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Overview by Peter Perlmann, Stockholm

The Malaria Workshop held in Kasumigaseki, Tokyo, on June 15-16, 1997, was chaired by Masamichi Aikawa and organized by him together with Yoshitsugu Matsumoto, Koichi Nagakura and Yamaji Nakano. It covered a wide spectrum of malaria research and gave an excellent overview of its broad range in Japan. In the morning session on Sunday, June 15, Ruth Nussenzweig (New York, USA) discussed "Sporozoite ligand and hepatocyte receptors of malaria parasites." This was followed by a lecture of Peter Perlmann (Stockholm, Sweden) on "Selected problems of malaria blood stage immunity." In the morning session on Monday, June 16, Michael R. Hollingdale (Leeds, UK) lectured on "Malaria liver stage immunity" followed by Sornchai Looareesuwan (Bangkok, Thailand) who discussed "Research on new antimalarial drugs in the Bangkok Hospital for Tropical Diseases".

In the following, a summary will be given of the lectures held during the afternoon sessions on June 15 and 16.

Afternoon session, June 15, 1997

Antigenic diversity and variation are some of the major problems which have to be accounted for in any attempt to construct efficient subunit vaccines against the malaria parasite. Toshihiro Horii (Osaka University) reported on studies of the *P. falciparum* blood stage antigen SERA, which may induce protective immunity in monkeys. Allelic diversity caused by deletions or insertions was found to be restricted to a molecular domain comprising ≦5% of the amino acid sequence of this molecule. Hence, SERA appears to be a suitable vaccine candidate requiring only a few allelic variants to be included in a vaccine.

One of the *P. falciparum* vaccine candidates for asexual blood stages is the major merozoite surface protein, MSP1. Here, intragenic recombination has been found to generate allelic diversity. Kazuyuki Tanabe and coll. (Osaka Inst. of Technology) performed extensive studies to establish the extent of allelic diversity in field isolates from South East Asia, using PCR strategy. They found 24 different MSP1 gene types and about half of the isolates had multiple gene types which were evenly distributed in different populations. The results suggested both selection of certain gene types and the importance of recombination events underlying the allelic diversity observed.

Development of vaccines to control malaria transmission requires knowledge of the sexual and sporogonic stages of the *Plasmodium* parasite. Takafumi Tsuboi and coll. (Ehime University) reported on the results of detailed structural studies of novel ookinete antigens from the rodent parasites *P. yoelii* and *P. berghei*. Several such proteins have been cloned, sequenced and analyzed for secretory signal sequences. The analyses also established the presence of EGF-like domains typical for proteins of the sexual stages of the malaria parasite. Comparison of overall sequence similarities for certain of these antigens indicated closer homologies between the parasite species than with other subfamily members within the species.

The influence of male sex hormones on T cell function in immune protection of mice to the rodent malaria *parasite P. chabaudi chabaudi* was discussed by Zhi-Hui Zhang and coll. (Yamagata University). By using IFNγ R and IL4 knock-out mice they found both IFNγ and IL4 to be important for protection against lethal infection in male mice but the situation appeared to be more complex in female mice. IFNγ appeared to be a critical factor controlling parasitemias but there was no evidence for sex differences with regard to regulatory T cell functions when assessed as differences in parasitemia.

Yoshitsugu Matsumoto and coll. (Tokyo University) reported on plasma levels of circulating adhesion molecules in relation to severity of disease in *P. falciparum* infection. As some of these molecules act as adhesion receptors for infected erythrocytes and are upregulated in severe malaria, analysis of

circulating (=shed) adhesion molecules provides a measure of the severity of inflammation in *P. falciparum* malaria. In general, plasma levels of these molecules were elevated in malaria patients compared to uninfected controls. Among malaria patients, levels of circulating ICAM1 and ECAM1 were correlated to disease severity, suggesting a direct role of these adhesion molecules in pathogenesis.

Kenji Hirayama and coll. (Saitama Med. School) investigated host genetic factors affecting the course of *P. falciparum* malaria in Myanmar adults with special emphasis on the influence of HLA-B polymorphism. While they found no particular HLA-B associations in patients with either low or high parasitemias, there was a certain overrepresentation of B1301 in patients with mild anemia. Interestingly, there also was a significantly increased frequency of B4601 in patients with cerebral malaria. On the other hand, the enhanced natural resistance associated with B53 seen in Gambian children was not found in the present investigation of a resistant population of Malaysian aborigines (Orang Asli). However, in this population the related factor B1513 may play a similar role.

In ongoing studies of the importance of the MHC system, Nobuo Ohta and coll. (Nagoya City University) are screening MSP1, a major *P. falciparum* vaccine candidate antigen, for HLA-DR restricted T helper cell epitopes, by measuring the binding of oligopeptides to some relevant purified HLA-DR molecules and comparing binding with their capacity to induce human T cell responses. Both HLA-DR allele specific and non-specific binding was found, with a majority of the binding peptides coming from the dimorphic region of MSP1. Although not all HLA-DR binding peptides functioned as helper T cell epitopes, such activities were always associated with binding peptides. The results reflect an evolutionary selection due to immune pressure.

