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Development of Sporozoite Vaccines [and Discussion]

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Development of sporozoite vaccines

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Protective immunity against malaria has been achieved in hosts ranging from birds to man by repeated inoculation of irradiated sporozoites. The main antigens involved in protective immunity to sporozoites are the circumsporozoite (CS) proteins, which are part of a family of proteins, covering the whole surface membrane of the parasite, and which have similar physico-chemical and antigenic properties. Monovalent fragments of monoclonal antibodies to CS proteins neutralize sporozoite infectivity. All monoclonal antibodies recognize a single immunodominant region within the various CS proteins, and this region contains repetitive epitopes. The recurrent immunodominant epitope of the CS protein of *P. knowlesi* has been identified, and shown to consist of 12 tandemly repeated subunits of 12 amino acids. The dimer of the dodecapeptide was coupled to protein carriers, emulsified in Freund's complete adjuvant, and injected into rodents and monkeys. All animals made anti-peptide antibodies, and most of the antisera reacted with *P. knowlesi* CS protein.

Introduction

In this review we will discuss only the rationale for the development of a malaria vaccine containing sporozoite antigens. Such a vaccine, if successful, would be truly prophylactic: it would prevent disease by inhibiting the penetration or development of sporozoites into the host's hepatocytes.

In mammalian hosts, sporozoites develop exclusively in hepatocytes. The penetration of parasites into these non-phagocytic cells is remarkably fast and efficient. Between 10^1 and 10^3 sporozoites can infect susceptible hosts and liver uptake occurs in a few minutes. It seems very likely therefore not only that specific surface recognition processes take place, but also that the membrane molecules of the parasite involved in this interaction would be good candidate antigens for the development of vaccines.

Vaccination with X-irradiated sporozoites

Immunization with sporozoites was first undertaken by Mulligan *et al.* (1941), using an avian malaria system. They injected birds with ultraviolet-irradiated sporozoites, and obtained a substantial degree of protection against an otherwise lethal malaria infection. They also made the important observation that immunity was stage-specific; that is, it conferred protection against challenge with sporozoites, but not with blood stages. Mulligan's findings were ignored for over 20 years, perhaps because they were in direct conflict with the widely accepted dogma that sporozoites could not induce an effective immune response, since they were present in blood only transiently, and the dose inoculated during the bite of mosquitoes was small.

Experimental evidence proved that this view was incorrect. Sporozoites are, at present, known to be highly immunogenic. Intravenous injection of small numbers of these parasites

into rodents and monkeys induces a very effective antibody response (Vanderberg *et al.* 1969; Chen *et al.* 1976). Furthermore, it was shown that over 90% of adults living in The Gambia, West Africa, in an area of high malaria endemicity, had detectable levels of antisporozoite antibodies (Nardin *et al.* 1979) (figure 1). It is well known that adults are more resistant than children to malaria infection. The acquisition of natural resistance to malaria with age is accompanied also by the development of antibodies to blood stages of the parasite. It is difficult to evaluate the respective roles of antibodies to sporozoites and to blood forms in this increased resistance.

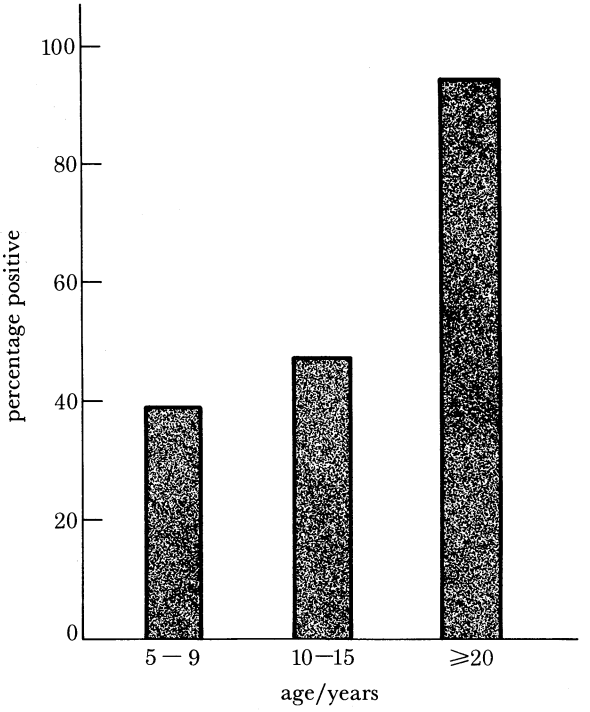

FIGURE 1. Serum samples of West Africans were tested for antibodies to sporozoites of *P. falciparum* by indirect immunofluorescence. More than 90% of serum samples from adults were positive while most samples from children gave negative or low positive reactions. (Adapted from Nardin *et al.* 1979.)

