electrophoresed antigenic polypeptides of *P.falciparum* our study revealed that the reactivity of the patients' sera were corresponding to their severity. Our results using an immuno-epidemiological approach will provide new suggestions for determination of parasite molecules as candidates for vaccine development.

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