

Malaria Transmission-Blocking Immunity Induced by Natural Infections of *Plasmodium vivax* in Humans

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Immunity to malarial infections in human populations is known to affect the development of the asexual blood stages of the parasites in the human host and to be capable of conferring significant protection against morbidity and mortality due to the disease. In this study we show that during acute infection with *Plasmodium vivax* malaria, one of the two main malarial pathogens of humans, most individuals also develop immunity that suppresses the infectivity of the sexual stages of the parasite to mosquitoes. The immunity is antibody mediated and is directed against the parasites in the mosquito midgut shortly after ingestion of blood by a mosquito. This immunity could be expected to have significant effects on the natural transmission of *P. vivax* malaria.

Transmission of malaria from humans to the mosquito vector is mediated by gametocytes; these are sexual stages that are present as intracellular parasites of erythrocytes and that are ingested by a mosquito during a blood meal. In the mosquito midgut the gametocytes become extracellular, form gametes, and become fertilized, leading to their subsequent development in the vector. By immunization (1, 9, 10, 13, 16) and use of monoclonal antibodies (11, 18, 20), it has been shown that antibodies against gametes and zygotes of malarial parasites can block transmission of the parasites to mosquitoes. In the chicken malaria parasite *Plasmodium gallinaceum* (2, 3) and the murine parasite *Plasmodium yoelii* (14), antibodies to gamete surface antigens may develop during a blood infection without previous immunization, although it is uncertain what effect these antibodies have on the infectivity of the parasites to mosquitoes. In *Plasmodium knowlesi* in rhesus monkeys (10), no evidence for the production of transmission-blocking antibodies was found during infection in unimmunized animals. A previous attempt to determine whether anti-gamete transmission-blocking immunity occurs during human malarial infection was inconclusive (4).

The existence of acquired immunity to malaria in humans, however, has been noted, at least since the observations recorded by Ross (19) and Christophers (5). Development of protective immunity against the pathogenic asexual blood stage parasites takes place slowly during several years of repeated infection (5, 6, 15). Eventually, some individuals may also develop immunity against the sporozoite stage of the parasite, which is inoculated by an infected mosquito (17). Results of this study show that serum-mediated, transmission-blocking immunity against the sexual stages of the parasites is induced in a high proportion of acute *Plasmodium vivax* infections in adults in Sri Lanka.

MATERIALS AND METHODS

Patients. The study group consisted of 40 patients attending the General Hospital in Colombo, Sri Lanka. Malaria transmission in Sri Lanka is unstable and it is characterized by moderate endemicity and frequent epidemics. The city of

Colombo itself is malaria-free, and the majority of the patients were adult males returning from visits to regions of Sri Lanka where transmission of *P. vivax* malaria occurs. All patients in the group were diagnosed as blood smear positive for *P. vivax* and had been symptomatic for an average of 7 to 8 days prior to examination. The number of previous malarial attacks was recorded from verbal statements; clinical records existed for several of the patients, and these were confirmed by the verbal statements.

Infection of mosquitoes with *P. vivax*-infected blood. Following informed consent and prior to treatment, 4 ml of blood was drawn from a patient without anticoagulant, and 2 ml was allowed to clot for preparation of serum; the remainder was diluted immediately in 20 ml of suspended activation solution (10 mM Tris, 170 mM NaCl, 10 mM glucose [pH 7.4]) (1); in this solution gametocytes can be washed and maintained for several hours without being stimulated to undergo gametogenesis or losing infectivity to mosquitoes. Meanwhile, autologous serum samples were prepared, with half being inactivated for 30 min at 56°C. Normal human serum (nonimmune) from type AB blood was prepared similarly. The washed parasitized blood was divided into three parts, and each part was suspended to a 50% hematocrit in either normal human serum or native or heat-inactivated serum from patients. These preparations were fed to laboratory-reared *Anopheles tessellatus* mosquitoes (a vector indigenous to Sri Lanka) through a water-jacketed membrane feeding apparatus that circulated water at 40°C. The blood-fed mosquitoes were maintained for 7 days at 25 to 26°C and a relative humidity of 70 to 80%. The mosquitoes were then dissected, their midguts were examined, and the numbers of oocysts (products of malarial gamete fertilization) were counted.

