Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. *87*, Suppl. III, 69-70, 1992

ROUND TABLE 1 — SUMMARY

BIOCHEMISTRY, CELL AND MOLECULAR BIOLOGY

Chairman: *Luiz Pereira da Silva**
Co-Chairman: *Joseph Schrevel***

An intensive research activity has been developed in many laboratories throughout the world, in the last ten years, in order to develop vaccines against malaria. Important progress has been obtained in identification and cloning of a series of genes coding for antigens of the different developmental stages of malarial parasites, particularly *Plasmodium falciparum*. These efforts have already provided promising results in the experimental immunization of primates and human volunteers with a series of malaria antigens. However, no safe and useful vaccine for human use has yet been obtained. Two main problems have been found which complicate the accomplishment of this goal, namely: (1) genetic polymorphism of parasite antigens; (2) absence of information concerning the functional role of parasite molecules defined as possible target antigens.

These problems and others have induced many research groups to shift their interest to more fundamental studies on the parasite. An increasing number of publications have appeared in the last few years concerning basic aspect of biochemistry, metabolism and molecular biology of the malaria parasites.

In the present workshop of the IV INTERNATIONAL CONGRESS OF MALARIA AND BABESIOSIS, among a series of important communications concerning basic research of *Plasmodia* and Babesia parasites, we have selected three topics: (1) Chromosome size polymorphism of *Plasmodia* parasites; (2) Merozoïte and red blood cells molecules related to cell/cell interaction; (3) News in the genome structure of *Plasmodium*.

Three communications were presented on the theme of the first topic. All of them dealt with the role of the sub-telomeric regions of the chromosomes in the generation of polymorphism and rearrangement of the genetic material.

Dr David Kemp (Walter & Eliza Hall Institute, Melbourne, Australia) presented evidence on the role of sub-telomeric structures in originating chromosome size polymorphisms during meiotic and mitotic cycles of the parasite development. Heterologus recombination implicating sub-telomeric regions of different chromosomes of *P. falciparum* during meiosis have been described. Sub-telomeric regions are also frequently affected by deletions in the mitotic multiplication of the parasite in culture. As a general conclusion Dr Kemp proposed the hypothesis that sub-telomeric regions present important loci for chromosome rearrangements and, consequently, that the genes located in this site are more frequently submitted to genetic variation. This could be an important mechanism to explain the antigenic polymorphism of the parasite related to the escape from the host immune response. An example was given with the deletions of chromosome 9 in correlation with loses of cytoadherence.

Dr Arthur Scherf (Institut Pasteur, Paris, France) also discussed the role of sub-telomeric regions in the origin of size polymorphism of chromosomes. A large >30 Kb deletion, affecting the chromosome 10 of *P. falciparum* was isolated in culture. The deletion eliminated most of the coding sequences of the PF11.1 gene located sub-teleomerically. The remaining fragment of the 5' end of the gene was amplified by PCR, in combination with a primer corresponding to the telometric *P. falciparum* repeats. The deletion site in the PF11.1 gene is directly followed by a large number of telomeric repeats. Analysing the "broken site" in comparison with other cases of gene deletions in *P. falciparum* (Ravetch et al.), Scherf could recognize, in all the cases, the presence of the dimer CA at the transition to telomere repeats. The conclusion is that the parasite telomerase, which repairs the broken chromosomes, seems to recognize a short sequence in the primer molecule. The enzyme is able to add a large number of telomeric repeats which, according

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to what is known from other systems, would be synthesized using the internal RNA template of the telomerase.

Dr Barend Mons (University of Leiden, Netherlands) described in the rodent parasite *P. berghei,* the role of a 2.3 Kb sub-telomeric repeat in the generation of chromosome rearrangements. Size polymorphism is generated by addition, or deletion of these repeats. A mutant was found (generated during the asexual reproduction of the parasite) with a translocation of chromosome 7 to chromosome 13/14. The internal junction of the fusion also contains the 2.3 Kb sub-telomeric repeats which seem therefore, to participate in non homologous recombination events.