Vaccination with sporozoites can lead to sterile immunity. A large proportion of mice injected with X-irradiated sporozoites of *P. berghei* resisted challenge with the viable parasites (Nussenzweig *et al.* 1969*a*) (table 1). Rats and mice could also be vaccinated with viable sporozoites if a curative chemoprophylactic regimen was instituted soon after injection of parasites (Verhave 1975; Orjih *et al.* 1982). The findings in rodent malaria were confirmed in primate systems. Immunization of monkeys with irradiated sporozoites of *P. knowlesi* and *P. cynomolgi* resulted in complete protection of several animals (Chen 1974; Gwadz *et al.* 1979).

TABLE 1. PROTECTION PRODUCED IN MICE BY MULTIPLE INJECTIONS OF 5×10^3 X-IRRADIATED SPOROZOITES OF *P. BERGHEI*

(Adapted from Nussenzweig *et al.* 1969*a*.)

	number of animals	number of deaths
vaccinated	147	23 (15.6%)
controls	149	145 (97.3%)

A small number of human volunteers have been successfully vaccinated by the repeated bite of *P. falciparum*- and *P. vivax*-infected, irradiated mosquitoes (Clyde *et al.* 1975; Rieckmann *et al.* 1979). Of interest and relevance for vaccine development was the finding that immunization with one isolate of the parasite-induced protection against challenge with sporozoites of different isolates of the same parasite species.

Characteristics of protective immunity

We determined the basic characteristics of sporozoite-induced immunity in rodents and primates. Protection is stage-specific: sporozoite-immunized mice, protected against sporozoite challenge, are fully susceptible to inoculation of blood stages of the same parasite strain (Nussenzweig *et al.* 1969*b*). The protection, in most instances, is species-specific. Immunization with sporozoites of one malaria species does not generally confer protection against infection with sporozoites of other malaria parasites. This has been shown in human (Clyde *et al.* 1975) and monkey (Gwadz *et al.* 1979) malarias. However, immunization of mice with *P. berghei* sporozoites protects them against challenge with sporozoites of some other rodent malaria species or subspecies (Nussenzweig *et al.* 1969*b*; and N. Yoshida, unpublished observations).

The most effective routes of immunization are intravenous inoculation, and the bite of infected, irradiated mosquitoes. In vaccinated mice, sporozoites are cleared more rapidly from the peripheral blood than in normal mice (Nussenzweig *et al.* 1972).

Immunization does not require adjuvants (Spitalny & Nussenzweig 1972; Clyde *et al.* 1975; Gwadz *et al.* 1979). Although the protection obtained under these conditions is brief, lasting approximately three months, immunity can be boosted by the repeated bite of infected mosquitoes. By using this procedure, we were able to maintain mice resistant to *P. berghei* challenge for more than one year. In endemic areas the repeated bites of mosquitoes and periodic injection of malaria sporozoites probably has a similar effect; namely, to increase resistance to infection.

Also of interest is the observation that sporozoite-immunized adult female mice transfer their immunity to the litters. The offspring acquire antisporozoite antibodies from their mothers through the milk (Orjih *et al.* 1981). The passive transfer of antibodies against *P. falciparum* sporozoites was also detected in infants in The Gambia (Nardin *et al.* 1981).

Some of the antibodies produced in the course of immunization are directed against the outer sporozoite membrane. Incubation of viable sporozoites with the serum of vaccinated rodents, monkeys or humans results in the appearance of prominent deposits on the surface of the parasite, and a tail-like precipitate which increases in size with time of incubation. This was designated as the circumsporozoite precipitation, or CSP reaction (Vanderberg *et al.* 1969). Ultrastructural studies showed that the antigen was distributed over the entire outer membrane of the parasite (Cochrane *et al.* 1976) (figure 2).

More important, antisporozoite antibodies have neutralizing activity. Sporozoites incubated with the sera of immunized, protected animals lose their infectivity (Nussenzweig *et al.* 1969*a*). When sera of vaccinated animals gave negative CSP reactions, lower levels of antibodies against sporozoite surface antigen or antigens were detected by more sensitive immunofluorescence assays performed with viable or glutaraldehyde-fixed sporozoites (Nardin *et al.* 1978).

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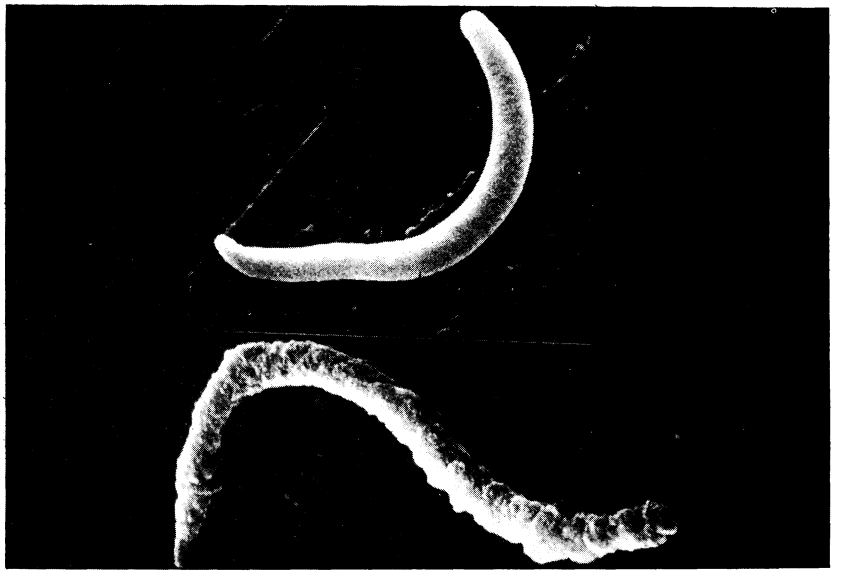

