Malaria transmission-blocking activity in experimental infections of *Anopheles gambiense* from naturally infected *Plasmodium falciparum* gametocyte carriers

Bert Mulder1,2, Timoléon Tchuinkam2,3, Koen Dechering1,2, Jan Peter Verhave1, Pierre Carnevale2, Joep H. E. Th. Meuwissen1 and Vincent Robert1,3

1University of Wageningen and University of Nijmegen, Department of Medical Parasitology, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands; 2Medical Entomology Service, ORSTOM/OCEAC, Yaoundé, Cameroon; 3Faculty of Sciences, University of Yaoundé, Cameroon

**Abstract**

Experimental infections of anopheline mosquitoes were carried out with *Plasmodium falciparum* gametocytes from 65 naturally infected patients in Cameroon. A comparison was made between infections with blood containing autologous plasma and blood in which the plasma was replaced with plasma from a donor without previous malaria exposure. A lower infection rate was observed in 50 of 65 autologous plasma samples. Transmission was significantly blocked in 3 infections. This indicates that, in a population living in an area endemic for malaria, blood plasma factor(s) can reduce the transmission capacity of gametocyte carriers to mosquitoes.

**Introduction**

Gametocytes, the sexual erythrocytic stages of *Plasmodium*, can become apparent in the bloodstream during malaria infection. Transmission of the parasite depends on ingestion of gametocytaemic blood by the *Anopheles* vector. In the mosquito, gametocytes first develop into gametes and then into gametocytes. The development of gamete sporozoites is therefore linked to gamete development. Some gamete antigens interfere with gamete development before fertilization occurs.

One such antigen is the 230 kDa Mab which, when there is a lower number of gametocytes in the blood meal, is highly associated with a lower transmission rate (CARTER et al., 1988). Its transmission-blocking activity is thought to be relatively reduced when the number of gametocytes in the blood meal is high (PONNUDURAI et al., 1986). Plasma from donors without previous exposure to malaria can reduce the transmission-blocking activity of the 230 kDa Mab (GRAVES et al., 1991). In birds, transmission-blocking immunization has not yet been achieved, although the 25 kDa protein, a neoantigen of *P. falciparum* gametocytes, remains a major candidate antigen for transmission-blocking vaccine (KASLOW et al., 1991). While much progress has been made in immunological and molecular biological laboratory studies, a great need for specific epidemiological studies in endemic malaria areas remains (MEUWISSEN, 1989). The development of natural transmission-blocking immunity in endemic populations may be important in malaria transmission and its understanding will be critical for transmission-blocking vaccine studies. Field studies have been conducted to estimate the effectivity of *P. falciparum* gametocytes to the vector in West Africa (MURHEAD-THOMSON, 1957), Papua New Guinea (GRAVES et al., 1988a, 1988b) and East Africa (LINES et al., 1991; GITHEKO et al., 1992).

Transmission-blocking antibodies against *P. falciparum* were demonstrated in serum from a missionary returning from Africa, where he had lived for 28 years. Purified antibodies reacting with blocking epitopes of the 48/45 kDa proteins were obtained with monoclonal antibodies (Mabs) (CARTER et al., 1988) and with serum factors (NAOTUNNE et al., 1991; MEUWISSEN et al., 1992). The Purified antibodies reacted with blocking epitopes of the 48/45 kDa proteins (MEUWISSEN et al., 1992). The 48/45 kDa proteins have been identified as autoantigens of the mosquito midgut wall, preventing penetration of the oocyte by the microgamete, while it has been suggested that the latter interacts with a ligand of the oocyte for a receptor on the mosquito midgut wall, preventing penetration of the oocyte (CARTER et al., 1988). The transmission-blocking activity of the 48/45 kDa Mab appears to be relatively reduced when the number of gametocytes in the blood meal is low (PONNUDURAI et al., 1987). Transmission-blocking Mabs against *P. falciparum* are not effective against *P. vivax* (GRAVES et al., 1988b).

Transmission-blocking antibodies against *P. falciparum* have been demonstrated in serum from a missionary returning from Africa, where he had lived for 28 years. Purified antibodies reacting with blocking epitopes of the 48/45 kDa proteins were obtained with monoclonal antibodies (Mabs) (CARTER et al., 1988) and with serum factors (NAOTUNNE et al., 1991; MEUWISSEN et al., 1992). The Purified antibodies reacted with blocking epitopes of the 48/45 kDa proteins (MEUWISSEN et al., 1992). The 48/45 kDa proteins have been identified as autoantigens of the mosquito midgut wall, preventing penetration of the oocyte by the microgamete, while it has been suggested that the latter interacts with a ligand of the oocyte for a receptor on the mosquito midgut wall, preventing penetration of the oocyte (CARTER et al., 1988). The transmission-blocking activity of the 48/45 kDa Mab appears to be relatively reduced when the number of gametocytes in the blood meal is low (PONNUDURAI et al., 1987). Transmission-blocking Mabs against *P. falciparum* are not effective against *P. vivax* (GRAVES et al., 1988b).

