Chimpanzees and Supporting Models in the Study of Malaria Pre-erythrocytic Stages

AW Thomas, B Slierendregt, B Mons, P Druilhe*

Laboratory for Parasitology, Biomedical Primate Research Center-TNO, Postbox 2215, 2280 HV Rijswijk, The Netherlands *Institut Pasteur, Paris, France

Chimpanzees are being used in the study of immune response to Plasmodium falciparum *malaria pre-erythrocytic stages (MPES). Responses induced by immunisation with recombinant/synthetic antigens and by irradiated sporozoites are being evaluated in a model system that is phylogenetically close to humans and that is amenable to limited manipulation not possible in humans. The value of chimpanzees for the in-depth study of immunological mechanisms at work in MPES-induced protection are discussed. A total number of 7 chimpanzees have been used to evaluate the immune response to recombinant antigens, and 5 have been challenged with large numbers of sporozoites, followed by surgical liver-wedge resection, in order to generate infected liver tissue for histological and immunological studies. As a complementary model, SCID mice carrying live, transplanted human and primate hepatocytes have been inoculated with sporozoites and infection of transplanted cells has been monitored by histological and immunological methods. In ongoing experiments chimpanzees are being immunised with MPES-derived lipopeptides that have been shown to overcome MHC restriction in mice, and with irradiated sporozoites.*

Key words: malaria - pre-erythrocytic - primates - immunology

PRIMATES IN MALARIA RESEARCH

The use of non-human primate models in malaria research and vaccine-evaluation is a complex issue that cannot be fully addressed in a short paper. Results obtained in non-human primates require careful interpretation, involving an understanding of the limitations of any model, such as the use of artificial host/parasite combinations, the need for splenectomy, the use of adapted strains, and also the phylogenetic relationship between the experimental primate host and humans. This last aspect may be of crucial importance when complex localised interactions between antigen presenting cells, T-cells and cytokines are studied. The MHC genes of the new world monkeys that are generally used for malaria vaccine studies (*Aotus* and *Saimiri* spp.) are distantly related to the human MHC genes, with an evolutionary distance from man of over 40 million years. The old world monkeys such as the rhesus are closer to humans in this respect (30 million years) and have their own natural parasites, but are not susceptible to the major human malaria parasites. The evolutionary split between chimpanzees and humans is dated at approximately 5 million years ago (Sibley & Ahlquist 1984, Hasegawa et al. 1987), and data from comparative MHC studies shows similarities between the species that may enable the experimental generation of immunological data with a highly predictive value for the human situation. In the chimpanzee, the only ape presently available for malaria experiments, it has been shown that cytokines, hormones and their specific receptors, including the extensive collection of molecular markers available for immune cells, are very similar (if not identical) to the human equivalents.

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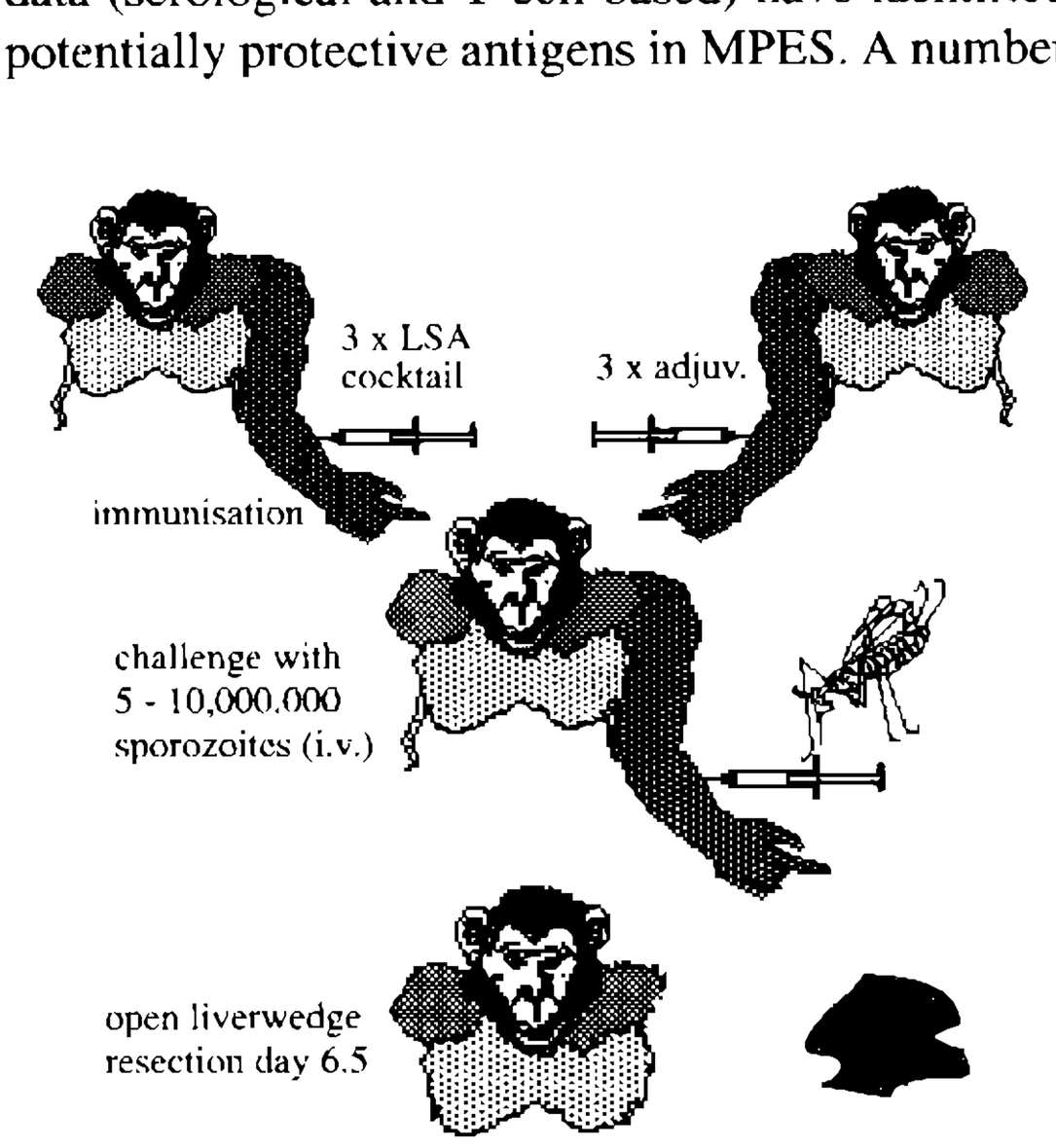
PRIMATES IN THE MALARIA PRE-ERYTHROCYTIC STAGES (MPES) NETWORK