FIGURE 2. Scanning electron micrographs showing the alterations of sporozoites following incubation with serum from animals vaccinated with X-irradiated sporozoites. Top: sporozoites incubated in normal serum. Note that the surface is smooth. The anterior end of the sporozoite is narrow and can be clearly distinguished from the rounder posterior end. Bottom: sporozoites incubated in immune serum. The whole surface of the parasite appears rough, and a tail-like precipitate with irregular surface extends a considerable distance posteriorly. This is called the circumsporozoite (CSP) reaction. (Pictures taken by M. Aikawa.)

Identification of the protective antigen

Taken together, these findings suggested that protective immunity was at least in part antibody-mediated, and that the antigens involved in protective immunity and in the circumsporozoite reactions, might be identical.

That this was indeed the case was first shown with *P. berghei*, a rodent malaria parasite. A monoclonal antibody (3D11) raised against the surface of *P. berghei* sporozoites displayed all the properties of polyclonal antisera obtained from animals vaccinated with whole X-irradiated sporozoites. 3D11 not only identified a M_r 44000 stage and species-specific protein (figure 3) which covers the surface membrane of sporozoites, but also neutralized their infectivity and mediated the CSP reaction (Yoshida *et al.* 1980). Passive transfer of as little as 10 μg of purified monoclonal antibodies per mouse induced complete protection against sporozoite challenge (Potocnjak *et al.* 1980).

Identical results were obtained when malaria parasites of monkeys (*P. knowlesi*) and humans (*P. vivax* and *P. falciparum*) were studied (Cochrane *et al.* 1982; Nardin *et al.* 1982). One important observation was that sporozoites could be neutralized not only by the native monoclonal antibodies to CS proteins, but also by monovalent Fab fragments. This implied that the simple binding of antibody to the parasite surface somehow interfered with their infectivity. In fact it was found that in the presence of specific Fab fragments, sporozoites of several species of malaria parasites do not attach to target cells *in vitro*, leading to the conclusion that CS proteins play a role in the process of penetration of the parasite into the host's cells (Hollingdale *et al.* 1982; Hollingdale *et al.* 1984).

Although these results clearly implicate antibodies as mediators of the protective immunity

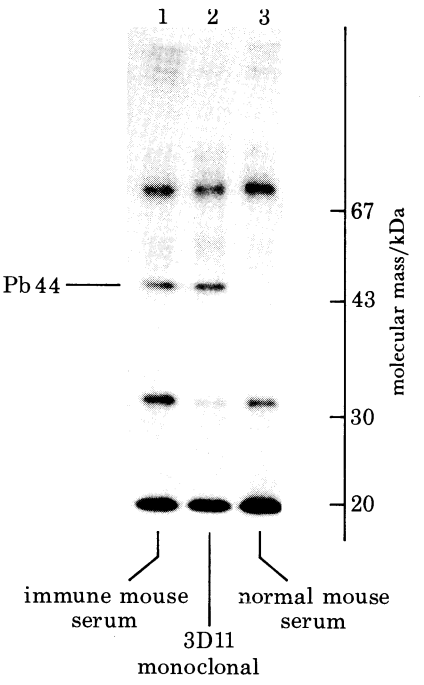

Figure 3. Radioautograms of extracts of *P. berghei* sporozoites subjected to immunoprecipitation and electrophoresis on SDS-polyacrylamide gels. Sporozoites were purified from salivary glands and radiolabelled with ^{125}I by the lactoperoxidase method. The extracts were immunoprecipitated with polyclonal antiserum from mice immunized by the bite of X-irradiated infected mosquitoes (track 1), with the culture supernatants of a hybridoma (3D11) (track 2), and with a normal mouse serum (track 3). The same band of M_r 44000 was specifically immunoprecipitated by 3D11 or by the polyclonal antiserum.

observed in animals vaccinated with X-irradiated sporozoites, they do not exclude a role for cell-mediated reactions in the destruction of the parasite. In this regard the earlier studies trying to correlate protective immunity with the CSP titres are not conclusive because the CSP reaction is only observed when the serum antibody levels reach 10–20 μg ml^{-1}. Moreover we have found some monoclonal antibodies that bound to CS proteins without producing CSP reactions (Cochrane *et al.* 1982), probably because they do not cross-link the antigen. In any case, in view of the extraordinary immunogenicity of the CS proteins, these molecules most likely would be the target antigen of putative cellular reactions.