Yusuke Wataya and coll. (Okayama University) described a new diagnostic method for malaria, based on PCR amplification of the conserved regions of the gene encoding the small 18S ribosomal RNA subunit, using species specific oligonucleotide probes immobilized in the wells of microtiter plates. This "microtiter plate hybridization" method made possible a rapid, reliable and highly sensitive diagnosis of the species of the infecting parasite without the need of prior DNA extraction. It was also useful for mass-screening in epidemiological investigations and permitted the detection of new parasite variants.

Afternoon session, June 16, 1997

Parasites may exhibit considerable diversity in energy metabolism, reflecting adaptations to the complexity of their different hosts. Kiyoshi Kita and coll. (The University of Tokyo) studied these aspects in *Plasmodial* mitochondria using molecular-biological approaches. Studies of the cytochrome c oxidase gene of *P. vivax* suggested that this gene is functional in the parasite's erythrocytic stages. Similar structural studies of another mitochondrial marker enzyme (succinate-ubiquinine oxidoreductase/fumarate reductase) revealed amino acid composition typical for mitochondrial targeting structures, containing both sequences which were well conserved in comparison with other species but also some which were unique for *P. falciparum.*

Exposure to stress such as parasitic infection induces expression of heat shock proteins (HSP) by parasite as well as host. These proteins have a role both for the parasite's adaptation to the host and for the host's defense against the parasite. K. Himeno and coll. (Tokushima University) reported on the role of HSP65 for host parasite interaction in malaria infection. They have earlier shown that this HSP is expressed in γ/δ T cells in *Toxoplasma gondii* infection, by NK-T cells in *Leishmania major* infection and by NK cells in *Trypanosoma cruzi* infection. In all instances it has a crucial role in resistance. In contrast, in malaria infection (mice) expression of HSP65 in γ/δ T cells, NK T- or NK-cells was poor. Another HSP, HSP90, appeared to be associated with virulence as it was found to be strongly expressed in highly virulent L-strains of *P. yoelii* as compared to NL strains of low virulence, suggesting that some HSPs may have a role in the parasite's escape mechanism.

Hisashi Fujioka and coll. (Case Western Reserve University, Baltimore) presented new data on cloning, structure and localization in *P. falciparum* of another heat shock protein, HSP60. In *P. falciparum* this protein appeared to be constitutively expressed with a mitochondrial localization in all life cycle stages of the parasites. Together with its apparent interactions with other parasite proteins the findings suggest a chaperonin like function for HSP60.

Together with centers in other countries, Japan is participating in the worldwide efforts to sequence the entire genome of *Plasmodium falciparum*. Junichi Watanabe and his associates. (The University of Tokyo and Kyushu University) reported on the strategies employed to prepare genomic libraries utilizing the P1 phage and to stabilize the malarial DNA. The studies of the Japanese group have been focused on preparing a high resolution map of chromosome No 4. To obtain information about gene expression, a method for full-length cloning of mRNA has been established. The notable gene-duplications seen in *P. falciparum* are studied to elucidate mechanisms of its adaptation to the different hosts.

Shigeyuki Kano and coll. (Gunma University) reported on their structural and serological studies of a 47kDa antigen from *P. falciparum*. The polypeptide was identified as an enolase, a key enzyme in the glycolytic pathway, with about 70% homology with the corresponding human enzyme. A monoclonal antibody against this enzyme inhibited schizont maturation *in vitro* and it is suggested that an antibody mediated inhibition of this enzyme could improve the hypoglycemia associated with severe infection. The importance of the antibodies found in patients with acute infections is under investigation.

Shusuke Nakazawa and coll. (Nagasaki University) used *in vitro* culturing of *P. falciparum* to investigate the important issue of recrudescence after chemotherapy. Pyrimethamine sensitive and pyrimethamine resistant strains were exposed to the drug for four days and were subsequently cultured in the absence of the drug. In a different treatment the parasites were exposed to sorbitol. In both instances recrudescence was observed but the recrudescent parasite did not differ from the original cultures in drug sensitivity or sensitivity to sorbitol. Similar results were obtained after treatment with chloroquine, mefloquine, quinine or combinations of these. The results suggest that the cultures include small populations of dormant parasites which are not affected by treatment but may become active different times after cessation of treatment.

Satoru Kawai and coll. (Dokkyo University) reported on the development of severe (cerebral) malaria in Japanese macaques, experimentally infected with *P. coatneyi*. This lethal disease was characterized by sequestration of infected erythrocytes in cerebral microvessels, similar to what is seen in human cerebral malaria after *P. falciparum* infection. Investigation of T lymphocytes, cytokines, soluble adhesion molecules and rosette formation as well as light and electron microscopic investigations after autopsy showed remarkable similarities with severe human illness. The findings suggest that this monkey infection constitutes an important model for human disease, much more relevant than the mouse models presently available.