Tokai J Exp Clin Med., Vol. 23, No. 2, pp.97-98, 1998

Serological Evaluation of Malaria Patients in Thailand, with Particular Reference to the Reactivity against a 47KD Antigenic Polypeptide of *Plasmodium falciparum*

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Quite a few antigenic polypeptides of *Plasmodium falciparum* (*P.f.*) have been investigated for their potentiality to serve as vaccine. Applying SDS-PAGE and Westerblotting technique, we have also studied an antigenic polypeptide, a relative molecular mass of 47kD, which may be related to the development of a protective immune response in the host.

First, the 47kD was subjected to the molecular characterization. Preparatively electrophoresed *P.f.* antigen was blotted onto the PVDF membrane and the 47kD band was cut off from the membrane. The polypeptide was eluted, treated with lysil-end proteinase, subjected to reversed phase chromatography, and blotted again on PVDF membrane by micro blotter. The chromatogram showed that several fractions of the 47kD polypeptides were obtained and small pieces of membrane were cut according to the respective peak and subjected to the peptide sequencer. The sequencing of the peak molecules revealed that obtained amino acid sequences were parts of *P.f.* enolase. This enolase is a key enzyme of the glycolytic pathway and catalyzes the conversion of 2-phosphoglycerate to phosphoenol pyruvate, which is the ninth reaction of the eleven-step pathway from glucose to lactic acid, and the only dehydration reaction in this series. The degree of identity of the *P.f.* enolase to that from human host is 68.4%, so that the enolase would not be a favorable target for chemotherapeutic intervention because of the relatively higher identity. Katakai's analysis based on the standard Chou-Fasman method proved only α helices and did not give reliable β-Sheet predictions, however, the similarity to the 8-fold $\beta + \alpha$ -Barrel structure as determined by that of yeast can be predicted.

Although it is not clear if immunity of the host can be established by the antibody biding to the antigen, consequently inhibiting glycolysis of the parasite, so that we have raised a monoclonal antibody against the 47kD antigenic molecule and investigated some of the potentiality of the molecule *in vitro*. Optical section images of the confocal micrograph of the IFAT demonstrated that this monoclonal antibody reacted specifically at schizont stage of the parasite. Incubation of the monoclonal antibody with the synchronized erythrocytic forms of *P.f.* for 30 hours *in vitro*, inhibited the maturation of the schizonts in a dose dependent manner. And also, cultivation of the asynchronous parasites with the monoclonal antibody inhibited the growth rate of the parasites. Therefore, the determination of the active site of the *P.f.* enolase and neutralization or inhibition of enolase activity will be of great importance. It is known that parasitized erythrocytes increase their utilization of glucose as much as 100 times the rate of uninfected host red cells, and consequently made the host hypoglycemic. Inhibition of this enzymatic activity could also contribute to the betterment of the host's symptom.

It was already proved that this 47kD antigenic polypeptide of *P.f.* 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,

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Malaysia and the Philippines. We also studied the reactivity of the sera from malaria patients who had been hospitalized in Faculty of Tropical Medicine, Mahidol University, Thailand. We also compared the reactivity of the sera according to the severity of the patients suffering from *P.f.* malaria, of which results is to be shown in this presentation. The fact that specific antibodies to this molecule were commonly found in sera from acute patients and humans in endemic areas suggested that they may be playing a certain role at an acute stage of the infection.