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ROUND TABLE 3 — SUMMARY

CHARACTERIZATION OF CANDIDATE ANTIGENS

Chairman: *Richard Carter**
Co-Chairman: *Elizabeth U. Canning***

This session covered studies on molecular and immunological aspects of some of the antigens, or classes of antigen, which are under consideration as vaccine candidates for protection against babesial infection in cattle or malarial infection in man. The main these dealt with in this session were (1) molecular characterization and cellular location of antigens (11) immune responses to the native or recombinant antigens and (iii) expression and immunogenicity of "recombinant" or molecularly cloned antigens in expression systems in eukaryotic cells. In view of the great number of such antigens under investigation those discussed here inevitably represented a small selection.

Two presentations dealt with culture derived exo-antigens of *Babesia bovis*. J.H. Patarroyo and colleagues have used culture supernatants from an attenuated strain of *B. bovis* of Brazilian origin as a source of antigen. The supernatants were fractionated by gel filtration on Sephacril S-200 followed by FPLC with a Mono Q HR 5/5 column the purification of protein fractions being monitored on SDS-PAGE and Western blotting using bovine immune serum from *B. bovis* infected cattle. Two proteins of 45 and 54 kDa were isolated by this means and used to immunize cattle providing a degree of protective immunity against challenge with a heterologous isolate of *B. bovis*.

S.A. Hines and co-workers have studied the products of a multigene family in *B. bovis* which encodes a series of merozoite surface antigens of the parasites. North American isolates of *B. bovis* showed considerable geographic diversity in the expression of these antigens which were designated Variable Merozoite Surface Antigens (VMSA). As these antigens could be important candidates for the development of a subunit vaccine against *B. bovis* their polymorphic nature presents an obvious problem. Results were reported which demonstrated that monospecific antibodies which recognize both native and recombinant versions of a VMSA designated D. 42, inhibited invasion of bovine RBCs by merozoites of a homologous line of parasite *in vitro*. VMSA, including D. 42, have yet to be tested as protective immunogens in live challenge experiments in cattle. It is not clear whether any of the culture derived exo-antigens, described above to be involved in protective immunity, correspond to members of the family of Variable Merozoite Surface Antigens of *B. bovis*.

Four papers were contributed on antigens of asexual, blood stage parasites of *P. falciparum*. *D.M* . Banic and colleagues in a paper presented by D. Camus described studies on the p126 parastiophorous vacuole protein of *P. falciparum* schizonts. This protein induces partial protection against *P. falciparum* in *Saimiri* as well as *Aotus* monkeys and is proteolytically cleaved during merozoite release. It was reported during this session that the antibody response against the p126 protein could be under a MHC restricted control as C57B1/6 mice immunized with p126 from schizonts or its processed fragments from culture supernatants failed to make antibodies to the molecule while Balb/c and CBA mice did. However when C57B1/6 mice were immunized with a synthetic peptide corresponding to the six repeat octapeptide located at the N-terminus of the molecule an antibody response to p126 was made. Thus the synthetic peptide may not be subject to the immune non-responsiveness that affects the naturally produced p126. Moreover the synthetic peptide was widely recognized by human sera from either chronically exposed individuals or those recovered from a primary infection of *P. falciparum*.

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These results are encouraging for the development of a p126 based vaccine.

B. Knapp (unfortunately unable to attend in person) and coworkers submitted a paper on the 113 kDa Serine Rich Protein of *P. falciparum* (SERP) now known to correspond to the p126 protein described above. Together with sequences representing parts of the Histidine Rich Protein II (HRPII) of *P. falciparum*, amino acids 631 to 764 of SERP were included in recombinant hybrid proteins each of which induced protective immunity against *P. falciparum* in Aotus monkeys. The polymerase chain reaction was used to amplify the DNA encoding this region of SERP from 3 Brazilian and 3 African isolates of *P. falciparum*. The 631 to 764 region was shown to be almost invariant among these isolates with only two conserved amino acid substitutions being identified. This observation is encouraging for the possible deployment of the recombinant protein as a vaccine candidate against *P. falciparum* malaria.

