3. From sporozoite to liver stages: the saga of the irradiated sporozoite vaccine

P. Druilhe and C. Marchand

The story starts forty years ago, when two important discoveries were made. The location in the liver of the exo-erythrocytic (EE) phase of the malaria life cycle was demonstrated for the first time (Shortt & Garnham 1948) and it was shown that UV irradiation of sporozoites made them no longer infectious, as animals inoculated with them were resistant to challenge with infectious parasites (Mulligan et al 1941). Twenty years later, R. Nussenzweig, in the laboratories of Meir Yoeli, extended this observation and began a systematic programme for the development of a sporozoite vaccine using first gamma-irradiation rather than UV as it was easier to deliver regulated doses and thereafter all tools of modern biology as soon as they became available (see review by Cochrane et al 1980).

In retrospect, it is amazing that a single observation, the effects of UV irradiation could give rise to such extensive studies, bringing with it high hopes, disillusionments and controversies. No attempt is made here to review the whole literature; rather we shall present a personal view of some of the critical steps of this fascinating story, and give some of our own recent results.

Ever since Garnham's discovery, the liver cycle has remained – particularly from an immunological standpoint – 'a big black box'. Little interest was shown in the antigens of that stage, and assessment of immunity induced by sporozoites was measured only by detection of erythrocytic stages. This impedes the interpretation of earlier experiments because there is no indication of what occurred in the stage between sporozoite and blood form, i.e. within the 'black box'. Therefore all the most critical and significant studies now need to be re-done with assessment of the fate of the parasites within hepatocytes.

Some of the initial experiments which led to the choice of the circumsporozoite protein (CS) as a major vaccine candidate need first of all to be recalled. Gamma-irradiated sporozoites (IRR-SPZ) proved protective to mice only when injected intravenously. It is important to stress that the protection achieved was total, allowing animals to resist challenge with millions of sporozoites. The same antigen (IRR-SPZ), injected by all other routes — intramuscularly, with or without a variety of adjuvants including Freund's complete adjuvant (FCA), intraperitoneally, subcutaneously, even orally — gave little protection, whatever the number of immunizing doses given. Conversely killed sporozoites injected intravenously, or by other routes, gave almost no protection (Table 3.1). The method of killing

Table 3.1 Immunization of mice against sporozoite challenge

Immunization regimen	*Protection (%)*	*CS-precipitation*
(i) X-irr. SPZ i.v.	90–100	+
(ii) Live SPZ + chloroquine		+
(iii) X-irr. SPZ i.m. + FCA	0– 25	+
(iv) X-irr. SPZ s.c.		+
(v) X-irr. SPZ i.p.		+
(vi) Killed SPZ i.v.		+
(vii) Killed SPZ i.m./s.c.		+
(viii) SPZ antigens	0	+
(ix) No antigen	60–100	–
C. parvum/BCG/Poly I:C		

X-irr. = X-irradiated
SPZ = sporozoites
FCA = Freund's complete adjuvant

may influence the integrity of an antigen, but results were the same whether parasites were killed by heat, formaldehyde, glutaraldehyde, alcohol or iodoacetamide. In addition we are now aware that CS is a stable molecule, able to resist heating to 100°C. It is well established that antigen presentation to the immune system is crucial, but it seems difficult to explain the above discrepancy only in terms of antigen presentation, especially when one considers that, in the protected as in the non-protected animals, antibodies to the sporozoite surface were produced.

Another critical experiment was reported by Beaudoin and colleagues. It was shown that gamma-irradiated sporozoites are living organisms, able to invade hepatocytes and to transform into young trophozoites. They are unable to divide, probably because of DNA damage, do not develop and remain, at least in the mouse model, for unknown periods of time as uninucleate intrahepatic bodies (Ramsey et al 1982).

These data pose several questions. Irradiation may have modified the antigen physically, increasing its immunogenicity, but why is it ineffective when injected by routes other than intravenously? Furthermore, sporozoites exposed to higher doses of irradiation were no longer effective immunogens. Alternatively, the intrahepatic parasite resulting from injection of irradiated sporozoites and unable to continue its development, could constitute an antigen depot, increasing the immunogenicity. However, live non-attenuated sporozoites, injected into mice treated with chloroquine in order to prevent death due to the blood stages of infection, also induced a high degree of protection — as high as that due to irradiated sporozoites.

