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Gamete vaccines and transmission-blocking immunity in malaria*

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We have recently proposed an approach to malaria control based on immunization of the host against extracellular malarial gametes, the stage in the mosquito guts, in order to block transmission by the mosquito vector. Our studies with avian and primate models have demonstrated that immunization of the host with extracellular gametes totally suppresses infectivity to the mosquito of a subsequent blood meal. Gametocytes within the erythrocytes are unaffected by the immunity, since resuspending the gametocytes in serum from normal nonimmune animals restores their infectivity to mosquitos. Immunity is mediated by antibodies that are ingested with the blood meal. These antibodies interact with extracellular gametes and prevent fertilization (the fusion of male and female gametes). Thus the infection in the mosquito is blocked, and in this way transmission is interrupted.

We have proposed an approach to the control of malaria, namely the immunization of the host against a stage of the parasite, the gamete, that appears in the mosquito gut. The immunity would block infection of mosquitos and thus interrupt transmission of the infection. In this paper we review the results of studies on gamete immunity in avian malaria, in simian malaria, and in falciparum malaria in man.

EXPERIMENTAL FINDINGS

Transmission-blocking immunity in the Plasmodium gallinaceum*/chicken system*

Our studies on immunization of chickens with various preparations containing sexual stages of *P. gallinaceum* were the first to demonstrate that the infectivity of subsequent blood infections to mosquitos could be effectively blocked by this means (*1*, *2*, *4*, *5*). The results of these studies established the following facts in regard to the *P. gallinaceum*/chicken model.

(1) Effective immunization depended on the release of the extracellular gametes from the gametocytes in the material used for immunization.

(2) Purified preparations from which gametes of either sex were absent were less effective in inducing transmission-blocking immunity than were preparations containing a mixture of gametes of both sexes.

(3) Intravenous inoculations without use of adjuvants of preparations of mixed gametes led to almost total suppression of infectivity to mosquitos of subsequently induced blood infections but had virtually no effect on the asexual parasitaemia or the number of gametocytes present during such an infection.

(4) When parasitized blood from immunized birds was washed free of their own plasma, resuspended in normal serum, and fed to mosquitos through a membrane, the gametocytes recovered full infectivity to the mosquitos.

In reciprocal experiments in which parasitized blood from nonimmunized birds was fed to mosquitos with serum from immunized birds, infection in the mosquitos was completely suppressed.

(5) Gametocytes induced to exflagellate *in vitro* failed to produce ookinetes in culture if immune serum was added before the time of fertilization (about 20 min after initiation of exflagellation); when immune serum was added after this time, ookinetes were formed in numbers comparable to those formed in the presence of normal chicken serum.

(6) Observations of the behaviour of exflagellating gametocytes in the presence of immune serum showed that microgametes were immobilized on the glass slide and agglutinated within seconds of their release. Immobilization of microgametes by serum from immunized birds was shown to be associated solely with the immunoglobulin fraction of immune serum.

(7) Using a test based on the immobilization of microgametes, a close correlation was found between this immobilizing activity of serum and effective transmission-blocking immunity in the birds from

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which the serum was derived.

(8) Transmission-blocking antigamete antibodies appeared at low titres following recovery from infection in nonimmunized birds; these were never present, however, during the main peak after the primary blood infection and did not interfere with the infectivity of the birds to mosquitos during the infection.

(9) The blood infection provided a dramatic boosting effect on titres of antigamete antibodies in immunized birds. These findings demonstrated that highly effective transmission-blocking immunity could be induced in chickens by immunization with preparations containing male and female gametes of *P. gallinaceum*. Such immunity was mediated by the elaboration of antigamete antibodies, which were ingested by the mosquito during a blood meal and interacted with the extracellular gametes of the malaria parasite released into the mosquito gut. As a result of this interaction, fertilization was prevented and the infection sterilized in the vector. Gamete immunization had no effect on asexual parasitaemia or gametocyte production or on the ability of the gametocytes to undergo gametogenesis in the mosquito.