Indirect immunofluorescence tests. Serum from each patient was also tested by the indirect immunofluorescent antibody test (IFT) against air-dried preparations of gametes and asexual blood stages of *P. vivax*. The IFTs were conducted with preparations of extracellular female gametes or asexual blood stages of *P. vivax* isolates that were air dried on glass slides. Dilutions of sera in phosphate-buffered saline (PBS) were incubated with a 1:20 dilution in PBS of fluorescein-conjugated goat anti-human immunoglobulin (Cappel Laboratories, Cochranville, Pa.) for 30 min. The

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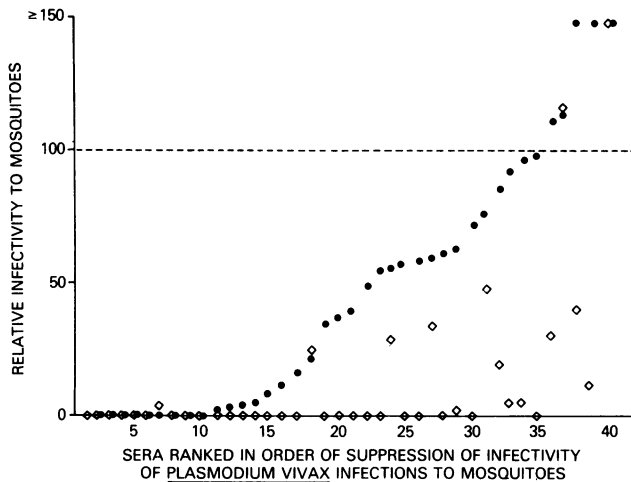


FIG. 1. Human sera from acute *P. vivax* cases ranked in the order in which they suppressed the infectivity of their autologous parasites to *A. tessellatus* mosquitoes with complement active (\diamond) and with complement inactive (\bullet). The intrinsic infectivities (the mean number of oocysts per gut in mosquitoes fed on patient blood with normal AB type human serum) in the experiments reported here varied from 2.2 to 149 oocysts per gut. A minimum of 7 and a maximum of 32 mosquitoes were dissected and counted for each parasite-serum combination. The relative infectivity of a serum sample is the infectivity of the parasites in a patient serum sample expressed as a percentage of the infectivity of the parasites fed simultaneously in normal human serum.

slides were again rinsed in PBS, overlaid with glass cover slips, and examined by UV microscopy. By this method antibody reactions with both internal and cell surface antigens were detected.

IFT reactions were also conducted against surface antigens of gametes of *P. vivax*. Living extracellular gametes of *P. vivax* were prepared, and immunofluorescence was done as described previously (16) with a 1:10 dilution of human serum in PBS and a fluorescein-conjugated goat anti-human immunoglobulin G antibody (Cappel) at a 1:20 dilution.

RESULTS

The results of the membrane feeding experiments are shown in Fig. 1. Sera from about two-thirds of the acutely infected patients completely suppressed infectivity of the autologous parasites to mosquitoes when native serum (i.e., serum freshly prepared and not heat inactivated) was used. In about half of these suppressive serum samples the suppression was independent of complement, i.e., the heat-inactivated sera suppressed as effectively as the native sera; in the other half suppression appeared to be complement dependent to various degrees; i.e., heat-inactivated sera suppressed infectivity less effectively than did the native sera. Most of the remaining sera mediated partial suppression of infectivity with or without complement; in three heat-inactivated serum samples infectivity was considerably greater than in controls.

By the Spearman rank correlation test, the degree of suppression of infectivity by the sera correlated with an increase in IFT titers against air-dried gametes of *P. vivax* (Fig. 2A) more strongly than with IFT titers against asexual parasites (Fig. 2B). With air-dried parasite preparations, antibody was able to penetrate the cells and react with intracellular as well as cell surface antigens. Therefore, the

serum samples were also tested for reactivity with live, intact female gametes of *P. vivax* by IFT to detect the presence of antibodies that reacted only with cell surface antigens. Acute sera, which strongly suppressed infectivity of the parasites to mosquitoes, gave positive reactions with gamete surface antigens (Fig. 3).

Immunoglobulins from sera from two patients that completely blocked infectivity were purified on protein A-Sepharose and reconstituted to their original volume in Tris hydrochloride (pH 8.0)-buffered saline; a normal human serum AB blood type was treated similarly. Relative to immunoglobulins from the normal serum, the purified immunoglobulins from patient serum blocked infectivity of *P. vivax* isolates as effectively as the original heat-inactivated sera. These data support the conclusion that the suppressive effects of the sera are antibody mediated.

Several native serum samples that effectively blocked infectivity of autologous parasites were tested against heterologous isolates of *P. vivax* (Table 1). In these experiments test sera and heterologous parasitized blood were matched for blood type antigens. In most cases sera suppressed the infectivity of heterologous parasites as effectively as they had done for the autologous parasites. The suppressive effects are not, therefore, narrowly isolate specific. One serum sample (no. 450) which appeared to completely suppress infectivity of the autologous parasites was only partially effective against a heterologous infection. Diversity in target antigens of transmission blocking immunity has already been found among different isolates of *Plasmodium falciparum* (7). The possibility of antigenic diversity in sexual stages of isolates of *P. vivax* could account for the apparent failure of a transmission-blocking serum sample to suppress infectivity of a heterologous isolate of this species.