To analyse results which can be thought of as quantitatively but not qualitatively different it is also necessary to recall that sporozoite challenge can be to some extent combated by administration to mice of cytokine inducers such as polynucleotides (Poly I:C), *C. parvum* or BCG (Jahiel et al 1968) (Table 3.1). This may explain the partial protection achieved in some of the animals receiving, for example, sporozoites with FCA intramuscularly.

Since live sporozoites, either irradiated or non-irradiated, get into the liver, these experiments have been highly suggestive to us that it may be the newly

formed liver trophozoite that is responsible for the immunity induced and more precisely the antigens appearing at that stage. In other words, it may be that the immunogenic molecule is not even carried by the sporozoite itself!

Let us now focus on a statement that has been encountered in more than one paper. 'Animals and man can be effectively protected against malaria by immunization with gamma-irradiated sporozoites'. We will also use this to illustrate the problem of models, which is certainly a very crucial one in the field of parasitology. As stated earlier, results in mouse models were excellent and protection achieved reached 100%. We even learned very recently that a single injection of only 1000 irradiated sporozoites, could induce protection in BALB/c mice for 5 months (Gordon et al 1989). In contrast, the results obtained in primate malarias, including humans, were far from being as convincing (see Table 3.2). Using *P. falciparum* sporozoites, 4 of 11 human volunteers injected were protected and only one of them resisted several challenges with various strains; 6–8 inoculations of irradiated sporozoites given intravenously were necessary to achieve this result which lasted for no more than 2 months. Four or fewer injections resulted in no protection. As in mice the intramuscular route was not effective (Bray 1976). Results obtained with *P. vivax* also highlight the problem of the number of doses: one group reported no protection in 3 volunteers receiving 4 doses of *P. vivax* IRR-SPZ, and another group protection in 2 volunteers receiving 7 inoculations. The results obtained with *P. cynomolgi* and *P. knowlesi* in monkeys were probably even worse. Up to 4×10^8 parasites have been injected in multiple doses for several months in monkeys to achieve either no protection or, in one experiment a low degree (20%) of protection (Table 3.2).

In contrast to mice which can be protected by 2 or 3 injections of non-irradiated sporozoites, data from the field suggest that the injection of live non-irradiated sporozoites in humans, does not induce any significant degree of protection whatever the number of injections. In areas of high endemicity, such as in the Congo, where individuals receive as much as 3 infective inoculations per day for life, that is nearly 100,000 immunizing doses of a few hundred sporozoites each,

Table 3.2 Comparison of protection achieved by immunization with attenuated or live sporozoites in various species

Hosts	*Plasmodium* spp.	Vaccine	Route and No. doses	Protection (% or n)	Duration (months)
Mice	*P. berghei*/*P. yoelii*	(i) X-irr. SPZ	i.v. × 2	100	6–20
		(ii) Live SPZ + chloroquine	i.v. × 3	90–100	
Monkeys	*P. cynomolgi*/ *P. knowlesi*	(i) X-irr. SPZ	i.v. × 5	0	
		(ii) X-irr. SPZ	i.v.–i.m. × 12	20	
Humans	*P. falciparum*	(i) X-irr. SPZ	i.v. × 3	0	
		"	i.v. × 6–8	1–4/7	2
		"	i.m. × 2	0	
		(ii) Live SPZ	i.v. × 10^{10}	±0	
	P. vivax	(i) X-irr. SPZ	i.v. × 7–10	2/2	
		"	i.v. × 4	0/3	

X-irr. = X-irradiated
SPZ = sporozoites

the incidence of new blood infection is high; at any given time, blood-stage prevalence reaches about 60% in adults (Trape 1987).

At that time the increasing problems of malaria control led perhaps prematurely to all hopes being directed to a new vaccine.