Properties of CS proteins

Further studies revealed many structural, biosynthetic and immunological similarities among the CS proteins of the various malaria sporozoites, and indicated that they belong to a family of homologous proteins (Santoro *et al.* 1983). CS proteins are found mainly in mature salivary gland sporozoites, and are uniformly distributed on their surface membrane. CS proteins have an apparent M_r between 40000 and 60000 as determined by SDS polyacrylamide gel electrophoreses under reducing or non-reducing conditions, and have isolectric points between 5 and 6 (table 2). They are absent, or present in very small amounts, on the non-infective sporozoites from oocysts, but constitute one of the main proteins synthesized by the mature parasites (Aikawa *et al.* 1981). It seems likely, therefore, that CS proteins have an important function for the development of the parasite in the mammalian host. Two intracellular

TABLE 2. MOLECULAR MASSES AND ISOELECTRIC POINTS OF CS PROTEINS AND THEIR PRECURSORS

(Santoro *et al.* 1983)

species	molecular mass kDa	pI (range)
P. berghei	44 (CS)	4.7
	53	5.2–5.5
	54	
P. knowlesi	42 (CS)	4.9
	50	5.3–5.6
	52	
P. cynomolgi	48 (CS)	4.9
	56	
	58	5.2–5.5
P. falciparum	58 (CS?)	5.3
	65	5–6
	67	

precursors of the CS proteins have been detected. They have a higher M_r and isoelectric point, indicating that the membrane proteins are generated from the precursors by sequential removal of basic peptides from these precursors. Comparison of tryptic digests of CS proteins of different malaria species revealed structural similarities between them. Several [^{35}S] methionine-labelled peptides from various CS proteins had identical retention times by reverse-phase high pressure liquid chromatography. The homology between CS proteins can also be shown by immunological methods. Monoclonal antibodies to *P. knowlesi* cross-react with *P. falciparum* and *P. cynomolgi*; antibodies to *P. yoeli nigeriensis* cross-react with *P. berghei*; antibodies to *P. malariae* cross-react with *P. brasilianum*.

CS proteins also display unusual immunological properties. The topological analysis of their antigenic determinants revealed that all monoclonal antibodies bound to the same site of the homologous polypeptides; that is, the analysis suggested that CS proteins have single immunodominant regions. This was unexpected because proteins as small as myoglobin (16900 M_r) have several immunoreactive areas. Another unique finding was that the monomeric CS proteins could simultaneously bind two or more monoclonal antibodies, and that they are multivalent with regard to the expression of a single epitope (Zavala *et al.* 1983).

Every monoclonal antibody produced to date in our laboratory against the surface membrane of sporozoites has been shown to be directed against this unique epitope. Its remarkable immunogenicity was confirmed by studying the properties of polyclonal antisera of animals and humans vaccinated with X-irradiated sporozoites. Preincubation of crude extracts of sporozoites with single monoclonal antibodies to the corresponding CS proteins strongly inhibited the subsequent binding of the polyclonal antibodies (Zavala *et al.* 1983) (figure 4).

Identification of the epitope reacting with the monoclonal antibodies

Since a single recurrent epitope contained most of the immunogenic activity of CS proteins, and the binding of monovalent fragments of monoclonal antibodies to these structures neutralized the infectivity of sporozoites, it seemed possible that the CS molecules could be used