The only immunisation protocol reproducibly leading to complete protection in humans remains that of multiple bites by irradiated mosquitoes. The potential importance of the hepatocytic stages in the protection thereby induced by irradiation-attenuated sporozoites (γSPZ) has recently been re-emphasised (e.g. Druilhe & Marchand 1989). However, major questions on the mechanisms involved in this protective immunity remain unanswered. Rodent studies with rodent parasites have shown that after

optimal irradiation, γSPZ enter the liver with only slightly reduced efficiency and express a number of antigens during their aborted development in the hepatocyte (e.g. Suhrbier et al. 1990). In vitro studies with human parasites have suggested a similar pattern of expression, although for obvious reasons human in vivo studies are not possible.

Cellular responses and associated cytokines are thought to play important roles in the immune response to MPES. Models enabling detailed *in vivo* analysis of such effector mechanisms are badly needed for the human malarias. Within the MPES network several models are under development. A model in *Aotus* monkeys is being developed in Colombia, and we are investigating the phylogenetic relationship between the chimpanzee parasite, *P. reichenowi*, and *P. falciparum* to help establish whether the natural *P. reichenowi*/chimpanzee host parasite relationship can usefully model the *P. falciparum*/human relationship. At present immunisation and evaluation studies for *P. falciparum* are being carried out in chimpanzees (Fig.).

Experimental data in small animals and field data (serological and T-cell based) have identified potentially protective antigens in MPES. A number of these molecules have been selected for evaluation as recombinant protein fragments and/or synthetic peptides in chimpanzees. Detailed descriptions of these molecules are being published in separate papers.



The standard protocol used for the evaluation of combined recombinant and synthetic peptide/lipopeptide antigens in chimpanzees and the collection of infected liver tissue from immunised and control chimpanzees for immuno-histological studies. Sporozoites for challenge are isolated from P. falciparum infected mosquitoes as described (1). During the immunisation period, sera and PBL are routinely collected and distributed to the network members involved. The liver wedge resections may be taken at day 6.5 when almost mature MPES are expected in the liver of the control chimpanzees.

COMPLEMENTARY MODELS

Ethical considerations and limitations on the numbers of animals available for experiment restrict the use of chimpanzees to only the most crucial experiments addressing the mechanisms of protection. Experiments requiring relatively large numbers of animals must therefore be performed in alternative systems. The *in vitro* culture of human hepatocytes expressing MHC molecules relevant to antigen presentation represents a valuable tool for such studies. T-cell studies are, however, limited in this system, because it is not possible to obtain hepatocytes from immunised human volunteers. Matching of target cells and effector cells is therefore very difficult. T-cell lines from immunised chimpanzees can be tested on *in vitro* cultures of hepatocytes from the same or an MHC-matched animal. However, the number of surgical biopsies that can be obtained from chimpanzees is limited, and primary cultures of hepatocytes can only be maintained for short periods before the cells lose essential properties required for the tests. Attempts were undertaken to "store" hepatocytes for prolonged periods in SCID mice as described by Sacci et al. (1992). In addition infection of hepatocytes was attempted in close collaboration with Dr. Sacci, who visited the primate center for a period of two weeks. Mice which had received prepared human hepatocytes under their kidney capsule several weeks earlier were i.v. injected with 10,000 *P. falciparum* sporozoites. 6 - 7 days after the injection, the kidneys were removed and fixed for histological examination. Several (immuno) histological techniques (Giemsa staining, IFA with MAbs and DNA in-situ hybridisation) were used in attempts to detect MPES in the transplanted liver tissue.

