RESTRICTED OR ABSENT IMMUNE RESPONSES IN HUMAN POPULATIONS TO *PLASMODIUM FALCIPARUM* GAMETE ANTIGENS THAT ARE TARGETS OF MALARIA TRANSMISSION-BLOCKING ANTIBODIES

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Vaccination against malaria is the goal of research in many laboratories around the world. Products of recombinant DNA technology or synthetic constructs representing malarial antigens have been tested as immunogens in human vaccination trials (1-3). These, however, have had only limited success in protecting against live parasite challenge. Among the problems that have emerged is the phenomenon of MHC genetic restriction of the immune response to malarial antigens. This has been shown to be critical in determining the immune response to the circumsporozoite protein (CSP)[1] of *Plasmodium falciparum* (4-7), which has been the object of two human vaccine trials (1, 2).

The goal of the human vaccine trials was to prevent or reduce infection with the asexual blood stage malaria parasites that are the direct agents of disease. Another rational objective of vaccination against malaria is to reduce or eliminate the spread of the disease within a community or human population. Transmission blocking immunity is against the sexual stages of malaria parasites that circulate in the blood and transmit the infection from man to mosquito (8-15). The principle mechanisms of transmission-blocking immunity are antibody mediated. After a blood meal on a malaria-infected individual, antibodies against gamete surface antigens come in contact with the gametes of the parasites as they are released from their surrounding host RBC in the mosquito midgut. Further development of the parasites in the mosquito is stopped due either to (*a*) prevention of fertilization by the antigamete antibodies, (*b*) lysis of the gametes or zygotes in antibody-dependent complement-mediated reactions, or (*c*) prevention of the fertilized zygote (ookinete) from penetrating the mosquito midgut wall after a period of ~24 h of development in the mosquito after fertilization.

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[1] *Abbreviations used in this paper:* CSP, circumsporozoite protein; PNG, Papua New Guinea.

Several different surface antigens of gametes and zygotes of *P. falciparum* have been identified as targets of transmission-blocking immunity using mAbs (16–18). Two gamete surface proteins, one of 230 kD (18) and another represented by a doublet of 48 and 45 kD (16, 17), are targets of antibodies that either prevent fertilization or kill the parasites by complement-mediated lysis. Antibodies against a 25-kD zygote, or ookinete, surface protein (identified as 21 kD in the present study) do not prevent fertilization but stop the late development of the parasite in the mosquito midgut (17).

In the present study we have examined the immune response in man to these and other surface and internal proteins of sexual stages of *P. falciparum* in populations in Papua New Guinea exposed to intensive malaria transmission. Our results indicate that human populations respond in a highly selective manner to antigens that are targets of transmission-blocking antibodies and they suggest that restricted immunogenicity may be a feature of natural target antigens of antimalarial immunity.

Materials and Methods

Human Serum and mAbs Used for Immunoprecipitation. The human serum samples used for immunoprecipitation were the same as those previously described (15). Venous blood samples of 5–10 ml were collected in August 1985 from a cross-sectional sample of individuals over the age of 4 yr attending malariometric surveys in the villages of of Agan (Madang Province, Papua New Guinea) and Tau (East Sepik Province). The crude parasite rate at the time of the survey in Agan was 30% (21.4% *P. falciparum*, 1.4% *P. vivax*, and 8.6% *P. malariae*, n = 70). In Tau the crude parasite rate was 50% (47.9% *P. falciparum*, 6.3% *P. vivax*, 12.5% *P. malariae*, n = 48). At Goroka Hospital, situated in the Eastern Highlands province at an altitude too high for malaria transmission, sera were collected from adults who had recently visited a coastal area and who presented to the outpatient's department with suspected malaria. In all cases blood was allowed to clot and the serum was collected and frozen at −20°C until shipped to the National Institutes of Health, Bethesda, MD, where it was heat inactivated and stored at −20°C.

Normal (nonimmune) human serum was obtained from blood samples drawn from U. S. donors and one serum was drawn from a donor (RC) infected 2 yr previously with the monkey malaria parasite *P. cynomolgi*.

mAbs used were protein A-purified preparations. Two mAbs, P5 E2-2F7-3B4 and P5 E2-2F7-1B3 (18), were against the 230-kD gamete surface protein of *P. faciparum* and one mAb, IIC5-B10 (16), was against the 48/45-kD gamete surface protein of *P. falciparum*. The mAbs were mixed together to give a final concentration of each of 0.5–1 mg/ml in PBS.