Transmission-blocking immunity in the P. knowlesi/*rhesus monkey system*

Studies of gamete immunization with *P. knowlesi* have demonstrated that antibody-mediated transmission-blocking immunity can be induced in rhesus monkeys by the injection of gamete-containing preparations. However, important distinctions exist between chickens and monkeys in the ways in which immunity can be induced. These distinctions appear to relate to fundamental differences in the responses of these two hosts to their respective malaria parasites. Our studies have established certain facts regarding immunization of monkeys with gametes of *P. knowlesi* (*3*). The following is a brief summary of these findings:

(1) In complete contrast to the results obtained in chickens, intravenous injection of monkeys with a preparation of gametes and trophozoites of *P. knowlesi* had no effect on the infectivity to mosquitos or on the severity of a subsequently induced blood infection (Table 1).

(2) When such a preparation was injected intramuscularly in Freund's complete adjuvant (FCA), on the other hand, infectivity to mosquitos was completely suppressed during a subsequently induced blood infection (Tables 2 and 3). Moreover, most monkeys immunized in this way developed only low-grade parasitaemias when challenged with the homologous strain of parasites.

(3) Challenge with a heterologous strain of *P. knowlesi* usually produced a fulminant infection, although such animals were still completely unable to infect mosquitos. Transmission-blocking immunity was thus shown to be effective against antigenically heterologous strains.

(4) Failure of immunized monkeys to infect mosquitos was due solely to the presence of antigamete-antibody, which sterilized the infections in the mosquitos. Thus, parasitized blood from monkeys with high- or low-grade parasitaemias readily infected mosquitos when the blood was washed free of immune plasma and fed to mosquitos.

(5) The spleen, which plays an important role in protective immunity against malaria in monkeys, does not appear to be involved in the development of transmission-blocking immunity. Thus, when gamete-immunized monkeys were splenectomized and challenged all were unable to control their blood infections, regardless of the challenge strain involved. Nevertheless, in spite of the large numbers of gametocytes produced none of the monkeys was infectious to mosquitos.

(6) Antigamete antibodies were readily detected *in vitro* in serum from monkeys immunized with gametes

Table 1. Effect of intravenous immunization of rhesus monkeys with *P. knowlesi* exflagellated parasitized blood, as measured by oocyst development in the guts of mosquitos fed on these monkeys

Monkey No	No of weekly immunizations	No of challenges: Spleen intact	No of challenges: After splenectomy	Time of last challenge (weeks after immunization)	Maximum parasitaemia [a] (%)	Mean no. of oocysts per feeding in 10 guts [b]
808	7	2	–	10	34	250, 460
142	5	2	–	10	23	390, 16
146	4	1	–	5	20	180
147	4	1	–	5	48	55

[a] All infections in spleen-intact monkeys developed rapidly and were cured with chloroquine at the parasitaemia level indicated

[b] 2–4 feedings per infection

Table 2. Effect of a single intramuscular immunization of rhesus monkeys with semi-purified *P. knowlesi* gametes in FCA, as measured by oocyst development in the guts of mosquitos fed on these monkeys

Monkey No.	Type of immunization	No. of challenges: Spleen intact	No. of challenges: After splenectomy	Time of last challenge (weeks after immunization)	Maximum parasitaemia (%)	Mean no. of oocysts per feeding in 10 guts[a]
533	FCA + 10^7 microgametes	1	5	86	0.1	0, 0, 0[b], 0.04, 0, 0.03[b]
434	FCA + 10^7 microgametes	6	–	98	5	0, 0, 0[b], 0, 0, 0
420	FCA alone	1	2	73	31[c]	29, 350, 28[b]
622	FCA alone	1	–	5	18[c]	170
426	None	1	3	23	8[c]	12, 400, 480[b], 400
348	None	4	–	27	40[c]	63, 56, 16[b], 130

[a] 2–4 feedings per infection

[b] Challenge with heterologous strain of *P. knowlesi*

[c] Treated with chloroquine when parasitaemia reached level indicated

Table 3. Effect of intramuscular immunizations of rhesus monkeys with various quantities of semi-purified *P. knowlesi* gametes in FCA, measured as infectivity of gametes to mosquitos fed on these monkeys