Let us now examine how the effect of antibodies, and particularly the biological effect of monoclonal antibodies (MAbs), has influenced this research on a sporozoite vaccine. Based on a morphological alteration which appears as a tail-like precipitate, called the circumsporozoite precipitation reaction, but which can be seen only in a percentage of the sporozoites incubated with immune sera, it was thought that protection induced by irradiated sporozoites was antibody-mediated. Although the time course of protection and that of antibody production were not parallel, the biological effect of mAbs reinforced very strongly the idea that antibody could act either by destroying the sporozoite or by preventing its penetration into liver cells. However, it has now become clearer that on certain occasions the biological effects of a MAb cannot be reproduced by the polyclonal response to the corresponding antigen.

MAbs identified the CS as the sole antigen on the sporozoite surface and also established firmly the basis of an antibody-mediated effect which now appears debatable. This prevented a complete analysis of the antigenic content of the sporozoite which still has to be performed. In view of the undoubted blocking effect of mAbs, a report of successful protection induced in B cell-deficient mice by gamma-irradiated sporozoites (Chen et al 1977) and the much less significant effect in chimpanzee of MAbs to *P. falciparum* CS were not taken into consideration.

Since the amount of CS antigen in sporozoites was considered to be too limited to attempt any direct immunization with extracted proteins, vaccination trials had to await the identification of the corresponding gene and the production by recombination or synthesis of the mAb-defined epitopes. The problems which were later encountered in trying to raise an immune response to these molecules in man were in fact indicated by epidemiological studies of the immune response to the mAb-defined epitope. In several endemic areas of Africa, including the one quoted above, despite daily immunizations about 40–50% of the people did not mount an antibody response to the CS-repeats, or had extremely low titres (Brahimi et al unpublished results). The basis for this defective response was recently analysed at the molecular level by Good et al (1988).

Comparison of results obtained by sporozoite surface labelling in an immunofluorescent (IFA) 'wet' sporozoite assay, and CS-repeat recognition by ELISA, showed a clearcut discrepancy in about 30% of cases, indicating that antibodies to CS-repeats were only part of the whole antibody response to sporozoite surface epitopes. This was confirmed in competition assays using either synthetic or recombinant antigens or monoclonal antibodies to the repeats.

Finally, evidence for the occurrence in man of antibody to non-CS epitopes was obtained by producing human monoclonal antibodies by Epstein–Barr virus transformation of peripheral blood lymphocytes. HuMab have demonstrated the presence of several distinct epitopes on the sporozoite surface. Some parasites were defined by single human monoclonal antibody specificities (Galey et al submitted).

The low immune response recorded in individuals vaccinated with CS-repeats led to the study of its genetic restriction. However, this defective response prevented an evaluation of the degree of protection that can be expected if a proper anti-CS response is achieved, that is if means to overcome the genetic restriction are found. At that time our research on liver stages of *Plasmodium* led to the design of a culture method for *P. falciparum* in human hepatocytes. In such *in vitro* conditions the effect of antibody upon sporozoite penetration was found to be concentration-dependent up to a certain concentration, and thereafter remained high but never complete, even when using MAb concentrated 1000 to 10,000 times more than antibody concentrations reached in man (Mellouk et al 1986). Similarly, antibody from individuals exposed to daily infective bites and reaching IFA titres of 1/100,000 also showed an incomplete inhibitory effect in vitro. In vivo, despite these high antibody titres, at least some sporozoites managed to complete the exo-erythrocytic cycle since blood-stage infections occurred in the same individuals at the time of sampling for some of them, or during follow-up for the others.

The validity of this in vivo/in vitro comparison is also supported by recent results obtained with *P. yoelii* and *P. berghei*. Sera from mice receiving an anti-*P. yoelii* CS mAb, and fully protected, inhibited sporozoite invasion in vitro by 100%. Conversely, sera from mice immunized with several constructs based on a subunit CS vaccine, and not protected, were not fully inhibitory in vitro (Mellouk et al submitted). These experiments again show the difference between the rodent and the human models, full inhibition being achievable with mAbs in vivo and in vitro in one model and not in the other.