Studies in Sri Lanka by MENDIS et al. (1987) showed that transmission blocking with *P. vivax* was correlated with titres of antibodies against air-dried gametes and that heat-labile factors, presumably complement, were involved. Transmission-blocking immunity was boosted by frequent reinfection (KANAWAKA et al., 1988). ZOYSA et al. (1991) compared the observed parameters of a *P. vivax* epidemic with those calculated in a mathematical model and concluded that an effect of transmission blocking immunity was essential to describe this malaria epidemic.

Here we report on the natural occurrence of factors that reduce transmission in an African population. Experimental infections of *Anopheles gambiense* with *P. falciparum* were performed by membrane-feeding of blood collected from local gametocyte carriers. Simultaneous membrane-feeding of blood containing autologous plasma and blood in which the plasma was replaced with plasma from a donor without previous malaria exposure was performed. Transmission-blocking activity was estimated in 3 infections. This indicates that, in a population living in an area endemic for malaria, blood plasma factor(s) can reduce the transmission capacity of gametocyte carriers to mosquitoes.
Materials and Methods

Gametocyte carriers

Each morning thick films were prepared from blood of patients reporting at the dispensary in Messa, an urban quarter of Yaoundé, the capital of Cameroon. After a rapid 20 min 8% Giemsa staining procedure, slides were examined for the presence of gametocytes.

Experimental infections

A strain of A. gambiae s.s., originating from Yaoundé, was infected by membrane feeding with blood of the P. falciparum gametocyte carriers. In each experiment 2 feedings were performed: (i) feeding on blood with autologous plasma (OWN) and (ii) feeding on blood, the plasma of which had been replaced by universal donor plasma from Dutch donors without any malaria exposure (AB). Donor plasma was obtained in Holland and stored at -20°C until use.

Two lots of 2 mL of venous blood from each gametocyte carrier were collected in heparinized tubes. To avoid activation of gametocytes, the tubes, the AB plasma and the syringes used to fill the membrane feeders were kept at 37°C during all manipulations. The tubes were centrifuged for 5 min at 540 g. In one of the tubes the plasma was replaced by AB plasma, which had been tested previously in transmission blocking assays at the University of Nijmegen (PONNIDURAI et al., 1989) and found to have no transmission blocking or enhancing activity. Membrane feeders were filled with blood from each tube and the mosquitoes were allowed to take a blood meal for 15 min. After feeding, blood-fed mosquitoes were counted and kept at 26-28°C with permanent access to a 10% sucrose solution without further blood meals.

The remaining heparinized blood was diluted 1:10 in phosphate-buffered saline and stored at -20°C for evaluation of chloroquine levels by enzyme-linked immunoabsorbent assay (ELISA) as described by WITTE et al. (1990). At the moment of venous blood collection another thick film was made, stained for 45 min with 4% Giemsa’s stain and examined. Gametocyte density was estimated from the parasite:leucocyte ratio by counting against 1000 white blood cells and assuming an average number of 8000 leucocytes/μL.

Detection of oocysts

After 7 d, the surviving mosquitoes were dissected and their midguts were stained with 2% mercurochrome in distilled water to facilitate examination for the presence and number of oocysts by light microscopy.

Enrolment criteria

Experimental infections were included in the study if the following enrolment criteria were met: samples of at least 20 surviving mosquitoes in both the OWN and AB groups could be dissected on day 7 and at least one mosquito with oocyst(s) was observed in either group.

Statistical analysis

Data were analysed using the SPSS® statistical software. A monofactorial analysis of the percentage of infected mosquitoes in both groups was performed with gametocyte density or age of the gametocyte carrier as independent variables. The Wilcoxon two-sample test was used for statistical analysis of the differences between the experimental groups. Differences in group means were analysed according to distribution by the t test or the non-parametric test (Mann-Whitney U test). Differences in proportions were determined by χ² with Yates’s correction.

Results

Gametocyte carriers

Sixty-five P. falciparum gametocyte carriers met the enrolment criteria. Their mean age was 18.9 years (range 6-36) and their mean gametocyte density was 212/μL (range 8-1208).

![Fig. 1. The relationship between P. falciparum gametocyte density and the percentage of infected A. gambiae mosquitoes in 65 experimental infections in autologous plasma (OWN) and after plasma replacement with plasma from Dutch blood bank donors (AB).](image)

![Fig. 2 A. Percentage of infected A. gambiae mosquitoes resulting from feeding with P. falciparum gametocytes in autologous plasma (OWN) in a malaria endemic area or feeding with gametocytes in replaced AB group plasma from European blood bank donors without malaria exposure; 65 experiments ranked in increasing order of infection percentage in the AB group. B. Mean number of P. falciparum oocysts per infected A. gambiae gut in 65 experiments, ranked in the same order as in A.](image)
mosquitoes in the AB group were dissected. The difference between the mean numbers of examined mosquitoes in these groups (33-43 and 33-21, respectively) was not significant (paired samples t test; t = 0.19, degrees of freedom (df) = 64, P > 0.05). There were 244 mosquitoes with oocysts in the OWN group and 387 in the AB group. The difference in the proportion of success in the 2 groups of infections was statistically significant (x² = 28.66, P < 0.001). The overall mean percentages of infected mosquitoes were 12-10 and 18-88, respectively, and the overall mean numbers of oocysts per midgut were 1-41 and 2-64, respectively.