No. of microgametes per inoculation	No. of monkeys	No. of challenges: Spleen intact	No. of challenges: After splenectomy	Oocysts per mosquito gut: Spleen intact[a]	Oocysts per mosquito gut: After splenectomy
10^7	4	9	5	0 (9)	0 (3) 0.04, 0.03[b]
10^6	4	7	6	0 (7)	0 (5) 0.7[b]
10^5	9	28	2	0 (23) 1, 5, 25[b] 0.03, 3[b]	0, 0.1
Non-immunized controls	8	17	7	mean 136 range 12–460	368 28–480

[a] No. in parentheses = no. of times monkeys were infected giving oocyst counts indicated.

[b] Challenge with heterologous strain of *P. knowlesi*.

by their ability to immobilize microgametes and in fluorescent antibody (FA) tests. FA tests failed, on the other hand, to demonstrate the presence of anti-gamete antibody in serum from monkeys repeatedly infected with *P. knowlesi* or from monkeys immunized with *P. knowlesi* merozoites or sporozoites (Carter et al., unpublished results, 1978).

(7) Various adjuvant alternatives to FCA have been tested. The results of these tests are listed below:

(*a*) Immunization using Freund's incomplete adjuvant (FIA) was found to be effective, although less so than the complete adjuvant, in immunizing against challenge with *P. knowlesi*. Four monkeys received the semipurified antigen emulsified in FIA (Table 4). Two were challenged with the homologous strain of parasites and two with heterologous parasites. All developed rapidly rising infections and were drug treated; nevertheless, two monkeys showed complete transmission-blocking immunity, while the other two showed only low levels of infectivity.

(*b*) Two monkeys were immunized with the semi-purified *P. knowlesi* gamete antigen in DPT vaccine. As a control, two monkeys received DPT alone. At the same time, two monkeys, as a positive control, received an aliquot of the same antigen preparation in FCA (Table 5). After the initial challenge, the 4 monkeys receiving DPT, or DPT plus antigen, developed high parasitaemias but showed only low-level

Table 4. Effect of one or two intramuscular immunizations of rhesus monkeys with semi-purified *P. knowlesi* gametes in Freund's incomplete adjuvant (FIA), as measured by oocyst development in the guts of mosquitos fed on these monkeys

Monkey No	No of microgametes per inoculation	No of inoculations	No. of challenges: Spleen intact	No. of challenges: After splenectomy	Time of last challenge (weeks after immunization)	Maximum parasitaemia[a] (%)	Mean no of oocysts per feeding in 10 guts[b]
2786	10^6	1	2	–	17	27	0, 0 6
479	10^5	1	1	–	5	20	4
506	10^5	2	1	–	6	24	0
507	10^5	2	2	–	17	32	0 3, 12

[a] Monkeys treated with chloroquine starting when parasitaemia reached level indicated.
[b] 2–4 feedings per infection

Table 5. Effect of two intramuscular immunizations of rhesus monkeys with semi-purified *P. knowlesi* gametes in DPT, as measured by oocyst development in the guts of mosquitos fed on these monkeys

Monkey No.	Type of immunization	No. of challenges: Spleen intact	No. of challenges: After splenectomy	Time of last challenge (weeks after immunization)	Maximum parasitaemia (%)	Mean no of oocysts per feeding in 10 guts[a]
98	DPT + 10^5 microgametes	2	1	34	35[b]	4, 6, 100
100	DPT – 10^5 microgametes	3	–	34	13[b]	4, 8, 12
498	DPT alone	2	1	34	30[b]	5, 67, 200
619	DPT alone	3	–	34	32[b]	6, 42, 96
592	FCA + 10^5 microgametes	3	–	34	0 4	0, 0, 0
614	FCA + 10^5 microgametes	3	–	34	3	0, 0, 0

[a] 2–4 feedings per infection
[b] Monkeys treated with chloroquine when parasitaemia reached the level indicated.

infectivity to *Anopheles balabacensis* mosquitos. Some antigamete activity persisted through the second and third challenge infections, but these immunized monkeys continued to infect mosquitos. At the same time, the monkeys receiving similar quantities of antigen in FCA failed completely to infect mosquitos. DPT did not appear to act as an effective adjuvant with this antigen mixture, although some enhancement of antigenicity was apparent.