In view of the rather disappointing results obtained in man both with recombinant and with synthetic CS vaccines – we learned recently that more than 700 subjects were included in such immunization attempts – research has shifted progressively towards the study of cell-mediated immunity. The genetic restriction of T helper cell responses was demonstrated in mice and, in man, since T cells from adults in one endemic area did not respond in 40% of cases to any of the T epitopes of the CS-protein (Good et al 1988). Furthermore, antigenic diversity was found to occur between parasite strains within the T-cell epitopes identified. Cytotoxic lymphocyte (CTL) studies are now being undertaken, but $CD8^+$ T epitopes are again searched for only within the CS molecule. The effects of various cytokines on the pre-erythrocytic phase were investigated in vivo and in vitro in several models, and among them gamma-interferon was the one with the most profound effect. However, in rodents, and probably in man, the degree of inhibition achieved by IFN-γ would seem again to be incomplete. In endemic areas we found an overall prevalence of 85% of circulating IFN at levels of 60 IU/ml on average (Druilhe et al 1982), while about 60% of adults had a blood-stage infection suggesting a successful liver cycle.

These findings at least modified views about γ-IRR-SPZ induced immunity by introducing the liver stages. Liver forms became a likely target for CTL but it has not yet changed the view on the triggering antigen, the CS-protein with the known restrictions associated with its T-cell sites.

From the comparison of results obtained using living sporozoites given by the i.v. route, versus living sporozoites inoculated by other routes, and killed parasites

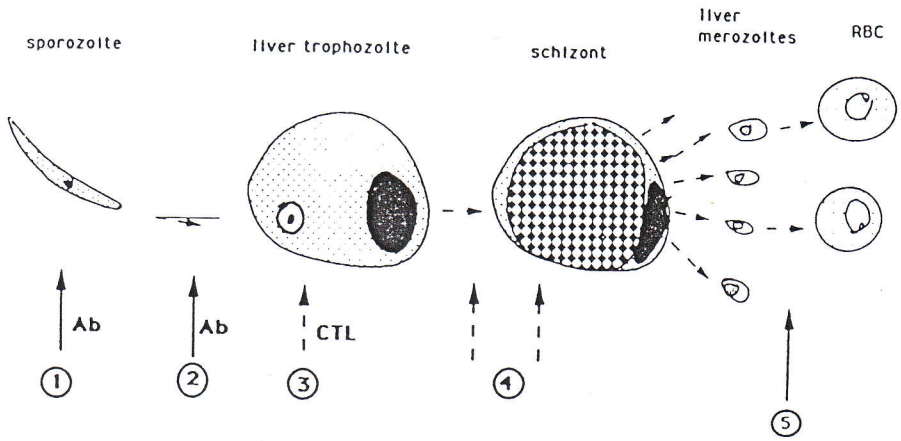

Figure 3.1 Schematic diagram of identified and non-identified but possible targets of defensive mechanisms to various stages of the pre-erythrocytic phase of malaria parasites

injected i.v., it always seemed likely to us that the transformation of sporozoites into liver forms was an obligatory requirement to achieve protection. In humans, this hypothesis was further reinforced by recent experiments. No protection was obtained in volunteers injected with sporozoites irradiated at 23 Krads instead of the 14 Krads used formerly (Herrington et al 1989). In vitro results obtained with sporozoites exposed at various irradiating doses demonstrate the lack of penetration of sporozoites exposed to the higher doses and therefore support our initial view that the production of young liver forms was critical in the induction of immunity.

For this and for other reasons we have focused our research on the characterization of liver stages or, more generally, on the pre-erythrocytic cycle (Fig. 3.1).

Despite initial progress which enabled production of *P. falciparum* liver forms both by in vitro and in vivo means, the output of those methods has remained too low to enable any immunochemical analysis (e.g. by electrophoresis), to have access to messenger RNA, or to induce an immune response in mice in order to prepare monoclonal antibodies. Human monoclonal antibodies specific for liver stages were produced but they turned out to be too unstable for screening purposes. The absence of probes for liver stage antigens (LSA), which was a major limitation, led us to use a more complex approach, which finally appears now to have been worthwhile.