Preparation of ^{125}I-labeled Antigen from Gametes of P. falciparum. Cultures of *P. falciparum*, clone 3D7, were grown in human O^+ RBC as previously described for production of gametocytes (19). The 3D7 clone is derived from isolate NF54 as described (20). At maturity, 14 d after setting up the cultures, the parasitized cells were collected and the gametocytes were stimulated to undergo gametogenesis for 30 min by resuspension at a 20% hematocrit in a solution consisting of nine parts of 7 mM Tris, 120 mM NaCl, 8 mM glucose, 30 mM $NaHCO_3$, one part of nonimmune heat-inactivated human serum adjusted to pH 8.3, and two parts of an extract of *An. freeborni* pupae (see reference 15 for preparation), which contains a potent stimulator of gametogenesis (21). The suspension was placed on a Nycodenz (Nycomed UK Ltd., Sheldon, Birmingham) gradient consisting of 3 steps of 16%, 11%, and 6% of Nycodenz in medium 199 (17) and spun at 10,000g for 10 minutes.

From the gradient 2.5 × 10^8 purified extracellular female gametes of *P. falciparum* were collected and washed two times in RPMI 1640 medium. The preparation was divided into two equal parts of 1.2 × 10^8 gametes each. One part was treated immediately as described below. The other part was incubated at a concentration of 2 × 10^6 gametes per milliliter for 15 h at 26°C in a solution consisting of equal parts of MEM and medium 199 supplemented with 0.1 mg glutamine per milliliter and 125 μg gentamycin per milliliter in 5-ml polystyrene

tubes (Sarstedt, FRG) exposed to atmospheric air. Culturing the gametes for a further 15 h under these conditions allows expression of the 21-kD ookinete surface antigen.

Both freshly prepared gametes (~3 h after gametogenesis) and those cultured for 15 h were each treated in the same manner as follows: (*a*) 4 × 10^7 gametes of each preparation were extracted in 50 µl of NETT (0.15 M NaCl, 5 mM EDTA, 50 mM Tris, 0.5% Triton X-100, pH 7.4) with protease inhibitors as previously described (22). The NETT extracted preparations (fresh and 15-h incubation) were combined and cleared of particulate material by spinning at 15,000 *g* for 5 min. The cleared extract was labeled with 0.3 mCi of Na ^{125}I by the Iodogen method (23). Excess unbound Na ^{125}I was removed by passing the preparation through a spin column of Sephadex G-25 previously equilibrated with PBS. (*b*) 4 × 10^7 gametes of each preparation were surface radio-iodinated by the lactoperoxidase method with 0.3 mCi of Na ^{125}I as previously described (16) and extracted with 500 µl of NETT (with inhibitors) as described above. The extracts of surface-labeled 3-h and 15-h preparations were combined and spun for 5 min at 15,000 *g* to remove particulate material.

Both preparations, the Iodogen-labeled Triton X-100 extract and the Triton X-100 extract of surface-labeled gametes were absorbed with protein A-Sepharose beads to remove nonspecifically binding material. The amount of TCA precipitable radioactivity in each protein A-Sepharose absorbed preparation was determined.

Immunoprecipitation and SDS-PAGE. For immunoprecipitation samples of labeled antigen extract equivalent to 200,000 TCA precipitable counts were used. Immunoprecipitations were done using 20 µl of human sera or of the mixture of mAbs and were carried out as previously described (15). Briefly, after incubation with antibody, 50 µl of a 25% suspension of protein A-Sepharose was added and incubated, with rocking, to absorb the immune complexes. The beads were washed twice with 5% low fat milk powder in NETT, once with NETT with 0.5 M NaCl, and twice with NETT in a fresh tube. The beads were extracted in SDS sample buffer (without reducing agent) and run on 5–15% SDS-PAGE as previously described. Gels were dried on Whatman CF11 filter paper and exposed for autoradiography.

Results

47 Papua New Guinea sera were used to immunoprecipitate from the following preparations of sexual stages of *P. falciparum*, clone 3D7. (*a*) A combined preparation of extracellular female gametes 3 and 15 h after gametogenesis extracted in Triton X-100 and the total protein extract labeled with ^{125}I. (*b*) The same combined preparation as in *a* but surface radiolabeled with ^{125}I before extraction with Triton X-100.