The degree of suppression of infectivity of autologous isolates by the acute *P. vivax* serum sample was strongly influenced by whether or not a patient had experienced a previous malarial attack; suppression of infectivity by sera from patients who had experienced one or more previous attacks was significantly greater than from those experiencing their first reported attack of malaria (Table 2).

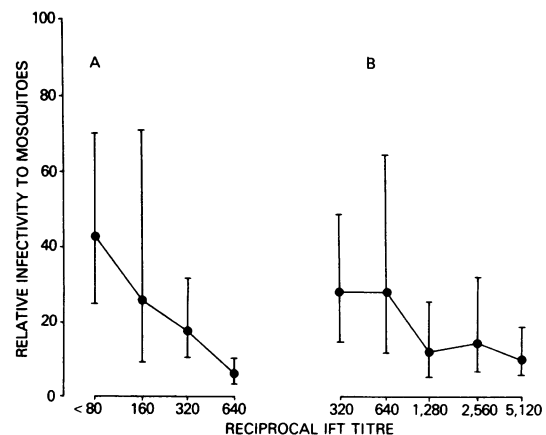


FIG. 2. Infectivity of *P. vivax* to mosquitoes in the presence of heat-inactivated autologous sera in relation to their IFT titer against air-dried parasites gametes (A) and asexual blood stage parasites (B) of *P. vivax*. Infectivity for the sera at each IFT titer is given as a geometric mean (\times/\div 1 standard error). The relative infectivity of a serum sample is the infectivity of the parasites in a patient serum sample expressed as a percentage of the infectivity of the parasites fed simultaneously in normal human serum.

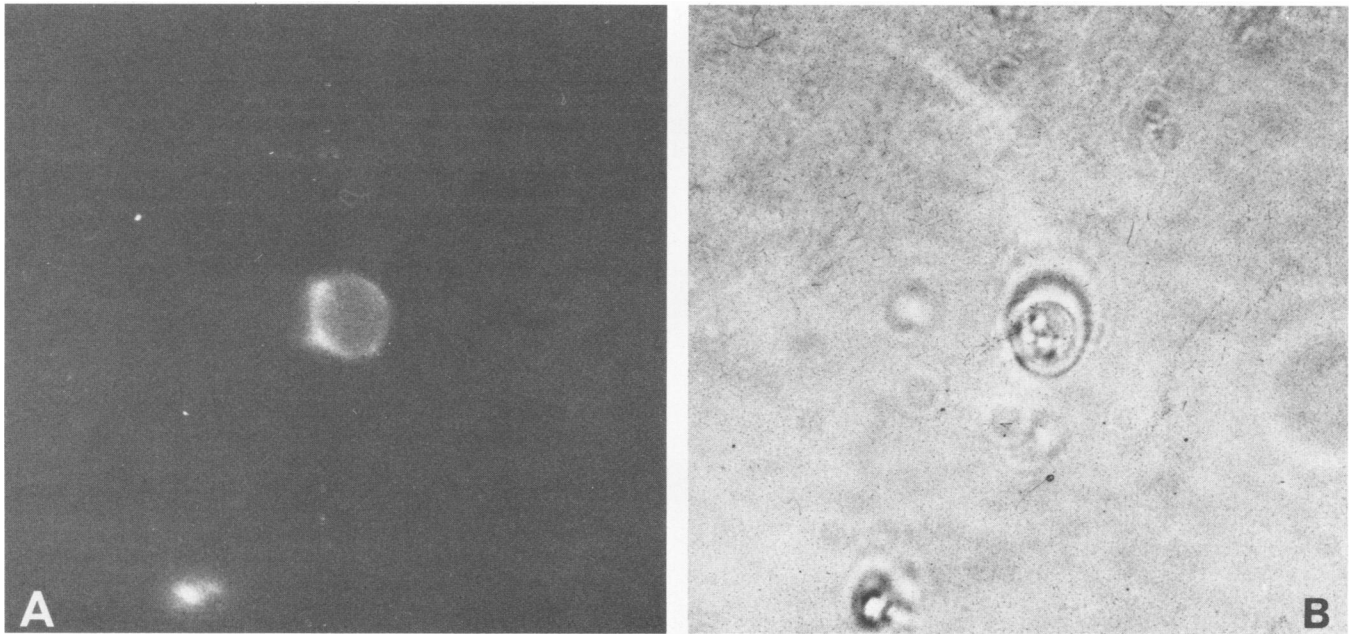


FIG. 3. Immunofluorescent reaction of transmission-blocking serum from an acute *P. vivax* infection with the surface of gametes of *P. vivax*. (A) Fluorescent micrograph; (B) phase-contrast micrograph of the same field shown in panel A.