We decided to try to select sera having mainly, and if possible only, antibodies directed against pre-erythrocytic antigens. Three sera from missionaries living in holoendemic areas, who had been taking uninterrupted chloroquine prophylaxis for 26 years, had very high titres of antibodies to sporozoite and liver stages while being almost negative for blood stages antigens. The screening of a genomic DNA library, cloned in an expression vector, by these sera allowed us to reject more than 85% of the antigen-expressing clones and to select about 120 clones thought to correspond to pre-erythrocytic stage antigens. We first picked up three of those most immunoreactive (Fig. 3.2). They encoded an epitope present only in

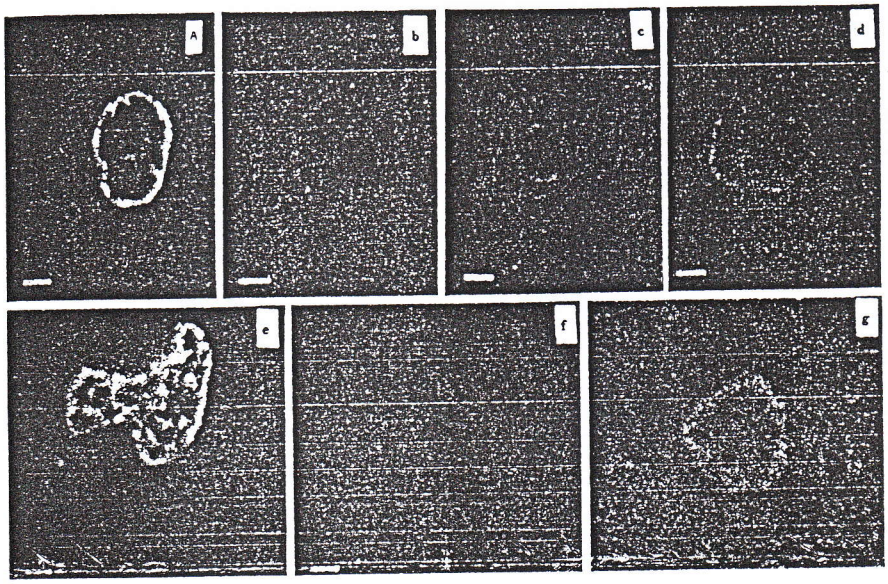

Figure 3.2 Reaction and localization of antibodies with liver-stage schizonts. *a*, Typical immunofluorescence using adult African serum diluted 1/2000 and reacted with 5-μm sections of Carnoy fixed liver fragments taken from *Cebus apella* monkeys infected with *P. falciparum*. Same antigen reacted with antibody eluted from protein expressed by: *b*, clone DG307; *c*, clone DG145; *d*, clone DG199, *e,f*, the same as *a* and *b*, using more mature schizonts to show the internal distribution of the antigen. *g*, Liver schizonts reacted with 1/250 dilution of a rabbit serum raised to clone DG307 fusion protein (one i.m. injection with FCA of the recombinant fusion protein isolated by preparative gel electrophoresis followed by four additional i.v. injections at 15-day intervals) (Marchand-Guerin et al, 1987, Nature 329: 164–167).

P. falciparum liver stages, made of 17-amino-acid repeats, organized in an α-helix, which was designated LSA (Marchand-Guerin et al 1987).

Further screening of the 120 clone-subset was performed by means of selected additional polyclonal sera, having high titres to sporozoite and liver stage native antigens, while being negative for CS and LSA. The pattern of reactivity with those sera allowed a first classification of clones into three categories.

One other means to select, preferentially, antigens well-conserved among isolates and with low, or no, restriction of immunogenicity, was to evaluate further the reactivity of each fusion protein with a complementary series of 8 immune sera, and retain the most consistently reactive.

Human affinity-purified antibodies were prepared from each clone and studied using as antigen, sporozoites, liver forms and blood forms of *P. falciparum* and from heterologous species, in order to determine the stage and species-specificity of the recombinant epitopes expressed. About 47 positive clones have been found so far.