Three communications were presented on the theme of the second topic, concerning merozoite/red blood cell interactions related to cell invasion.

Dr Joseph Schrevel (Muséum d'Histoire Naturelle, Paris, France) reviewed the work of his group on *Plasmodia* proteases and presented evidence on the role of a neutral cysteine protease in the red blood cell invasion by merozoites of *P. falciparum.* The protease is a 105 kDa protein which acts on Val-Leu-Gly-Lys (or Arg) fluorogenic substrate. Anti-serum raised again purified protease present in the schizont and in the merozoite strongly inhibits invasion.

Dr John Barnwell (New York University, N.Y., USA) reviewed recent results of his group at NYU and the group of the NIH N.I.A.I.D. (Bethesda, USA) concerning invasion of red blood cells by merozoites of *P. vivax.* The classic observations showing a correlation between Duffy- phenotype and resistance to *P. vivax* infection has now been demonstrated at the molecular level. A *P. vivax* protein of 135-140 kDa (DAPs) specifically interacts with the 43 ka Duffy glycoprotein (DAP for Duffys' adhesion protein). The interaction does not depend on carbohydrate structures since it is not inhibited by treatment of erythrocytes with endo-glycosidases. To explain the preferential binding of *P. vivax* merozoites to reticulocytes. Barnwell looked for parasite molecules binding specifically to reticulocytes and found two proteins of 250 kDa (RBP1 and RBP2 for reticulocyte binding protein). The cloned genes also hybridize to *P. cynomolgi* DNA, known to invade preferentially reticulocytes. As the MSA-1 protein of *P. vivax* also binds to erythrocytes, the merozoite/reticulocyte interaction, in the case of *P. vivax* seems to be a complex sequence of phenomena. On the parasite side four proteins were identified: the DAP (135 kDa), the RBP1, RBP2 add the MSA-1 proteins; in the reticulocyte, besides the Duffy receptor for the DAP, no receptors have been yet identified for the three others.

Dr Hernando Del Portillo (University of São Paulo, Brazil) presented a comparative analysis of the structure of the MSA-1 gene from *P. vivax, P. falciparum* and *P. yoelli.* Conserved regions were observed in the N-terminal moiety of the molecule, 4 among all sequenced genes and one conserved only in human malaria *P. vivax* and *P. falciparum.* Recombinant fusion proteins were produced with expression vectors in *E. coli* containing one or more of the conserved regions. These proteins were used to analyse the antibody response of humans from endemic area of malaria in Amazon region of Brazil. Some of the conserved regions contain epitopes recognized by antibodies present in sera of people infected by *P. vivax* or *P. falciparum.* Other epitopes seem to be specific to one or other parasite. No correlation was observed between the level of ELISA titers and the previous number of previous clinical episodes of malaria.

Dr V.S. Misra (University of Florida, Gainesville, USA) presented the molecular analysis of the merozoite surface antigen of *Babesia bigemina*, an immunodominant antigen of the parasite. An open reading frame of 1440 bp long corresponding to the P58 protein contains an hydrophobic (transmembrane?) domain and a signal peptide at the N-terminus. PCR analysis of gene sequences from different isolates indicate an important polymorphism of the gene which may be involved in antigenic variation.

In the third topic Dr Luis Osaki (Federal University, Porto Alegre, Brazil) presented an analysis of total DNA of *B. bigemina* in which was found an extrachromosome element of 6,2 Kb. This element was correlated to a virus like particle and was found by other authors in other species of the *phylum Apicomplexa.*

Dr F. Santoro (Medical School, University of Grenoble, France), using specific probes, demonstrated the presence in the DNA of *P. falciparum,* of sequences homologous to that of two DNA oncogene sequences: v-fms and v-Ha ras.