Each of these radiolabeled extracts was immunprecipitated with each of the Papua New Guinea sera as well as with a serum from a U. S. donor previously infected with *P. cynomolgi*, a serum from a nonimmune U. S. donor and a mixture of mAbs against the 230-kD and 48-/45-kD gamete surface proteins of *P. falciparum*. The immune complexes were adsorbed to protein A-Sepharose beads, washed extensively, as described in Materials and Methods, and separated under nonreducing conditions on SDS-PAGE. Positions and intensities of labeled immunoprecipitated proteins on the gels were visualized by autoradiography.

Visual Analysis of Immunoprecipitations on SDS-PAGE

A general inspection of the autoradiographs of gels representing immunoprecipitations with the three labeled parasite extracts showed the following.

(*a*) *Triton X-100 Extract Labeled for Total Gamete Protein (Fig. 1).* Almost all Papua New Guinea (PNG) sera immunoprecipitated numerous proteins from the Triton X-100 extract labeled for total protein, of which the most prominent was a protein of 27 kD. Other well-labeled and fairly consistently immunoprecipitated proteins from this extract were at 97, 70, and 55 kD. None of these, including the 27-kD protein, are represented on the gamete surface (Fig. 2). However, clearly recogniz-

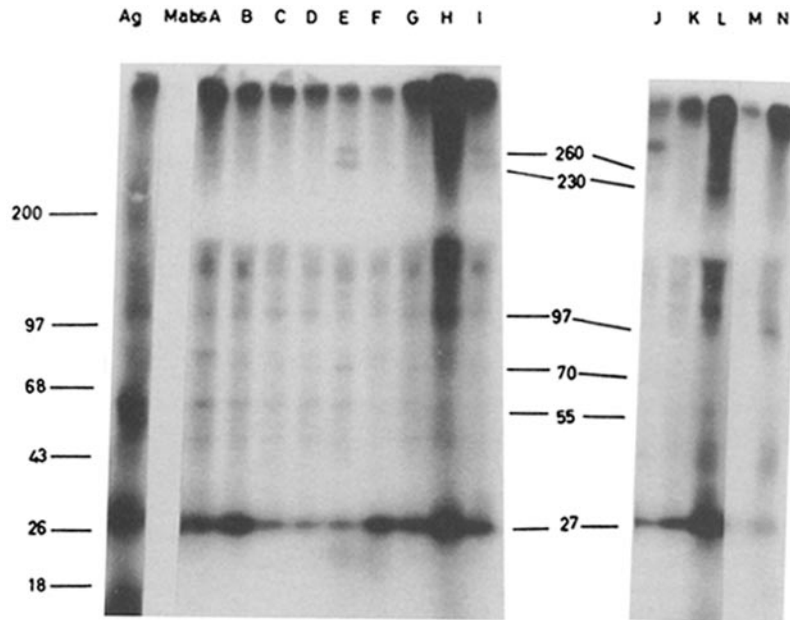

FIGURE 1. Autoradiograph of immunoprecipitations from a Triton X-100 extract of 3- and 15-h gametes of *P. falciparum* clone 3D7 in which total extracted protein was labeled with ^{125}I, and separated on 5–15% SDS-PAGE under nonreducing conditions. Total antigen extract is run in the first lane and a mixture of mAbs against the 230- and 48/45-kD gamete surface antigens in the second. The remaining lanes are immunoprecipitations with PNG human sera as follows: (*A*) T373, (*B*) T374, (*C*) T376, (*D*) T377, (*E*) T378, (*F*) T380, (*G*) T384, (*H*) G15, (*I*) T400, (*J*) G8, (*K*) G13, (*L*) G510, and with U. S. human sera, (*M*) nonimmune, (*N*) post-*P. cynomolgi*. Molecular mass markers ($\times 10^{-3}$) are indicated at the left and molecular masses ($\times 10^{-3}$) of specific parasite proteins are indicated between the two panels. The same antigen was used for each panel and the immunoprecipitates were done together but were run on separate gels.

able in many of the immunoprecipitates of the total labeled protein extract was the 230-kD gamete surface protein, generally associated with a higher molecular weight peptide of ~260 kD (cf., Figs. 1 and 2). Only trace amounts of labeled proteins were precipitated with normal serum from the total labeled protein extract. The post-*P. cynomolgi* serum, on the other hand, immunoprecipitated significant amounts of parasite protein; among these the 97- and 27-kD internal proteins were relatively prominent (Fig. 1). The mixed mAbs immunoprecipitated the 230-kD, and even more weakly the 260-kD, in barely detectable amounts from the total labeled protein extract.