One of them was called SALSA (sporozoite and liver stage antigen). The reactive epitopes are contained within an 87-amino-acid polypeptide which corresponds to a 70-kD protein in *P. falciparum* sporozoites.

The remaining clones were classified based on similarities in antigenic features and DNA structure. For the LSA 'family' and the SALSA clone a good

correspondence between the two methods was found. Analysis of results from cross-immunological reactivity studies allowed us to distinguish three situations.

1. A first group of clones, negative with the selection sera, correspond only to the LSA family, and represents nearly 20% of the clones, an expected event since the 5-kbp gene is known to contain many 51-bp repeats.
2. A second group of clones, positive with each of the selection sera, corresponds to antigens mostly shared between sporozoites and liver stages, but probably distinct from SALSA.
3. In the third group, showing variable results with the 5 sera, we found mostly, if not only, sporozoite surface-specific antigens.

One of the important characteristics of some of the above proteins is their high and consistent immunogenicity when presented by the parasite to the human immune system. For example, in one area of very low endemicity in Africa, the prevalence of antibodies to CS-repeats was 27%, to LSA 80%, and to SALSA 93%. This may not appear surprising since the identification of these antigens is based on methods which are exactly opposite to those used previously for CS and for several blood stages antigens, that is mainly on the quality of the response they generate in man.

Thus, at least some information is coming from the formerly 'black box' liver phase. This phase is currently considered a likely target of CS-induced CTL which is MHC class I restricted. We believe that several more steps are now required in considering these antigens.

First, from the point of view of IRR-SPZ-induced immunity, there is evidence from several lines of investigation that additional, non-CS, antigens are present on sporozoites and one has been characterized. More important may be the newly formed antigens in young liver trophozoites which may be more relevant to the type of resistance induced by IRR-SPZ. From studies on mouse malaria, the mechanisms mediating protection were thought for a long time to be solely due to antibodies but now they are considered to be mostly by CTL. So far there is no indication of the possible mechanism prevailing in man and the history of model studies makes us cautious about extrapolation.

Second, the finding that irradiated sporozoites can induce protective immunity does not mean that other ways of inducing a protective immune response to pre-erythrocytic stages do not exist. The steps of transformation from liver trophozoite to liver schizont provide another interesting target, especially since an inflammatory reaction, with cellular infiltrates, is known to occur upon challenge of LS-primed animals. Also the steps of liver merozoite release into the blood stream and penetration of red blood cells are probably critical and, possibly, vulnerable ones.

Our continued interest in LS is also supported by results from epidemiological surveys performed using whole parasites as antigens which: (i) showed that LS-specific antigens were very immunogenic; (ii) suggested that the immune response to sporozoite surface antigens has a regulatory rather than a blocking effect on sporozoite penetration, modulating the proportion of sporozoites transforming into liver forms and dependent on the level of endemicity; (iii) As a consequence of this regulation of LS load by anti-CS immunity no epidemiological situation has

allowed a maximal immunization with LS antigens. Therefore, the results of optimal immunization with such antigens cannot be derived, in contrast to sporozoites, from responses to naturally occurring challenges, and remain to be evaluated.

Until recently we were dealing with a single antigen triggering a single protective mechanism but now research has shifted towards liver stages and the range of antigens and of mechanisms is much wider. However, the lack of a relevant model, as is often the case in parasitology, is, in our opinion, likely to be the major limitation to vaccine development.

ACKNOWLEDGEMENTS

Some of the results quoted above are part of a team effort in which the following participated: B Galey, S Mellouk, E Bottius, K Brahimi, R L Beaudoin, A Londono, C Desgranges, O Puijalon, J F Trape. These studies were supported in part by WHO/UNDP/WB TDR programme, contract Nos. 840105 and 870121.

