STRAIN VARIATION AND GENE HUNTING IN MALARIA

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ABSTRACT

Malaria still remains uncontrolled affecting millions and killing many. We have found the high chloroquine-resistance in Rajasthan epidemic which contains multiple *P.falciparum* strains. Large number of variant *P.falciparum* strains exist in India which should be taken into account for future malaria control strategies. We have characterised several parasite molecules which are involved in the host-parasite interaction. They can be used to develop the molecular therapy or anti-malarial drugs for malaria. The parasite enzymes described by us will be quite useful in this regard, particularly in drug development.

KEY WORDS : *P.falciparum*, *P.vivax*, Knob protein, Heat shock protein, Metalloprotease, Alu elements.

INTRODUCTION

Malaria affects millions of people each year with large number of deaths. The disease still remains uncontrolled and causing epidemics in the country. The factors responsible for the situation are the chemical resistance shown by the parasite and its vector as well as non-availability of an effective malaria vaccine. The malarial parasite shows fluidity in its genome. This results in a high rate of antigenic variation as well as the emergence of newer strains. Here we review the existence of multiple *Plasmodium falciparum* strains in India. We also review our work on various parasite molecules involved in host-parasite interaction and they could be used in future malaria control programmes.

STRAIN VARIATION

We have used PCR technology to type the *P.falciparum* strains from clinical isolates obtained from different parts of the country. A total of 28 different genotypes and thus the *P.falciparum* strains were found among 79 clinical isolates tested. There seems to be a high rate of strain variation in some states than the others. Certain

strains were also found to be region specific whereas others were common to more than one state. We have also observed multiple strains among chloroquine resistant cases. Taken together, the data indicates the existence of multiple *P.falciparum* strains in India which should be taken into consideration for future malaria control programmes..

GENE HUNTING

We are working mainly on two human malaria parasites-*P.falciparum* and *P.vivax*. We have isolated several immunobiologically important molecules from these parasites by screening the gene libraries with nucleic acid or antibody probes.

(A) *P.falciparum*

This parasite causes the most fatal form of malaria in human. We have studied two molecules of this parasite. (I) The knob associated histidine-rich protein (KAHRP) also called as knob protein (KP) or *P.falciparum* histidine-rich protein I (PfHRPI), and (II) Heat-shock protein (HSP) also called as PfHSP70.

A.I Knob-associated histidine-rich protein

The protein is responsible for the induction of protuberances on the surface of *P.falciparum*-in-

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fectured erythrocytes. These structures are known as knobs and are responsible for the cytoadherence of mature parasite-infected RBCs to the host endothelial cells. This causes the sequestration of mature parasites in blood capillaries of internal organs and give rise to the complications. We have cloned the gene of KAHRP by using oligonucleotide probe (oligos for 5 contiguous histidines) to screen a cDNA library (1). Now we have cloned this gene from various isolates to show the existence of its three allelic forms (2,3). The information on the allelic forms of KAHRP has been used in the above mentioned experiments for strain typing. We have also cloned the KAHRP gene from a monkey malaria parasite *P. fragile*. We are currently developing the molecular strategies to inhibit the protein synthesis by cleaving its mRNA by ribozyme and taking monkey malaria as a model.

A.II Heat Shock Protein

The discovery of this HSP gene was serendipitous while we were screening the cDNA libraries with the *P. falciparum* patient's serum. We have shown that this gene is not only homologous to the HSP70 family but it is also heat-inducible at high temperatures (4). We have also found the protective nature of this protein to the parasite during elevated temperatures. More than 70% *P. falciparum*-infected individuals produced antibodies against this antigen, but these antibodies were having short half life and did not inhibit the parasite growth in-vitro. Our results, therefore, did not support its vaccine candidature as proposed by others.

(B). *P. vivax*

This is the most common human malaria parasite in India and poses a great socio-economic threat to the country. Unfortunately, the parasite is not yet continuously cultivable in the lab and thus poorly characterized at molecular level. We have circumvented this problem by constructing the expression gene libraries and screening them with the patient's antibodies. This way we have isolated several antigen genes of this parasite (5,6). They are briefly reviewed here.

B.I. Heat-shock protein with metalloprotease motif:

Partial genomic clone, named Pv9 with 188

bp insert size, was isolated from *P. vivax*-gt₁₁ library by immunoscreening. The complete gene has recently been isolated from lambda EMBL3 library. The gene encodes a 483 amino acid long polypeptide with calculated mol. wt. of 51.2 kDa. It is rich in Ala, Leu, Pro and Arg, contains two transmembrane domains, one RGD motif of adhesive proteins and a N-linked glycosylation site. The antibody raised against 188 bp encoded peptide recognized a 55kDa parasite protein on western blots. This is close to the calculated mass considering the presence of a glycosylation site. The same antibody also reacted to all blood stages of the parasite during IFA. We have also shown experimentally the enhanced expression of this parasite protein at higher temperature indicating it to be a HSP. This is further confirmed by its high sequence homology to the small HSP of *E. coli* called HtpX. Interestingly the sequence also contains the zinc metalloprotease motif 'HEXXH'. Our preliminary results indicate that the 55 kDa protein is a proenzyme which is processed by the parasite to give rise to the mature 28 kDa protease. This is the first malarial HSP to have such biological function.

B.II Alu elements in a *P. vivax* antigen gene:

The another sero-reactive *P. vivax*-gt₁₁ clone, named Pv12, was also isolated with patient's antibodies. This clone was also partial and thus the complete gene was isolated from EMBL3 library as described above. The gene encodes a 200 amino acid long polypeptide which is highly hydrophobic and contains strong transmembrane domains. There were Alu elements in this gene which showed a sequence homology to the host sequences. Despite the high sequence homology to host Alu elements, PCR amplification was specific to the parasite sequences. The expression of this gene in parasite is experimentally proved by RT-PCR and also by the antibodies. The expression of Alu elements in the parasite is interesting since they are generally not being expressed in the host. Presence of Alu elements in parasite and in host may show their co-evolutionary pattern.

B.III Calcium ATPase:

Like above mentioned two clones, this clone (named Pv14) was also a partial clone obtained from gt11 library of *P. vivax*. Only 18 amino acids

were encoded from this 450 bp insert. The respective EMBL3 clone also did not extend this ORF except for 2 more amino acids towards N-terminus. Although we have sequenced a total of 2.3 kb DNA segment in continuation of Pv14, no further extension of ORF or presence of another exon was found for this gene. Therefore, it could be a smallest gene. However, in the upstream region there was a putative ORF for 101 amino acids which showed homology to P-type ATPases. But this ORF is too small to represent any P-type ATPase gene size. Furthermore, no RNA transcript was detectable to this ORF. Therefore, we considered this a pseudogene for P-type ATPases. In order to isolate the real gene, we carried out RT-PCR for calcium ATPase by using degenerate primers from the phosphorylation and energy transduction domains. This amplified cDNA (~500bp) was cloned and sequenced which showed high homology to *P. falciparum* calcium ATPase (4). This homology

was more at amino acid level than the nucleotide. This is because of the codon bias. This enzyme is involved in the calcium ions influx. The calcium ions are required for parasite growth.

B.IV. Cytochrome oxidase subunit III

This gene is cloned from the 6kb circle of the parasite. The 6kb circle is the smallest genome of parasite containing three ORFs. Although parasite seems to have rudimentary mitochondria, we found all the three genes for the respiratory chain enzymes being expressed during blood stages. Currently, we are sequencing the entire 6kb circle.

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