(*b*) *Triton X-100 Extract of Surface-labeled Gametes (Fig. 2).* The mixed mAbs and some of the PNG sera immunoprecipitated the 230-kD (but not the 260-kD) and 48/45-kD gamete surface proteins from the Triton X-100 extract of surface radioiodinated gametes, including minor proteolytic fragments of the 230-kD protein. Other PNG sera gave weak or totally negative reactions for one or both the 230-kD and the 48/45-kD proteins.

There were other proteins of 60, 40, and 21 kD that were prominently labeled on the gamete surface but that were not readily detectable at normal autoradiographic exposure in immunoprecipitates by any of the PNG sera (Fig. 2). Even when autoradiographs of the immunoprecipitates were heavily overexposed (Fig. 3) the 21-kD protein was conspicuously absent; barely detectible amounts of the 40-kD

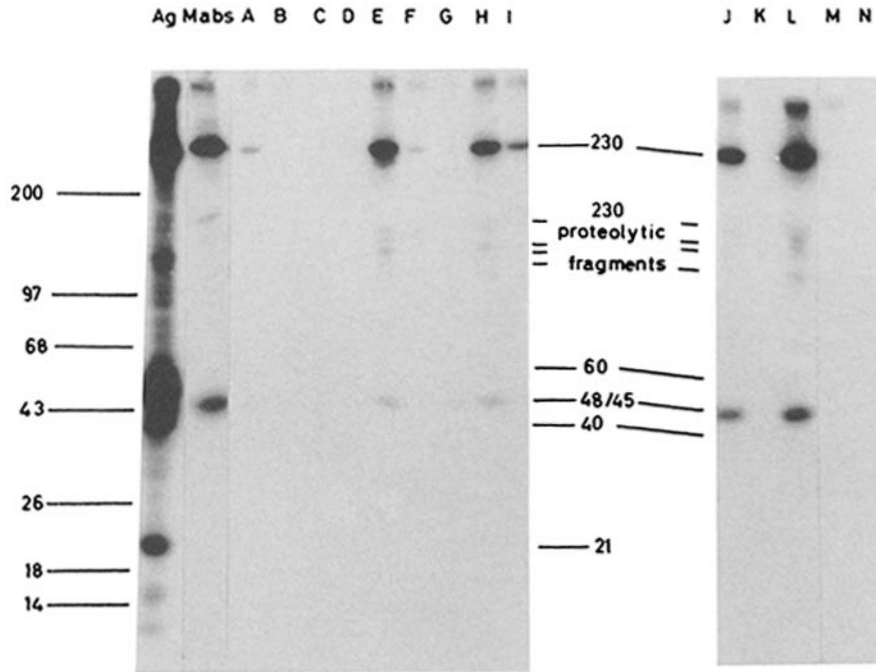

FIGURE 2. Autoradiograph of immunoprecipitations from a Triton X-100 extract of 3- and 15-h gametes of *P. falciparum* clone 3D7 that had been surface labeled with ^{125}I before extraction. Conditions of electrophoresis, layout, and identification of lanes as in Fig. 1.