REFERENCES

Bray R S 1976 Vaccination against *Plasmodium falciparum*: a negative result. Trans R Soc Trop Med Hyg 70: 258

Chen D H, Tigelaar R E, Weinbaum F I 1977 Immunity to sporozoite-induced malaria infection in mice. I. The effect of immunization of T and B cell-deficient mice. J Immunol 118: 1322–1427

Cochrane A H, Nussenzweig R S, Nardin E H 1980 Immunization against sporozoites. In: Kreier J (ed) Malaria, Academic Press, London, 3: 163–202

Druilhe P, Rhodes-Feuillette A, Canivet M, Gentilini M, Peris J 1982 Circulating interferon in patients with *Plasmodium falciparum, ovale* and *vivax*, malaria. Trans R Soc Trop Med Hyg 76: 422

Galey B, Druilhe P. Ploton, I et al. *Plasmodium falciparum:* evidence for diversity of sporozoite surface antigens derived from analysis of antibodies elicited in man. (submitted)

Good M F, Pombo D, Quakyi I A et al 1988 Human T-cell recognition of the circumsporozoite protein of *Plasmodium falciparum*: immunodominant T-cell domains map to the polymorphic regions of the molecule. Proc Natl Acad Sci USA 85: 1199–1203

Gordon D M, Egan J E, Arthur J D et al 1989 Subunit vaccines designed to induce protective antibodies against the circumsporozoite (CS) protein of *Plasmodium berghei*: Pre-erythrocytic stage malaria vaccine development: current status and future prospects, Bethesda 12–15 April, WRAIR/AID/WHO meeting. Am J Trop Med Hyg 1989 (in press)

Herrington D, Clyde D, Davis J et al 1989 Clinicals trials of sporozoite vaccine at the University of Maryland. Pre-erythrocytic stage malaria vaccine development: current status and future prospects. Bethesda 12–15 April, WRAIR/AID/WHO meeting. Am J Trop Med Hyg 1989 (in press)

Jahiel R I, Vilcek J, Nussenzweig R S, Vanderberg J 1968 Interferon inducers protect mice against *Plasmodium berghei* malaria. Science 161: 802–804

Marchand-Guerin C, Druilhe P, Galey B et al 1987 A liver stage antigen of *P. falciparum* characterized by gene cloning. Nature 329: 164–167

Mellouk S, Mazier D, Druilhe P, Berbiguier N, Danis M 1986 *In vitro* and *in vivo* results suggesting that anti-sporozoite antibodies do not totally block *Plasmodium falciparum* sporozoite infectivity New Engl J Med 315: 648

Mellouk S, Charoenvit Y, Berbiguier N, Beaudoin R L, Druilhe P 1989 High but sub-total inhibition of transformation of *P. falciparum* sporozoite to liver stage by monoclonal and human polyclonal antibodies to sporozoite surface antigens. (submitted)

Mulligan H W, Russell P F, Mohan B N 1941 Active immunization of fowls against *Plasmodium gallinaceum* by injections of killed homologous sporozoites. J Malar Inst India 4: 25–34

Ramsey J M, Beaudoin R L, Hollingdale M R 1982 Infection of tissue-culture cells with Co gamma-irradiated malaria sporozoites. In: Nuclear techniques in the study of parasitic infections, International Atomic Energy Agency, pp 19–25

Shortt H E, Garnham P C C 1948 Pre-erythrocytic stage in mammalian malaria parasites. Nature 161: 126

Trape J-F, Zoulani A 1987 Etudes sur le paludisme d'une zone de mosaïque forét-savane d'Afrique Centrale, la région de Brazzaville II – Densités parasitaires. Bull Soc Path Ex 80: 84–99

Discussion of paper presented by P. Druilhe

Discussed by A. Holder
Reported by G. A. T. Targett

The discussion began by considering to what extent the nature of the immune responses in malaria has been revealed by how patients with agammaglobulinaemias or AIDS cope with infection. Agammaglobulinaemic patients are certainly highly susceptible but, so far, mechanisms of resistance to either pre-erythrocytic or blood stage infections have not been clarified from studies on groups of patients whose immune competence is impaired. With AIDS patients it was suggested that the group to examine is infants below 1 year when they are exposed to malaria infection and are beginning to respond immunologically.

Circulating gamma-interferon (IFN-γ) is demonstrable in malaria patients and the question of whether the levels are high enough to be (partly) responsible for symptoms was raised, as occurs with influenza-A. The levels detectable are, however, not so high and may last several months.