(*c*) Another group of monkeys was used to test the efficacy of BCG vaccine as an adjuvant (Table 6). Two monkeys were given BCG with the semipurified *P. knowlesi* gamete antigen; this material was administered intradermally and subcutaneously in the hip and upper arm. Two other monkeys received BCG alone, while the positive control group received the gamete antigen with FCA intramuscularly. With the exception of one monkey (No. 737), which received BCG without antigen, all animals developed rapidly rising infections and required drug treatment. In spite of these high asexual and sexual parasitaemias, mosquitos fed on the BCG-plus-antigen group showed significantly reduced infections. Only 6 of 40 mosquitos fed on 1 and 13 of 30 mosquitos fed on the other immunized monkey had oocyst-positive guts. Infections of mosquitos fed on the BCG only group were low, but within the limits expected of nonimmunized control infections. Moreover, mosquitos were uniformly infected; almost all had oocysts. The two monkeys immunized with the same gamete antigen preparation in FCA showed a delay in patency, but eventually developed high parasitaemias and were drug treated; neither was infectious to *Anopheles balabacensis*. However, when 1 animal in each pair was rechallenged 10 weeks later, both the BCG and BCG/antigen animals were highly infectious to mos-

Table 6. Effect of two intradermal immunizations of rhesus monkeys with semi-purified *P. knowlesi* gametes in BCG, as measured by oocyst development in the guts of mosquitos fed on these monkeys

Monkey No.	Type of immunization	No. of challenges: Spleen intact	No. of challenges: After splenectomy	Time of last challenge (weeks after immunization)	Maximum parasitaemia[a] (%)	Mean no. of oocysts per feeding in 10 guts[b]
723	BCG + 10^5 microgametes	2	–	17	18	1, 121
724	BCG + 10^5 microgametes	1	–	5	12	1
734	BCG alone	1	–	5	11	30
737	BCG alone	2	–	17	8	6, 48
731	FCA + 10^5 microgametes	1	–	5	25	0
733	FCA + 10^5 microgametes	2	–	17	15	0, 0

[a] Monkeys treated with chloroquine when parasitaemia reached the level indicated

[b] 2–4 feedings per infection

quitos; only the FCA/antigen animal blocked transmission.

All three adjuvants, FIA, DPT, and BCG, showed some effect on the development of an antigamete response. However, none was as effective as FCA in producing persistent transmission-blocking immunity. Moreover, none produced an effect against the development of asexual parasites.

Antigamete antibodies and transmission-blocking immunity in human populations in an area of holoendemic malaria

Our observations on antigamete antibodies during and following malarial infections in nonimmunized chickens and monkeys have shown that these are elaborated not uncommonly in chickens, following recovery from infection, but apparently not at all in monkeys. It was of interest, therefore, to determine whether antigamete antibodies with transmission-blocking properties occurred during or following malarial infections in man. We have made such a study among native inhabitants of the Gambia, an area of holoendemic *P. falciparum* malaria (Carter et al., unpublished results, 1977).

Using antihuman Ig fluorescent antibody, we found high titres of antibody to air-dried preparations of asexual *P. falciparum* in most of the sera that we collected. A proportion of sera also gave moderate antibody titres against air-dried gametes and gametocytes of *P. falciparum*. However, when tests were performed on living material in wet preparations, even sera showing the highest antibody titres against the dried gamete preparations failed to show any reaction against the living microgametes. Wet preparations of merozoites and segmenting schizonts, on the other hand, continued to react strongly.