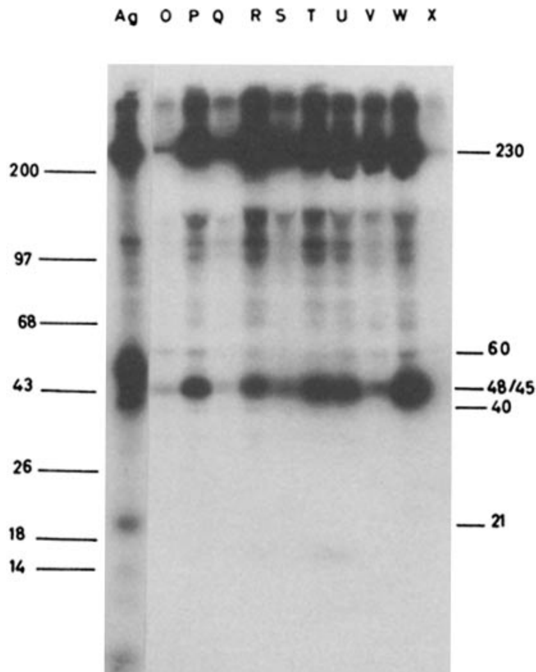

FIGURE 3. Autoradiograph of immunoprecipitations from a Triton X-100 extract of 3- and 15-h gametes of *P. falciparum* clone 3D7 surface labeled with ^{125}I before extraction as in Fig. 2. Immunoprecipitations and electrophoresis as before but autoradiograph heavily overexposed. Total antigen extract (not subject to overexposure in autoradiograph) in first lane; the remaining lanes are immunoprecipitates with PNG human sera as follows: O, A41; P, A34; Q, G5; R, G6; S, G11; T, A21; U, A29; V, A3; W, A5; X, A20. Molecular weight markers on left; molecular weights of specific parasite proteins on right.

protein appeared in some immunoprecipitates. Most sera were seen to immunoprecipitate small amounts of the 60-kD gamete surface protein in the overexposed autoradiographs. The nonimmune and post-*P. cynomolgi* sera were negative for all gamete surface proteins in the Triton X-100 extract (Fig. 2 and data not shown).

Semi-Quantitative Analysis of Immunoprecipitations on SDS-PAGE. Visual inspection of the autoradiographs of the immunoprecipitations on SDS-PAGE indicated that certain proteins, most notably the 230- and 48/45-kD gamete surface proteins, were being selectively immunoprecipitated by certain PNG sera only, as had been noted in our previous study with these sera (Table I). To analyze this observation more critically we wanted to be able to express the amount of a particular protein immunoprecipitated, e.g. the 230-kD gamete surface protein, relative to the general antibody response to gamete antigens. To do this three proteins, which were easily identified and which seemed to be fairly consistently immunoprecipitated by the PNG serum, were chosen for reference. The proteins, 97, 70, and 55 kD, labeled after Triton X-100 extraction, appear to be exclusively internal proteins of the parasites (cf., Figs.

TABLE I

Values for Amounts of Proteins Immunoprecipitated

	Internal proteins		Gamete surface proteins			
	TX-100 extracts		TX-100 extracts		SDS extracts* previous study	
Serum sample	Reference proteins	27 kD	230 kD	48/45 kD	230 kD	48/45 kD
T353	3	1	3	0.5	14.6	3.0
T362	6	3	0	0	0.8	1.5
T381	1	1	0	0	0.7	2.6
A67	6	2	3	3	19	28
A68	3	1	0	0	0.5	5.0
A41	3	2	0	0	1.0	24.8
A34	3	4	2	0.5	9.6	4.3
G5	0	1	0.5	0	1.2	0.8
G6	3	2	2	0.5	12	3.7
G11	4	3	0.5	0	3.3	9.1
A21	1	2	2	0.5	14.7	10.0
A29	6	3	1	0.5	17.3	9.2
A3	2	3	1	0	14.3	4.4
A5	4	3	1	2	13.8	20.0
A20	3.5	2	0	0	0.4	2.7
A40	2	2	0	0	0.4	1.4
A65	3	4	0	0	0.6	2.2
T314	0	1	0	0	0.6	1.5
T315	2.5	2	0	0	0.4	1.9
T333	5	4	0	0	1.1	3.4
T336	2.5	2	0	0	0.4	2.3
T350	5	3	0	0	0.9	3.4
T370	2.5	3	0	0	0.7	3.5
T373	3	2	1	0.5	2.3	3.2
T374	2.5	3	0	0	0.6	2.2
T376	2	1	0	0	0.4	2.1
T377	2	1	0	0	0.3	1.8

continued

1 and 2). These proteins and the 27-kD internal protein and the 230- and 48/45-kD proteins immunoprecipitated from surface-labeled gametes, were given numerical values in each immunoprecipitate based on intensity of labeling from a visual inspection of autoradiographs (see for example, Figs. 1 and 2; and cf. Table I). The scoring system is described and the data are presented in Table I. For the reference proteins, 97, 70, and 55 kD, the sum of scores of individual proteins was used to give a reference score for each serum.