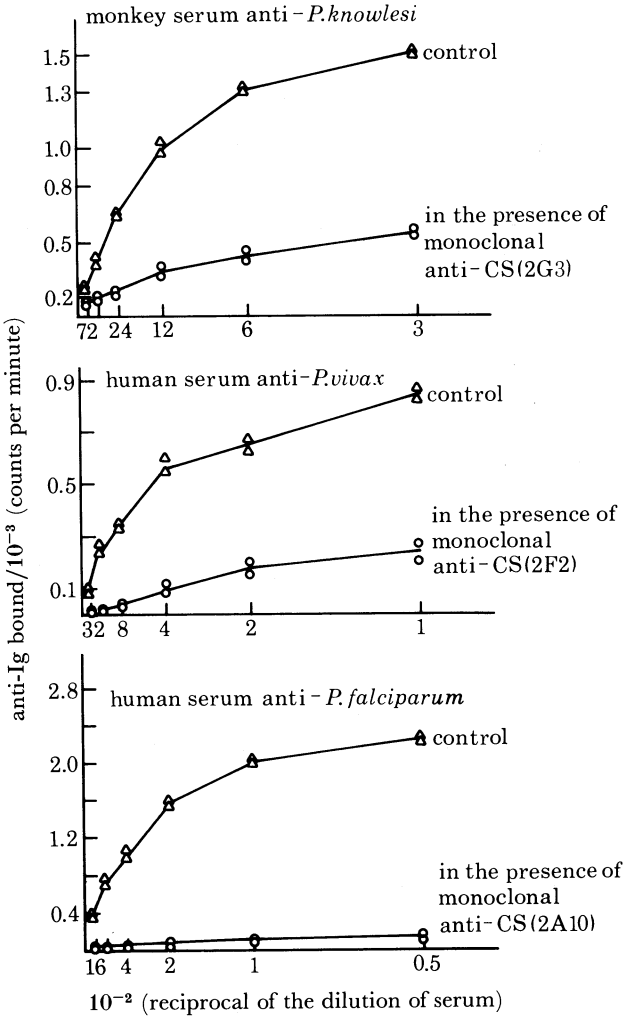

FIGURE 4. Inhibitory effect of monoclonal antibodies on the binding of polyclonal antibodies to sporozoite extracts. The experiments represented above were performed in two steps. First, serial dilutions of polyclonal antisera were incubated with a crude extract of sporozoites in the presence or absence of a monoclonal antibody to the corresponding CS protein. Then, the amounts of the polyclonal Ig that bound to the solid-phase associated antigen were measured using an excess of radiolabelled affinity purified anti-Ig of the appropriate specificity. As shown, the monoclonal antibodies inhibited 70–95% of the binding of the polyclonal antibody to antigen. (Reproduced from Zavala *et al.* (1983).)

as vaccines. However, a major practical problem was that the only source of CS proteins was salivary gland sporozoites. To overcome this difficulty there were two possible approaches: genetic engineering or chemical synthesis of the portion of the molecule that contains the relevant epitopes.

Using mRNA from sporozoites of *P. knowlesi*, J. Ellis, while in Dr N. Godson's laboratory, cloned the *CS* gene fragment which expressed the epitope recognized by monoclonal antibodies (Ellis *et al.* 1983).

The sequence of nucleotides of PEG81 was read by Dr N. Godson and P. Svec, and was found to contain several repeats of 36 base pairs. As explained in figure 5, the correct reading frame could be deduced by immunological methods; that is, by comparing the properties of the epitope and of the various candidate dodecapeptides whose composition was derived from the

reading frames (strand 1)

1 Cys-Ile-Cys-Ser-Ile-Ser-Leu-Cys-Leu-Trp-Leu-Ser

2 Leu-Lys-Leu-Leu-His-Leu-Leu-Val-Leu-Val-Val-Cys

3 Pro-Ala-Phe-Ala-Pro-Ser-Pro-Cys-Ala-Cys-Gly-Cys

rejected: peptides too hydrophobic; epitopes unlikely to contain *S-S* bonds, because they react with antibody after complete reduction and alkylation.

reading frames (strand 2)

1 Met-Glu-Gin-Met-Gln-Asp-Asn-His-Lys-His-Lys-Val

2 Asp-Gly-Ala-Asn-Ala-Gly-Gln-Pro-Gln-Ala-Gln-Ala

3 Trp-Ser-Lys-Cys-Arg-Thr-Thr-Thr-Ser-Thr-Arg-STOP

frame 1: rejected because the epitope is trypsin-resistant.

frame 2: acceptable because the peptide is hydrophilic and the epitope is elastase-sensitive.

FIGURE 5. Deduction of the reading frame of the cDNA clone from the *P. knowlesi* CS protein.

nucleotide sequence. For example, one of the possible reading frames coded for a hydrophilic polypeptide rich in alanine and devoid of lysine and arginine. We reasoned that, if it was the correct reading frame, the epitope should be resistant to trypsin but sensitive to elastase, an enzyme that cleaves peptide bonds at alanine residues. This was confirmed experimentally by subjecting the *in vivo P. knowlesi*-synthesized CS protein, as well as the *E. coli*-derived fusion protein, to enzymatic digestion, followed by an immunoassay to detect the epitope. To prove that this reading frame was correct, the corresponding dodecapeptide was synthesized and tested for reactivity with the monoclonal antibodies. The synthetic dodecapeptide (Gln-Ala-Gln-Gly-Asp-Gly-Ala-Asn-Ala-Gly-Gln-Pro) at concentrations of 10^{-12} M inhibited the interaction of all the monoclonal antibodies with the authentic *P. knowlesi* CS protein (Godson *et al.* 1983).

The specificity of four of these antibodies was studied further, in collaboration with Dr D. Schlesinger, who synthesized several peptides corresponding to overlapping regions of the repeats. By measuring their ability to inhibit the specific interaction between the antibodies and the CS protein of *P. knowlesi*, we found that three antibodies had very similar patterns of reactivity with this series of peptides, and defined an epitope of eight amino acids: Gly-Asp-Gly-Ala-Asn-Ala-Gly-Gln. The fourth antibody recognized a configurational epitope formed by a tandem repeat of the dodecapeptide (Schlesinger *et al.* 1984).

The structure of the *CS* gene has been elucidated and is described elsewhere in this volume. We know now that the *P. knowlesi* CS protein contains 12 tandemly repeated subunits of 12 amino acids. Data recently obtained on the structure of the *P. cynomolgi CS* gene also show the presence of multiple tandem repeats (Enea *et al.* 1984). This explains the observation that monomeric CS proteins can simultaneously bind several molecules of monoclonal antibodies, and may also account for the extraordinary immunogenicity of this epitope. In fact, the CS protein covers the entire membrane of sporozoites, and close to one-half of each CS molecule consists of epitope repeats.