Sera with high fluorescent antibody titres against dried gametes failed to immobilize or in any other way affect the behaviour of living motile microgametes *in vitro*; nor did such sera reduce the infectivity of gametocyte-carrying blood to mosquitos.

We have concluded that sera that reacted with dried gamete preparations contained antibodies specific to internal antigens of *P. falciparum* found in both sexual and asexual parasites. We found no indication of antibodies specific to the surface of the microgametes; nor did we find evidence for antibodies capable of blocking malaria transmission in human beings exposed to infection in this area of holoendemic *P. falciparum*.

CONCLUSIONS

Our studies have shown that transmission-blocking immunity can be induced in chickens and monkeys by immunization with preparations containing gametes of their respective malaria parasites, *P. gallinaceum* and *P. knowlesi*. In both systems immunity is mediated by the interaction of antigamete antibody with the extracellular gametes of the malaria parasite as they are released in the stomach of the mosquito during a blood meal. Fertilization is prevented and the infection in the mosquito is promptly sterilized.

In spite of evidence that chickens and monkeys respond differently to immunization and infection, the two systems share an important feature. In both chickens and monkeys, transmission-blocking immunity can be induced with comparative ease, whereas

protective immunity against the asexual stages of the malarial infections is difficult to achieve. Moreover, in the monkey system, it has been demonstrated that transmission-blocking immunity is independent of splenic functions and is fully effective against antigenically heterologous strains.

Nevertheless, gamete immunization of monkeys is dependent upon the use of an adjuvant, of which FCA is the only one that is fully effective. Other adjuvants, such as FIA and BCG, but not DPT, have been found to be effective but less so than FCA.

In contrast to the situation found in chickens, malarial infections in nonimmunized monkeys and humans do not result in the production of transmission-blocking antibodies. It is not known, however, whether such infections in humans or monkeys would boost levels of transmission-blocking antibodies in gamete-immunized individuals as they do in chickens.

ADDENDUM

Since the paper was prepared, Mendis & Targett (*6*) have reported the successful immunization of mice against the sexual stages of *P. yoelii* using formalin-fixed parasites and no adjuvant. In addition to very effective transmission-blocking immunity, vaccinated mice were protected against the sexual stages of the parasite.

RÉSUMÉ

VACCINS À BASE DE GAMÈTES PERMETTANT D'INTERROMPRE LA TRANSMISSION DU PALUDISME PAR LE MOUSTIQUE VECTEUR

Les auteurs ont récemment proposé une nouvelle approche à la lutte contre le paludisme fondée sur l'immunisation de l'hôte contre les gamètes—formes extracellulaires du parasite se développant dans l'estomac du moustique—afin d'empêcher la transmission par le vecteur. Les études faites sur des modèles aviaires et simiens ont montré que l'immunisation de l'hôte au moyen de gamètes extracellulaires supprimait le pouvoir infectant pour le moustique de tout repas de sang ultérieur pris par celui-ci sur le sujet vacciné. Cette immunité n'a pas d'effet sur les gamétocytes présents à l'intérieur des érythrocytes puisque, après leur transfert dans le sérum d'animaux normaux non immunisés, ils font de nouveau preuve d'infectivité pour les moustiques. L'immunité est conférée par des anticorps ingérés avec le repas de sang. Par leur interaction avec les gamètes extracellulaires, ces anticorps empêchent la fécondation (soit la fusion des gamètes mâles et femelles). Le développement du parasite chez le moustique est ainsi bloqué, d'où l'interruption de la transmission.

REFERENCES

1. GWADZ, R. W. *Science*, **193**: 1150 (1976).
2. CARTER, R. & CHEN, D. H. *Nature* (*London*), **263**: 57 (1976).
3. GWADZ, R. W. & GREEN, I. *Journal of experimental medicine*, **148**: 1311 (1978).
4. CARTER, R. ET AL *Experimental parasitology*, **47**: 185 (1979).
5. CARTER, R. ET AL. *Experimental parasitology*, **47**: 194 (1979).
6. MENDIS, K. N. & TARGETT, G. A. T. *Nature* (*London*), **277**: 389 (1979).