The relationship between immunoprecipitation of the reference proteins and immunoprecipitation of the 27-kD internal protein is shown in Fig. 4; that between the reference proteins and the 230-kD gamete surface protein is shown in Fig. 5. The amount of 27-kD protein immunoprecipitated by the PNG sera correlated fairly well with the immunoprecipitations of the reference proteins (correlation coefficient 0.516; p = 0.0002) (Fig. 4). There were almost no sera in which the relative amount immunoprecipitated of the 27-kD protein deviated markedly from the amount of reference proteins immunoprecipitated. By contrast, there was a very poor correla-

TABLE I (*continued*)

	Internal proteins		Gamete surface proteins			
	TX-100 extracts		TX-100 extracts		SDS extracts* previous study	
Serum sample	Reference proteins	27 kD	230 kD	48/45 kD	230 kD	48/45 kD
T378	2.5	1	2	0.5	5.9	4.6
T380	0.5	2	0.5	0	1.4	2.1
T384	1	2	0	0.5	<2	<2
G15	5	4	2	1	23	7
T400	1	2	1	0	1.1	<4
T426	3	2	3	0	11	<4
T427	3	2	0.5	0	0.4	<4
T438	6	4	1	0	1.0	<4
T447	3	3	3	1	22	<4
T448	3	2	0	0	0.4	<4
T449	3	1	0	0	0.4	<4
T450	4	2	2	0.5	3.5	<4
T451	3	2	1	0	3.0	<4
T452	2	2	0	0	4.1	<4
T489	4	2	0	0	0.4	<4
G7	6	4	2	1	4.1	13
G8	0	1	3	2	6.7	14.4
G13	0.5	2	0	0	0	<4
T510	3	4	3	2	14.8	6.7
T547	1	4	2	0	43	5.6

Numerical values for the amount of protein immunoprecipitated from extracts of sexual stages of *Plasmodium falciparum* with human PNG sera. The values are derived from a visual inspection of autoradiographs of SDS-PAGE gels with immunoprecipitates of ^{125}I-labeled proteins. Each identified protein on a gel was awarded a value based on a −, +, + +, etc., score according to the intensity of label in the band on an autoradiograph. The numerical scores are derived from the −, +, + +, etc., score..

* The values in the last two columns are from our previous study (15) and represent radioactive counts from bands cut out of gels. Serum sample prefix T, Tau; A, Agan; G, Goroka.

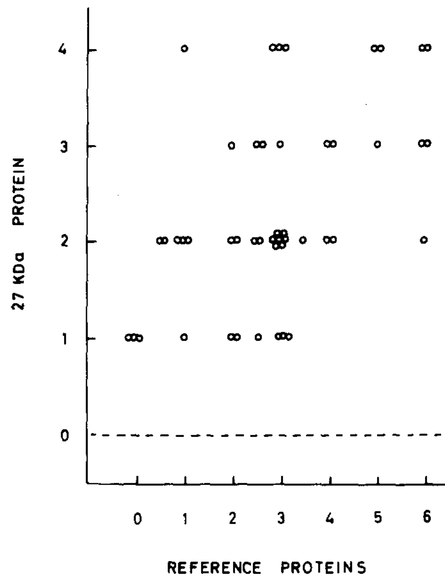

FIGURE 4. Correlation between immunoprecipitation of the 27-kD internal protein of gametes of *P. falciparum* and immunoprecipitation of the internal "reference proteins" of the gametes (97, 70, 55 kD) by 47 PNG human sera from *P. falciparum* gametes extracted in Triton X-100. The data is from Table I. The correlation coefficient for immunoprecipitation of the 27-kD protein and the "reference proteins" is 0.516 for these sera; $p = 0.0002$.

tion (correlation coefficient 0.123; $p = 0.4091$) between the amount of the 230-kD gamete surface protein immunoprecipitated from Triton X-100 extracts and the amount of the reference proteins immunoprecipitated (Fig. 5). Many of the sera were completely negative for the 230-kD protein in the Triton X-100 extracts, even though they were strongly positive for the reference proteins. About 18 of the 47 sera were in this category. Table I shows that a similar situation applies for the 48/45-kD proteins immunoprecipitated from the Triton X-100 extract in relation to the reference proteins (correlation coefficient 0.233; $p = 0.115$).