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Significance of the repetitive epitope

The function of this large domain of the CS molecule is unknown. One possibility is that the repeats are associated with some unique structural feature of the CS molecules. There is indirect evidence that CS molecules are organized in the membrane; that is, they may interact to form complex protein assemblies. The experimental finding supporting this higher order of organization is the CSP reaction. The sheath, which is eliminated from the parasite surface, and which contains CS proteins cross-linked by antibodies, maintains its morphology even after repeated washing of the parasite by centrifugation. This suggests that the antigen molecules may be organized in an orderly fashion, perhaps forming interlocking aggregates. However, the participation of the repeats in the assembly of this aggregate should not require the perfect conservation of their amino acid sequence, since conservative substitutions occur very frequently in other structural proteins which have sequence periodicities.

Another hypothesis to explain the conservation of the repeats is that the sequence itself is essential for a parasite function. For example, the dodecamer could be a ligand for a cellular receptor in the host. The presence of a very large number of closely packed ligands on the surface of the parasite would favour the encounter with the receptors, and if these are multivalent, the interaction with the ligands could be cooperative and much stronger.

Finally, it is also conceivable that the repeats are conserved to increase the immune response of the host to the CS molecule. Although this may seem paradoxical, this enhanced immunogenicity could favour the parasite development by protecting the host from additional and potentially lethal infections by the same parasite species. A similar reasoning has been invoked to explain the strong immune response to cercariae in animals bearing adult schistosome worms (concomitant immunity). On the other hand it could also be argued that the strong immunogenicity of the repeats serves as a lure to deviate the immune response of the host against other areas of the CS molecule of even greater significance for the development of the parasite. Perhaps some of these interesting issues will be resolved when the structure of other CS proteins and the corresponding genes is known.

Development of a malaria vaccine

Whatever the function of the repeats, there is strong evidence that the binding of antibodies to this region of the molecule interferes with parasite development. It is also clear that the immunodominant epitope recognized in the repeats is formed by a short peptide of 8–12 amino acids, which suggests that synthetic vaccines are possible. We found recently that mice and rabbits immunized with the synthetic dodecapeptide conjugated to a carrier protein have in their serum antibodies which bind to the surface membrane of sporozoites, give CSP reaction, and immunoprecipitate the CS protein. More important, sporozoites of *P. knowlesi* lost their infectivity when incubated with the serum of one of the rabbits. Squirrel monkeys have also been immunized with these peptide preparations and were found to produce anti-sporozoite antibody levels similar to those detected in monkeys immunized with irradiated sporozoites (Gysin *et al.* 1984).

An alternative approach to immunization with the malaria antigen is to use the vaccinia virus as a vector of the *CS* gene (Macket *et al.* 1982; Panicali & Paoletti 1982) and to construct a live recombinant virus to be administered in the same manner as smallpox vaccines. Tissue culture cells infected with such a recombinant virus, and containing the *CS* gene from *P. knowlesi*,

synthesized *CS* proteins. Rabbits vaccinated with the recombinant virus produced antibodies to the repetitive epitope (Smith *et al.* 1984).

These findings raise hopes that if the repeated epitopes of CS proteins from human malaria parasites have similar properties, vaccines can be developed for human use.

Would such vaccines be effective? It has been frequently argued that, since immunity to sporozoites cannot protect against blood stages, vaccination with sporozoites has to lead to sterile immunity to be useful. In our view, this idea may be incorrect. A vaccine that contributes to substantial reduction of the inoculum could diminish the severity of the disease, which seems to increase with the number of sporozoites injected during the mosquito bite (McGregor 1964). Moreover, for adults living in endemic areas, who already have circulating anti-sporozoite antibodies, such a vaccine will serve to boost their naturally acquired immunity.

Also encouraging is the observation that sterile immunity was achieved in experimental models, and in humans vaccinated with X-irradiated sporozoites. However, the degree of protection conferred by this vaccine is dependent on the dose of sporozoites used for challenge. The higher this dose, the less effective the protection. What is the size of the inoculum under natural conditions? It seems to vary according to the geographic area, and the vector, but in most instances the number of sporozoites in salivary glands of mosquitoes is low. A two-site immuno-radiometric assay has recently been developed to identify and quantify the sporozoites in individual mosquitoes (Zavala *et al.* 1982). By using this assay in The Gambia, West Africa, an area of high malaria endemicity, a large proportion (more than 40%) of the infected mosquitoes had less than 4000 sporozoites in their salivary glands (Collins *et al.* 1984). Since only a small proportion of the sporozoites present in the salivary glands are inoculated during mosquito bite, it is conceivable that a vaccine consisting of only sporozoite antigens could protect part of the exposed population, and that, in those cases where some sporozoites escape, the course of the disease would be milder.

