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ROUND TABLE 5 — SUMMARY

T CELLS AND PROTECTIVE IMMUNE RESPONSES

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The importance of both the regulatory role and direct effector function of T cells for immune protection in malaria is well established. Both aspects were discussed in this session and several interesting new results were presented.

The role of T cells in *blood stage* infections was discussed in several contributions. While passive transfer of malaria specific T cell clones makes it possible to evaluate the protective role of T cells in a relatively direct manner, in human malaria such evaluations usually rely on indirect evidence involving comparison of T cell responses *in vitro* with the malaria status of the cell donor. It was emphasized (*R. Pink*) that induction of lymphocyte proliferation *in vitro* by malaria antigens is a poor measure of protective immunity. Thus, as seen in many laboratories, T cells from donors who have never seen malaria may well proliferate when exposed to malaria antigen. Moreover, the decrease of malaria specific proliferation of T cells from the peripheral blood during an ongoing immune response has frequently been observed. Finally, the proliferating T cell may have no role in protection. Thus, $CD8^+$ T cell clones specific for conserved epitopes of the major *Plasmodium falciparum* schizont surface glycoprotein were induced by immunizing mice with a recombinant immunogen containing these epitopes. As these mice did not form IgG antibodies to the immunogen, it was speculated that specific $CD8^+$ T cells may suppress the corresponding antibody responses.

A measure of the possible role of antigen specific T cells in malaria immunity may be provided by determining their production of various lymphokines. In the rodent *P. chabaudi chabaudi* model, $CD4^+$ T cells of the IFN-gamma/IL-2 producing type (TH1) appear to predominate in the earlier phases and IL-4 producing cells (TH2) in the later phases of infection (*J. Langhorne*). However, although it was pointed out that antibody independent mechanisms are of great importance for clearing infection in this model, the role of IFN-gamma in this context remains unclear as removal of circulating interferon did not make resistant mice susceptible to infection and the protective effect of $CD4^+$ T cells could not be replaced by interferon administration. Moreover, experiments with immuno-deficient mice (SCID and nu/nu) also indicated that B-cells as well as $CD4^+$ T cells are required for the development of protective immunity in this mouse model. $CD4^+$ T cells have also been shown to be essential for the development of protection in another mouse malaria model (*P. vinckei*). However, malaria specific T cell lines producing either IL-4 (TH2) or IL-2 (TH1) or both (TH0) did not induce protection when transferred to syngeneic nu/nu mice while total spleen cells only induced partial protection in such recipients (*A. Krettli*). As antibodies are believed not to be important for protection in this model, additional undefined mechanisms may be involved in immunity to this parasite.

The regulation of the human immune response to *P. falciparum* was investigated by studying antibodies and T cell stimulation *in vitro*, using the merozoite antigen Pf155/RESA as a model. This antigen induces both antibodies and T cell responses in the majority of donors who have become immune through repeated natural infections. Both the total antigen and short synthetic peptides representing some of its immunodominant epitopes induce proliferation, IFN-gamma and/or IL-4 production in $CD4^+$ T cells of such donors. However, in individual donors, these T cell responses are not correlated, indicating that they represent the activity of different cells corresponding to the TH1 and TH2 cells described in mice (*P.*

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Perlmann). Of the three lymphocyte responses measured, only IL-4 production correlated with the donors' specific antibody levels, supporting the T-helper function of TH2 cells in this human system. Studies of monozygotic and dizygotic twins living under similar conditions of malaria exposure in Africa further showed that both the antibody- and T cell responses were genetically regulated. As no association between the immune responses and MHC class II alleles or haplotypes could be discerned, it is likely that the genetic regulation seen in these donors was due to factors encoded by genes outside the MHC locus and probably superimposed on MHC restrictions.