RESULTS

IMMUNISATION STUDIES

Three antigens which were selected for further evaluation in the chimpanzee model are briefly described here. Full description of the cellular location of the various antigens, the evaluation of the

immune response at the antibody and the T-cell level and the indications for protective effects is being submitted for publication elsewhere. LSA1, a fully sequenced 200 kDa molecule expressed in MPES has already been published and is being characterised and evaluated for its vaccine potential by different groups (e.g. Guerin Marchand et al. 1987, Zhu & Hollingdale 1991). SALSA is a 70 kDa antigen shared between sporozoites and liver stages to which antibody and T-cell responses have been found (submitted for publication). LSA3-729, is a further MPES-specific molecule which shows particular promise as a vaccine candidate. Various combinations of these molecules were tested using either aluminum hydroxide or acrylamide as adjuvants. Chimpanzees which were repeatedly immunised with LSA or SALSA cocktails showed antibody and T-cell responses to various epitopes in the native molecules.

Improved reproducibility of liver infections in *Aotus* monkeys, as is the aim of studies being carried out by the Colombian team, should further enhance the possibilities for protection studies. The chimpanzee studies may then optimally provide for the detailed analysis of the role of cytokines, T-cells and antibody-cell cooperation in effective anti-MPES immune responses. Cyclical laboratory transmission of the naturally occuring chimpanzee malaria, *P. reichenowi*, is also a goal of our research, where its extreme similarity to *P. falciparum* may enable less invasive protection studies in chimpanzees in the future. In collaboration with other EC networks several potentially important *P. falciparum* genes are currently being analysed in *P. reichenowi*, revealing its extremely close phylogenetic relationship to the human parasite.

The models applied within the network will also allow a detailed assessment of the protection induced by irradiated sporozoites, and the role of MPES in this protection. Chimpanzees have been immunised with irradiated *P. falciparum* sporozoites, and analysis of these responses is beginning. From PBL's isolated after immunisation, preliminary data suggest that cytotoxic responses are being induced and can be detected. Within the context of the newly formed Primate Vaccine Evaluation Network of the EU (see elsewhere in the proceedings), for which the BPRC/TNO is scientfic co-ordinator, we are developing techniques for the analysis of localised immune responses. Thus, to date CD8+ T-cell populations have been isolated and cloned from needle biopsies of liver infected with irradiated sporozoites. Characterisation of these cellular responses is ongoing.

MODEL STUDIES

The transplantation of human and primate hepatocytes in SCID mice has been successfully performed, and it appears that the cells may remain viable long enough to sustain MPES development. Although the reproducible infection of transplanted cells through i.v. injection of sporozoites has so far not been attained by any of the three MPES groups involved in the SCID work, the potential value of this model as a means to dissect critical aspects of chimpanzee immune responses to MPES is such that a continued and enhanced effort to establish the model within the network is required. Unfortunately, up to now the different techniques applied have not provided an unequivocal demonstration of significant MPES invasion and maturation in our hands.

DISCUSSION

The molecular analysis of hepatic stages of parasite development, and the immune responses against those stages is rapidly moving out of the "black box" era. It is clear that vaccines against MPES hold great promise, and it is important that determination of this promise proceeds rapidly. The MPES network has a philosophy of using and developing particular models to answer defined questions. In this way rodent and primate models are compared, contrasted, and the lessons from one model are where possible, applied to the others. Some aspects of the role and the limitations of the chimpanzee model have been outlined above. It offers a unique means of assessing immune reponse to *P. falciparum* at the site of infection in the closest species to humans. The numbers of animals available, and the limited number of experimental procedures that are ethically acceptable with such animals, restricts the scope of analysis. It also means that it is imperative that the maximum information is obtained from each experiment. In this context, the PVEN is intended to exist as a global network of interested associated Primate Centres and researchers. In this way experiments at any one site will produce resources that can, through collaborative interaction, be made available worldwide. The preliminary experiments described above have sug-

gested that the chimpanzee model will be of value in the determination of useful targets to induce immunity to *P. falciparum* MPES and in the analysis of the means by which such immunity is mediated. Current attention remains focussed on *P. falciparum*, and includes the development of new formulations of synthetic peptides that may efficiently induce the required immune responses, and on attempting to define the pathway(s) by which γSPZ induce protective immunity.

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