Also important, with regard to the development of sporozoite vaccines, is the recent finding that sporozoites of *P. falciparum* from nine widely different geographical origins, bear the same repetitive epitope. Western blotting of extracts of these sporozoites also revealed small differences in the apparent molecular masses of the various CS proteins. Similar results were obtained comparing sporozoites of six *P. vivax* isolates (Zavala *et al.* 1984). The differences in molecular mass may reflect the presence of different numbers of tandem repeats, or variability in other regions of the CS proteins. If the protective domain of CS proteins turns out to consist of tandem repeats, the lack of variability of this part of the molecule would ensure the broad applicability of a sporozoite vaccine in various parts of the world.

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Discussion

R. R. Freeman (*Wellcome Research Laboratories, Langley Court, Beckenham, Kent, U.K.*). Is there a role for anti-sporozoite immunity in the acquisition of resistance against malaria in endemic areas?

Ruth S. Nussenzweig. As we mentioned, the serum of most adults in one endemic area of Africa contain antisporozoite antibodies. It is difficult, however, to evaluate their role in the acquisition of resistance to malaria.

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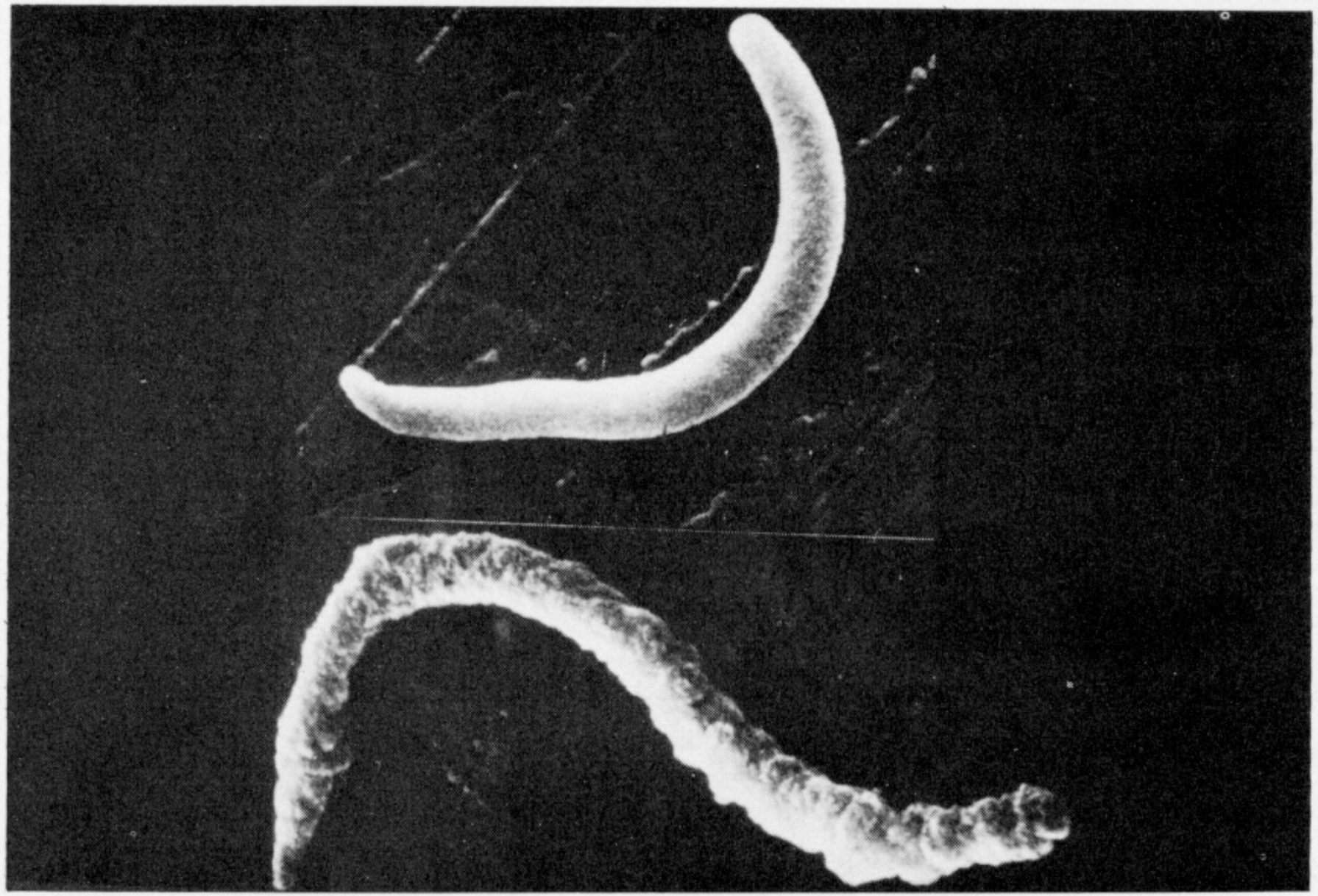

FIGURE 2. Scanning electron micrographs showing the alterations of sporozoites following incubation with serum from animals vaccinated with X-irradiated sporozoites. Top: sporozoites incubated in normal serum. Note that the surface is smooth. The anterior end of the sporozoite is narrow and can be clearly distinguished from the rounder posterior end. Bottom: sporozoites incubated in immune serum. The whole surface of the parasite appears rough, and a tail-like precipitate with irregular surface extends a considerable distance posteriorly. This is called the circumsporozoite (CSP) reaction. (Pictures taken by M. Aikawa.)