Several contributions dealt with the possible function in malaria infection of T cells bearing receptors of the γ/δ type (*M. Ho, P. Dubois, J. Langhorne*). Such cells which normally constitute a minor subpopulation in human peripheral blood have been found in elevated numbers (both relative and absolute) in patients with acute *P. falciparum* infection. The function of these cells which were HLA-DR$^+$ is presently unknown although their role as non-MHC restricted cytotoxic effector cells lysing parasitized erythrocytes was suggested as an attractive possibility (*M. Ho*). The cells did not respond to any known soluble malaria antigen. However, they were stimulated to proliferation by *P. falciparum* lysates (*P. Dubois*). This stimulation required antigen presentation by MHC class molecules and the T cell receptors of practically all responding cells were classified as Vγ9/Vδ2. These results suggested that the stimulating antigen was a "superantigen", i.e. an antigen binding to MHC class II molecules in a non-MHC restricted fashion and polyclonally activating T cells carrying receptors expressing particular V-gene products.

Important results on T cell functions in immunity to *malaria sporozoites* were presented in three contributions. By means of CD4$^+$ T cell lines and clones from human volunteers immunized by bites of mosquitoes infected with irradiated *P. falciparum* sporozoites, a new T helper epitope in the 5' region of the repeat block of the circumsporozoite (CS) protein was identified (*E. Nardin*). The efficiency of this epitope to help B cells to produce very high antibody titers to this antigen was confirmed by immunizing mice with synthetic peptide constructs ("multiple antigenic peptide system", MAP) containing 4 copies of both the new T cell epitope and the previously known repeated B cell epitope of the PfCS protein. These authors also identified an amino acid sequence which constituted an epitope for human cytotoxic T cell clones. The effector cells were MHC class II restricted CD4$^+$ T cells and the results suggested a direct role for CD4$^+$ T cells in protection against sporozoite infection, in addition to their established T-helper function.

The epitope seen by the cytotoxic CD4$^+$ T cells was distinct from that previously defined for CTL of CD8$^+$ type. The latter were studied in another investigation in which T cells from the peripheral blood of individuals primed to *P. falciparum* by repeated natural infections were screened for cytotoxicity to autologous target cells (PHA blasts) in the presence of synthetic peptides representing the relevant CS sequences (*D. Doolan*). A significant CTL activity was found reproducible in 3/43 individuals, all with extensive previous exposure to the parasite. The epitopes seen by either CD8$^+$ or CD4$^+$ T cells are all located in a polymorphic region of the CS protein and this polyorphism is believed by some (but not all) authors to reflect the parasites' response to the selective pressure by the hosts' immune system. In a comprehensive study of this CS diversity in a large number of isolates from different parts of the world it was found that there appear to be marked constraints in the parasites' ability to change the CS sequence in this region. This suggests that the extent of variation in the CTL domain may be limited and has practical implications for the development of subunit vaccines to *P. falciparum* sporozoites.

While the CS protein is the dominating immunogen of the malaria sporozoites, the success of achieving full protection of animals as well as humans by immunization with this antigen has been inconsistent, as contrasted to immunization with irradiated sporozoites. Therefore, it has been suspected for some time that additional sporozoite antigens may be important for the induction of efficient immunity. One such surface antigen, called SSP2, has now been identified in the rodent *P. yoelii* malaria system. Mice immunized with irradiated sporozoites developed both antibodies and CTL against SSP2 and were partially protected against parasite challenge when immunized with transfectants expressing either a SSP2 fragment or the CS protein (*S. Hoffman*). However, when the mice were immunized with a vaccine in which both immunogens were combined, full

protection was achieved. Protection was dependent on $CD8^+$ T cells, again emphasizing the importance of CTL for protection against sporozoite infection. The results point the way for the development of efficient polyvalent sporozoite vaccines.

There was no contribution at this session on the role of T cells in immunity to *Babesia* infection. However, some of the differences between the bovine lymphoid system and that of rodents and humans were pointed out, particularly with regard to the role of non-T/non-B type of cells (*W. Davis*). *B. bovis* also served as a model to illustrate the use of a new vital dye (hydroethidine) to monitor viability and replication rate of hemoparasites, a method of great potential for the analysis of the effects of the immune system on intra-erythrocytic parasites in general.