It is a common finding that attempts to prevent infection immunologically are never 100% effective. Druilhe had described experiments on blockade by monoclonal antibodies of liver cell invasion in which this was the case. Data recently presented by Anna Szarfman (NIH, Bethesda, USA) were described where sporozoites became non-reactive with an anti-CS protein monoclonal antibody, having shed the protein in a CSP reaction. This prompted questions on what happens to CS protein during and following invasion of liver cells, and how this is influenced by the presence or absence of high levels of anti-CS protein antibody. Druilhe found that sporozoites, either inside or outside cells, were detectable with the human monoclonal antibody tested. The incomplete protection occurred even when experiments were carried out with a cloned parasite, 7G8, which is evidence against the effect being due to polymorphism in antigenic structure. While sporozoites show some quantitative differences in their reactions with an anti-CSP monoclonal antibody, they are all labelled with it if the antibody concentration is sufficiently high.

A potentially important consequence of incomplete protection is the extent to which disease is dose dependent; what is the effect on parasitaemia or morbidity if 1, 10, 100 or hundreds of sporozoites successfully invade the liver? Clearly, if the parasite load in the blood can be reduced by 80–90%, the clinical situation will be improved. It has been claimed that reduction in the number of liver stage parasites would reduce the pathology, based on the hypothesis that a smaller load of

merozoites reaching the blood would allow the host to mount a more effective immune response. The evidence is generally against this view, and an infection induced by a single pre-erythrocytic parasite that had escaped effects of vaccination, would be as severe as the infection in those not vaccinated and caused by many more parasites. Also, first exposure to infection is not always the worst since many patients with cerebral malaria have a previous history of infection.

Claims that administration of the cytokines IL-1 or IFN-γ can be 100% effective against infection have been made. This appeared to be so from experiments by Schofield and colleagues (New York University Medical School) with IFN-γ where use of a DNA probe indicated 100% inhibition of parasite development in the liver. In due course, however, blood infections developed. The possibility that there are sites within the liver where parasites can escape the effects of cytokines should be considered.

The report, by Druilhe, of French missionaries from an endemic area who had taken chloroquine for 26 years prompted two questions, the first on the state of their retinas, and the second on their immunity to challenge infection. They were advised to stop using chloroquine prophylactically, and their immune status will be shown through exposure to natural challenge.

The exo-erythrocytic stages of *Plasmodium* express both sporozoite and asexual blood stage antigens and the discussant, Holder, described experiments in which the NANP repeat region of the *P. falciparum* CS gene was coupled to a C-terminus conserved region of the PMMSA (=MSA 1) merozoite antigen. This hybrid is immunogenic since it induced antibodies to both the CS sequence and the merozoite surface protein sequence (Holder et al 1988).

He described, too, a eukaryotic expression system used for the merozoite proteins and the hybrid, showing the importance of secretion of the construct protein on the surface of the eukaryotic cell. This can be achieved by removal of the anchor sequence. The conformation of the secreted protein was also important, underlining the need to consider very carefully not only which antigens to make but how they will be produced (Murphy et al in press).

REFERENCES

Holder A A, Lockyer M J, Hardy G W 1988 A hybrid gene to express protein epitopes from both sporozoite and merozoite surface antigens of *Plasmodium falciparum*. Parasitology 97: 373–382

Murphy V F, Rowan W C, Page M J, Holder A A 1989 Expression of hybrid malaria antigens in insect cells and their engineering for correct folding and secretion. Parasitology (in press)

4. Cerebra

P.-H. Lamb

INTRODUCTION

The most severe comp
from which more tha
(Noguer et al 1976, C
Health Organization
infected by malaria e
incompletely understo
possible mechanisms ir
model was developed.

Using this model, w
pathways of the immu
interactions between
cytokines, have been
the immune system ha
These data are prese
children with severe fa
also have a role in hur

HYPOTHESES FOR

Cerebral malaria (CM
death in severe malaria
falciparum malaria ac
Malaria Action Progra
poorly understood, alth
pathological feature of
containing mature for
greatest in the brain (I
such a prominent feat
associated with a 20%
theories have been pr
Malaria Action Progra