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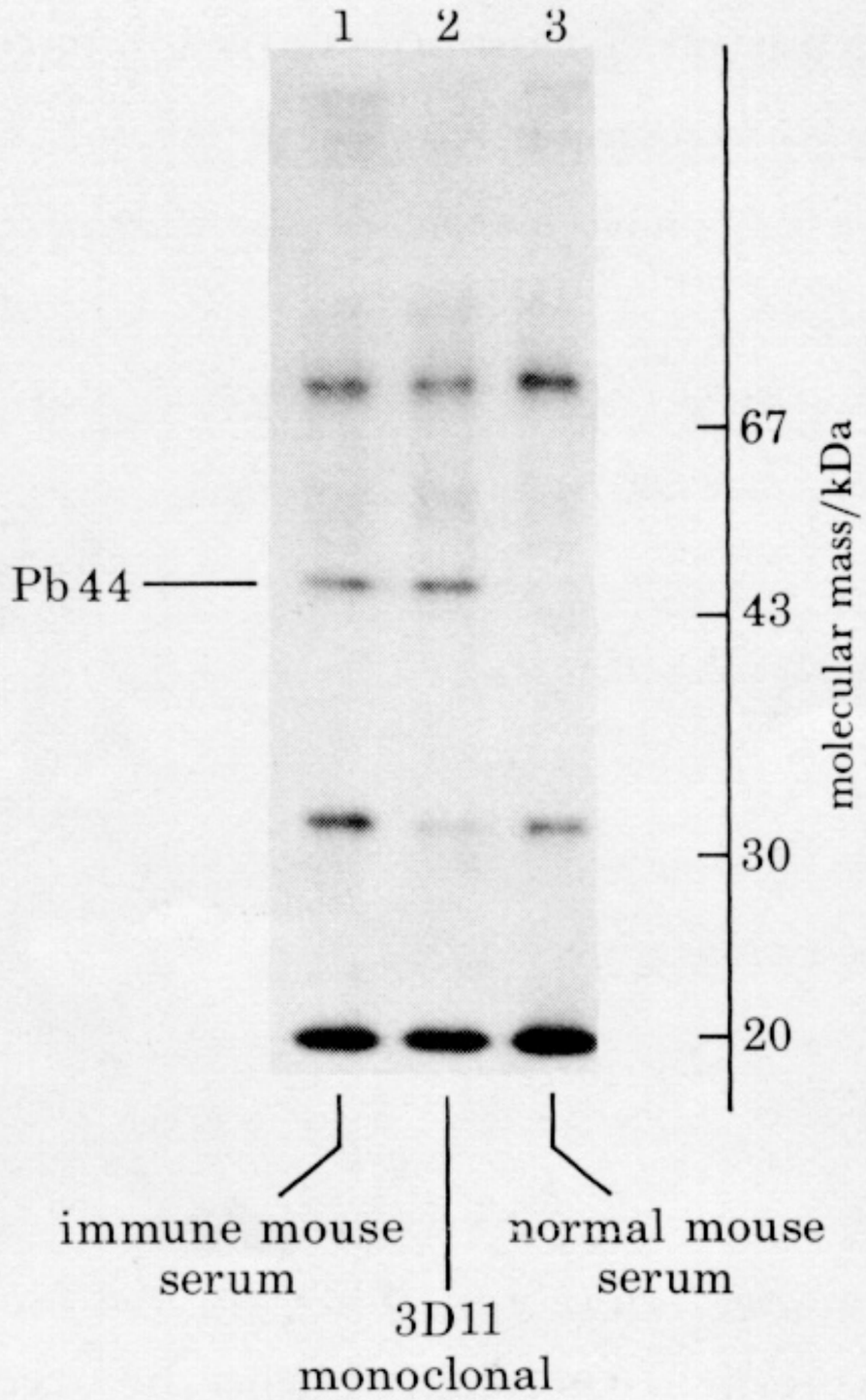

Figure 3. Radioautograms of extracts of *P. berghei* sporozoites subjected to immunoprecipitation and electrophoresis on SDS-polyacrylamide gels. Sporozoites were purified from salivary glands and radiolabelled with ^{125}I by the lactoperoxidase method. The extracts were immunoprecipitated with polyclonal antiserum from mice immunized by the bite of X-irradiated infected mosquitoes (track 1), with the culture supernatants of a hybridoma (3D11) (track 2), and with a normal mouse serum (track 3). The same band of M_r 44000 was specifically immunoprecipitated by 3D11 or by the polyclonal antiserum.