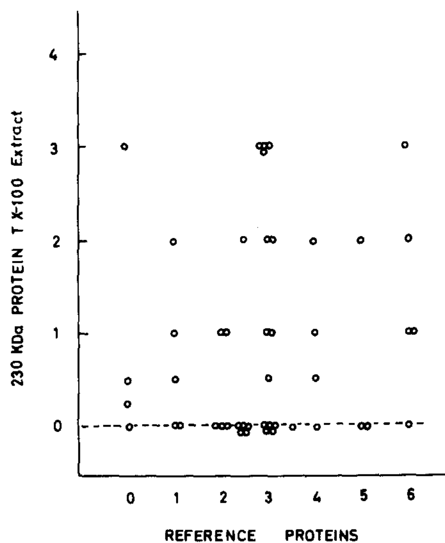

FIGURE 5. Correlation between immunoprecipitation of the 230-kD gamete surface protein of *P. falciparum* from Triton X-100 extracts of surface-labeled *P. falciparum* gametes and immunoprecipitation of the internal "reference proteins" from Triton X-100 gamete extracts by 47 PNG sera. The data are from Table I. The correlation coefficient for immunoprecipitation of the 230-kD protein from Triton X-100 extracts of gametes and the "reference proteins" is 0.123 for these sera; $p = 0.4091$.

Discussion

We have analyzed the immune recognition surface proteins and intracellular proteins of gametes of *P. falciparum* by antibodies in human PNG sera. All of the 47 sera studied reacted in varying degrees with intracellular antigens of gametes of *P. falciparum*. A prominent internal sexual stage specific (Carter, R., unpublished results) antigen of 27 kD was immunoprecipitated by almost all sera in similar amounts to a group of three internal proteins used as a reference. This demonstrates that all individuals from whom the sera were taken had been exposed to and had made immune responses to sexual stage malaria parasites. Nevertheless, immune recognition by the PNG sera of the gamete surface antigens was very variable. Many sera were completely negative for one or both the 230- and 48/45-kD gamete surface proteins. The 60- and 40-kD gamete surface proteins were scarcely detected by any of the sera, and no antibodies could be detected in any of the PNG sera to the 21-kD surface protein. Several points of discussion arise from these data.

Universal Antibody Response to the 27-kD Sexual Stage-specific Internal Protein. Our semiquantitative analysis of the antibody response to gamete proteins demonstrates that the response to the 27-kD internal protein correlates quite well with the mean antibody response to other internal proteins used as reference. Because the 27-kD protein is a sexual stage-specific protein the antibody response to this protein is probably a good indicator of exposure and general antibody response to the sexual stages of the parasites. It may not, however, be strictly representative of an antibody response to *P. falciparum*. The post-*P. cynomolgi* serum also recognized the 27-kD protein, which presumably, therefore, shares crossreacting epitopes with other parasite species such as *P. vivax* and *P. malaria*, both prevalent in Papua New Guinea (see Materials and Methods). Antibodies to the 27-kD protein may, therefore, represent exposure to sexual stages of malaria parasites in general and not to those of *P. falciparum* in particular.

Variable and Weak Antibody Responses to Gamete Surface Proteins. In contrast to the 27-kD protein, the antibody responses to the 230-kD and 48/45-kD gamete surface proteins were highly variable among the sera. There are several possible explanations for these observations.

(*a*) Those individuals whose sera failed to immunoprecipitate either the 230 or 48/45-kD gamete surface proteins may not have been exposed to *P. falciparum*, but rather to *P. malariae* or *P. vivax*. Individuals exposed to only to *P. vivax* to *P. malariae* might have antibodies to species-crossreactive antigens such as the 27-kD protein, but would probably not have antibodies to the species-specific 230- and 48/45-kD gamete surface antigens of *P. falciparum*. However, most individuals in this study were probably infected with *P. falciparum* several times a year and should have antibodies induced by immunogens of this species. Also some individuals made a strong antibody response to one gamete surface antigens of *P. falciparum* but not to another. Such individuals have clearly been exposed to *P. falciparum* gametocytes. Other explanations of our results are needed, therefore.

(*b*) The sera from different individuals may have been exposed to genetically different populations of *P. falciparum* with distinct antigenic forms of the 230- and 48/45-kD gamete surface proteins. Only some of these parasites may have given rise to antibodies capable of reacting with the corresponding molecules on the gametes of *P. falciparum* clone 3D7 used in these studies. However, the antibody epitopes on

the 230- and 48/45-kD proteins are mostly highly conserved among isolates of *P. falciparum* (Foo, A., and R. Carter, unpublished data). It would seem unlikely, therefore, that our results are due to a failure of the 230- and 48/45-kD gamete surface proteins of clone 3D7 to be recognized by polyclonal immune sera against these molecules.