The percentage of infected mosquitoes in the OWN and AB groups is shown in Fig. 2 A. A higher percentage of infected mosquitoes in the AB than in the OWN group was found for 50 of the gametocyte carriers (77%). While 8 feedings on OWN blood did not give rise to infected mosquitoes, all but one of the AB feedings infected at least one mosquito. The mean oocyst number per infected gut per experiment is shown in Fig. 2 B. A significant difference in the percentage of infected mosquitoes, as well as in the mean oocyst number per infected gut, was found between the OWN and the AB groups (Wilcoxon matched pairs test, P < 10⁻³ in both cases).

The relationship between age and reduction in infection, expressed as the difference between the AB group and the OWN group, is shown in Fig. 3. In 3 experiments the reduction in the proportion of infected mosquitoes was significant. Based on the OWN/AB ratio, the mean reduction in the percentage of infected mosquitoes was 36-8%. There was a highly significant difference in mean reduction of percentage with age. In the age group <15 years the mean reduction percentage was 17-6, while in the age group ≥15 years it was 51-4 (Mann-Whitney U test, P < 0.005). The reduction in the mean number of oocysts per infected gut was not significantly different in these 2 age groups (Mann-Whitney U test, P > 0.09); this could be expected since the overall median oocyst number in infected mosquitoes was only 1 (Fig. 2B).

High transmission reducers (more than 50% reduction in OWN infection in comparison with AB infection) appeared not to differ from the other gametocyte carriers in mean gametocyte density, prevalence of trophozoites, or prevalence of fever (i.e., body temperature ≥38°C) (Table).

Chloroquine (15–600 ng/mL) was detected by ELISA in the blood of 24 (44%) of 55 gametocyte carriers. There was no relation between infectivity in the OWN group and chloroquine levels, neither could a correlation be demonstrated between chloroquine levels and reduction percentage in the 2 groups (OWN and AB).
PERIS et al. (1988) reported enhancement of transmission of *P. falciparum* malaria by low concentrations of antibody, which was also dependent on the intrinsic infectivity of the parasite isolate (GAMAGE-MENDES et al., 1992). In *P. falciparum* infections we never observed sufficient enhancement of parasitemia in the own group compared to the AB group. Also, in transmission experiments with cultured *P. falciparum* enhancement was never observed (PONNUDURAI et al., 1987).

In order to investigate the role of antibodies, we are conducting experiments to compare the extent of transmission blocking, assessed by bio-assay (PONNUDURAI et al., 1989), induced by whole sera and isolated immunoglobulin G fractions of these sera. In these experiments we hope also to determine whether the factors involved influence the fertilization of gametes or the development of zygotes into oocysts.

Not only antibodies, but also cell-mediated immunity, have been reported to play a role in transmission blocking. Immune T cells can reduce gametocyte numbers (HARTE et al., 1985) and with *P. cynomolgi* it has been reported that loss of infectivity of gametocytes during the crisis is due to the presence of cytokines and crisis factors in the serum (NAOTUNNE et al., 1991). Cytokine levels are elevated in malaria parasitaemia (KWIATKOWSKI et al., 1990) and have pyrogenic activity (LE & VILCEK, 1987). Our observation that high transmission reducers, although recruited from patients who had experienced malaria-like complaints, did not differ from low transmission reducers in body temperature or prevalence of trophozoites, suggests that 'crisis' serum factors and/or cytokines may not play an important role in transmission blocking in *P. falciparum* infections. Because *P. falciparum* gametocytes appear in the blood stream about 10 d after the expedite of parasites, it is not likely that factors appearing in the acute phase of the attack have an epidemiologically significant influence on the transmission capacity of circulating gametocytes.

Acknowledgements

This work has never been possible without the invaluable inspiration and contribution of the late Dr Thivi Ponnudurai.

We thank the personnel and patients of the Messa dispensary, Yaoundé for their co-operation, Harneke Stoffels, Gerald de la Faille, Ernest Mooh and Ousmane Traore for excellent technical assistance, Mariel Droomers, Michel Cot and Johan Velena for statistical advice, and Ton Lensen and Christina Celluzzi for critical reading of the manuscript. Financial support was provided by the Economic Community of STDS and by the French Ministry of Research and Space.

References


Ponnudurai, T., van Gemert, G. J. A., Bensink, T., Lensen, A.


Received 28 October 1992; revised 5 April 1993; accepted for publication 13 May 1993

Corrections


The international code numbers of 2 of the strains of Leishmania infantum isolated from these patients were incorrectly printed on p. 705 (3 lines from the bottom of column 2) and p. 706 (line 13 of column 1); the correct numbers are MHOM/ES/91/LEM2298 and MHOM/ES/91/LEM2361, respectively. The editor apologizes for these errors.


The authors have pointed out that the word 'its', in line 4 of paragraph 5 of their letter, appeared as 'whose' in the original typescript, and that this more clearly indicates their meaning, that it is the replication of the plasmid which is an epiphenomenal self-perpetuating feedback process.