The Antigen 332 (Ag332) of *P. falciparum* is a protein belonging to that family of antigenically cross-reactive proteins originally recognised, or, as it were, not recognised, due to their cross-reactivity with the 155 kDa protein in the membrane of *P. falciparum* ring-infected erythrocytes designated Pf115/RESA. Like Pf155/RESA, Ag332 is the target of monoclonal antibody that inhibits the *in vitro* growth of *P. falciparum* and has thus been studied as a potential vaccine candidate antigen. D. Mattei and A. Sherf presented data which now identify Ag332 as a 1,000 kDa protein coded by *P. falciparum* but located primary in vesicles in the cytoplasm of RBC infected with mature asexual stages of the parasite. There is no significant sequence homology between Ag332 and pf115/RESA other than the frequent occurrence of the dipeptide E.E. (glutamate glutamate) which appears to account for the cross reactivities through this protein family. The gene coding for Ag332 is located in a subtelomeric position of chromosome 11.

In addition to the use os synthetic peptides representing sequences of parasite antigens, efforts have been increasing to express the genes coding such antigens in eukaryotic cells in order to achieve, if possible, proteins with post-translational modifications similar to those made by the parasite itself. C. de Taisne reported on such studies in which the p126 parasitophorous vacuole protein of *P. falciparum* (discussed above) and the major merozoite surface antigen precursor of *P. falciparum*, a highly polymorphic protein of Mr varying in the range of 200 kDa and now generally referred to as MSA1 (Merozoite Surface Antigen 1), were expressed as full length genes in vaccinia recombinants. Both parasite proteins are considered to represent important candidate antigens for a malaria vaccine. Each of the vaccinia-expressed proteins was recognized by monoclonal and polyclonal, monospecific antibodies against the native parasite proteins and post-translational glycosylation was shown to occur. In the parasite both of these proteins were subject to extensive, site-specific proteolysis. No such proteolytic cleavage was found for the vaccinia-expressed recombinant products indicating that parasite or host erythrocyte-specific proteases must be involved in the natural processing of these molecules.

The last two presentations of this session deal with immunity against the sexual stage antigens of *P. falciparum* and the identification and expression of a sexual stage antigen which shows promise as a candidate for a malaria transmission-blocking component of an anti-malarial vaccine.

The underlying mechanisms of transmission blocking immunity were reviewed by R. Carter. Most such mechanisms involve the direct action of antibodies against surface antigens of the sexual stages of the parasites when they are released into the midgut of a blood-feeding mosquito. Such immunity has been induced in a number of experimental animal systems by immunization with the sexual stage parasites themselves. Transmission-blocking immunity has also been shown to occur spontaneously in man following natural infection with either *P. falciparum* or *P. vivax* malaria. A striking contrast, however, appeared to apply between the immunity induced by active immunization and that spontaneously following infection. Whereas active immunization induced an anti sexual stage immunity with a strong memory response, that following natural infection was virtually without effective long term memory. Re-infection and life-long exposure to infection gave no more effective transmission-blocking immunity than that achieved following a primary malarial infection. It was questioned whether the immunogens and targets of natural trans-

mission-blocking were in fact protein in nature or, if protein, whether they were structurally modified in the natural infection in such a way that subverted the involvement of memory T cells.

A 25 kDa protein on the surface of zygotes of *P. falciparum*, designated Pfs25, has been previously shown to be a sensitive target of transmission-blocking antibodies. This protein may be expressed by the parasites only after they enter the mosquito mid gut in a blood meal although there is some evidence indicating a low level of expression in the gametocytes within the vertebrate host. In the case of Pfs25, therefore, issues of immunological memory and boosting by infection may or may not apply depending on how effectively it is expressed during a blood infection. The gene coding for Pfs25 has been cloned and sequenced. D. Kaslow reported here on the expression of this gene in vaccinia and in yeast and on the immunogenicity of the products in these two eukaryotic systems. The expression of Pfs25 in these systems was of particular concern as the protein is cysteine-rich and, as far as is known, all the target epitopes of transmission blocking antibodies against this protein are dependent upon the correct formation of disulphide bonds. Both vaccinia and yeast derived expression products induced *P. falciparum* transmission blocking antibodies in both mice and monkeys. The yeast product was, of the two, the more effective immunogen despite having only partial integrity of disulphide bonds and lacking glycosylation and the glycosylphosphatidyl-inositol anchor of the native parasite protein.

The antigens discussed, in this session represent a small selection of those potentially available as vaccine candidates against malaria or babesiosis. It is clear, however, from the work presented that new insights are continuing to be gained into the nature of these molecules *per se* and as immunogens, while new technologies are becoming available which will steadily move many of these molecules towards the goal of vaccine trials in their target populations.