(*c*) A third possible explanation of our results is that individuals exposed to the 230- and 48/45-kD gamete surface proteins differed in their ability to make an immune response to them. The basis for this restricted immune response is likely to be genetic differences between individuals in the MHC region controlling response to helper T cell epitopes, as already suggested (15). The weak or absent antibody responses to the 40- and 60-kD gamete surface proteins could represent extreme restriction of the immune response to these proteins in the human population. There is direct evidence for MHC restriction of the immune response to the 230-, 48/45-kD, and 40-kD *P. falciparum* gamete surface proteins in H-2 congenic mice immunized with gametes of this parasite (24). Our present results for human antibody response to the gamete surface antigens of *P. falciparum* accord well with these findings.

Absence of Antibody Response the 21-kD Ookinete Surface Protein. The ookinete surface protein recorded as 21 kD in the present studies, is a demonstrated target of transmission-blocking mAbs (17); its gene has been cloned (25) and the protein is now designated Pfs25. There are two general possibilities for the failure of the PNG sera to have antibodies to Pfs25: (*a*) the protein, although present in the blood stage parasites, is not immunogenic in the human host, or (*b*) it is not present in the parasite stages in the human host and cannot, therefore, act as an immunogen. The latter explanation probably applies to Pfs25. Biochemical studies suggest that Pfs25 is synthesized only after transformation of gametocytes into gametes (17, 26), an event that is believed to occur only after the gametocytes are removed from the blood circulation as in a mosquito blood meal. The absence of any definite antibody response to this protein in the PNG sera is certainly consistent with this view. Interestingly, there was little genetic restriction of the immune response to Pfs25 in the studies with the H-2 congenic mice (24). This accords well with the evidence that the protein is not expressed during the blood infection and cannot, therefore, be under selective pressure to undergo immune evasion.

General Conclusions. The target antigens of malaria transmission-blocking antibodies fall into two general categories: (*a*) Those that are expressed only on the sexual stages of the parasites in the mosquito blood meal and are not found in the blood stage parasites circulating in the vertebrate (human) host. Such antigens cannot be naturally immunogenic as they do not exist in the vertebrate host. Pfs25 appears to be an example of such a protein. (*b*) Those targets of transmission-blocking immunity that are present as potential immunogens during natural infection. These are represented by the gamete surface proteins that are already present in the gametocytes in the blood circulation. Antibodies against these proteins probably exert significant antiparasite transmission-blocking effects during natural infections of *P. falciparum* (15). The probable MHC-restricted immunogenicity of these target antigens, notably the 230- and 48/45-kD gamete surface proteins may be the result of this immune selection pressure.

As a vaccine candidate, Pfs25 may not have been under evolutionary selection pressure to be of restricted immunogenicity in human populations. Such a molecule

could be widely recognized as an immunogen but immunity would not be boosted by natural infections. Vaccines based on molecules such as the 230- and 48/45-kD gamete surface proteins may be immunogenic in only a proportion of the human population. This could be overcome by coupling the proteins to a universally recognized carrier molecule (27). However such vaccines would suffer a similar drawback to Pfs25 and in many individuals would not be boosted by natural infections.

Summary

We have studied the antibodies to sexual stage antigens of *Plasmodium falciparum* in human sera from Papua New Guinea where intense transmission of *P. falciparum* occurs as well as the less prevalent *P. malariae* and *P. vivax*. In extracts of gametes of *P. falciparum* we have studied the reactivity of serum antibodies with antigens labeled with ^{125}I on the surface of the gametes as well as intracellular gamete antigens. A prominent 27-kD sexual stage–specific intracellular protein was recognized more or less in proportion to the general antibody response to gamete proteins. The response to the gamete surface proteins, however, was quite unrepresentative of the general antibody response to the intracellular gamete proteins. No antibodies were detected against Pfs25, a 21-kD protein expressed on zygotes and ookinetes of *P. falciparum* and known to be a sensitive target of malaria transmission–blocking antibodies. The antibody response to two other target antigens of transmission-blocking antibodies on the surface of gametes of *P. falciparum*, a 230- and a 48- and 45-kD protein doublet, was very variable and independent of the response to the internal protein antigens. Several possibilities are discussed that may account for the variable response to these gamete surface antigens in individuals with otherwise good antibody responses to internal sexual stage proteins. Among these is the possibility that there is MHC restriction of the immune response to the gamete surface antigens in the human population. This interpretation accords well with evidence for MHC-restricted immune response to the same *P. falciparum* gamete surface antigens in studies with H-2 congenic mice (24).

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