DISCUSSION

Results of this investigation have demonstrated a high incidence of serum-mediated, malaria transmission-blocking activity among individuals infected with *P. vivax*. The immunity appears to be mediated by antibodies that are directed against the surface antigens of extracellular gametes or zygotes, as has been shown to occur in other systems (9, 11, 18, 20). In many serum samples the suppressive activity involved heat-labile factors (Fig. 1 and Table 2), presumably complement. More effective suppression of infectivity of malaria parasites to mosquitoes by antibodies in the presence of complement has been found in several examples

involving monoclonal antibodies against malarial gametes (7, 11, 18).

The serum-mediated, transmission-blocking immunity found in this study developed readily during primary infection and tended to be increased further during subsequent attack. The induction of antibodies to gamete surface antigens during malarial infection can be accounted for by the presence of these antigens in the gametocytes. Gamete surface antigens are known to be present in gametocytes of *P. falciparum* (12, 20). Nevertheless, it is remarkable that these antibodies should be so effective in blocking the infectivity of the parasites to mosquitoes in such a high proportion of primary infections and boosted further during subsequent infection; the relevant antigens must be highly immunogenic during malarial infection while parasite-mediated immunosuppression (8) must be of little signifi-

TABLE 1. Effect of native (untreated) sera from acute *P. vivax* infections on autologous and heterologous parasite infectivity to *A. tessellatus* mosquitoes^a

Test serum no.	Mean no. of oocysts/mosquito for:			
	Autologous combination in:		Heterologous combination in ^b :	
	Normal serum	Test serum	Normal serum	Test serum
442	21.7	0.8	71.7	0.0
445	41.4	0.0	26.8	0.0
447	22.1	0.0	63.3	0.1
449	63.3	0.0	22.4	0.0
450	26.8	0.0 ^c	50.8	19.6
452	22.4	0.0	18.6	1.6
454	3.4	0.0	5.8	0.1
455	18.6	3.4	30.7	7.4

^a For each serum-parasite combination tested the infectivity of the parasites to mosquitoes was determined in the presence of normal human serum from uninfected adults with no recorded experience of malarial infection. Autologous parasites indicate parasites and serum from the same patient. Heterologous parasites indicate parasites and serum from different patients.

^b Parasitized blood and test serum were matched for blood type.

^c Only five mosquitoes were dissected.

TABLE 2. Effect of previous malarial attacks on suppression of infectivity of *P. vivax* blood infections to *A. tessellatus* mosquitoes by autologous serum from acute infection^a

No. of attacks reported by patient	Relative infectivity (%) in:		No. of serum samples tested
	Heat-inactivated serum		
	Fresh serum	Heat-inactivated serum	
1	8.5 \times/\div 1.75	44.7 \times/\div 1.65	14
2	0.65 \times/\div 1.64	12.8 \times/\div 1.76	12
3	1.2 \times/\div 2.16	9.47 \times/\div 1.84	14

^a Infectivity data are given as a percentage of the infectivity of the parasites to mosquitoes (number of oocysts per mosquito) in simultaneous tests with normal human sera. Results are grouped into those from patients experiencing their first, second, or third reported attack of malaria; and in each category the geometric mean for the percentage infectivity is given \times/\div by 1 relative standard error of the geometric mean. The mean infectivity (in number of oocysts per mosquito) in normal human sera for patients experiencing a first, second, or third attack were 19.3 \times/\div 1.35, 17.7 \times/\div 1.45 and 17.2 \times/\div 1.35, respectively (\times/\div by 1 relative standard error). Among all categories an average of between 13 and 21 mosquitoes were dissected for each parasite-serum combination; at least seven mosquitoes were dissected for each combination.

cance to this response. These observations are encouraging for the development of a malaria transmission vaccine.

On the other hand, the high efficiency with which this immunity is induced in nature raises the question of how the parasites survive and, because they do, what effect further vaccination could be expected to have. That sufficient parasites escape to maintain transmission could be due to individuals who fail to mount an effective transmission-blocking response, to the presence of infectious gametocytes before adequate levels of transmission-blocking antibodies are achieved during an infection, or both. The goal of vaccination against gamete antigens, therefore, would be to achieve effective transmission-blocking immunity throughout malarial infection in a sufficiently high proportion of the human population that transmission of the parasites would be significantly reduced or eliminated.

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