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IMMUNE MODULATION OF PARASITE TRANSMISSION IN PLASMODIUM VIVAX MALARIA. ANTI-GAMETE ANTIBODIES CAN BOTH BLOCK AND ENHANCE TRANSMISSION[1]

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ABSTRACT

Antibodies directed against Plasmodium vivax gametes can block development of the parasite in the mosquito vector and interrupt transmission. Using monoclonal antibodies we have defined two components of molecular weights 36 and 42 kilo Daltons as target antigens of transmission blocking immunity in this parasite. A high level of transmission blocking activity can be detected in the sera of P.vivax infected individuals Anti gamete monoclonal antibodies as well as antibodies in immune human sera can have two opposite effects on transmission, blocking and enhancing.

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INTRODUCTION

Antibodies directed against the surface of sexual stages of the malaria parasite (gametes, zygotes or ookinetes) can block the development of the parasite in the mosquito vector and interrupt transmission. This has been shown by immunisation (1,2,3,4) and through the use of monoclonal antibodies (5,6). These observations have led to the concept of a transmission blocking vaccine. By using such a vaccine on a large scale in an endemic population, it may be possible to curtail transmission and protect individuals from the disease. We have previously demonstrated that Plasmodium vivax, a major human malaria parasite, is susceptible to transmission blocking immunity (7). In the present study we show that antibody can have two opposite effects on transmission, blocking and enhancing. This dual activity can be demonstrated both with monoclonal antibodies directed against gametes and with antibodies from vivax malaria patients.

MATERIALS AND METHODS

The Parasite and the Vector.

Parasite material was obtained from acute P.vivax malaria patients at the General Hospital , Colombo. Following voluntary informed consent, patients were bled for upto 15 ml of blood intravenously, and infected blood was used either for membrane feeding or for preparation of gametes (7). Anopheles tessellatus, a species indigenous to Sri Lanka which we have laboratory-adapted and currently maintain as a colony, was used as the vector.

Assessment of Infectivity.

Infectivity of parasite isolates from malaria patients was assessed by membrane feeding mosquitoes (7,8), or in a limited study by directly feeding mosquitoes on patients (9). The mean number of oocysts per mosquito mid gut was used as an index of transmission.

The infectivity of an isolate mixed with a given antibody was expressed as a percentage of its intrinsic infectivity (Intrinsic infectivity=infectivity in controls

eg. normal human serum or culture supernatant of myeloma cells). The transmission blocking ability of an antibody was expressed as the percentage block in transmission, that is: 100 - (infectivity in antibody/intrinsic infectivity X 100).

Isolation of Monoclonal Antibodies.

Mice were immunized with purified female gametes of P.vivax and hybridomas were established (10). Antibody-secreting clones were screened by indirect immunofluorescence using female gametes of P.vivax.

Indirect Immunofluorescence Test.

The indirect immunofluorescence test (IFT) was performed using P.vivax female gametes (7), either in a live unfixed state in suspension to detect antibodies which react with the gamete surface, or air-dried on slides, a method which exposes internal as well as surface parasite antigens.

Western Blot Analysis.

SDS extracts of purified gametes were run under non reducing conditions on 5-15% polyacrylamide gradient gels and transferred to nitrocellulose (11). Strips of nitrocellulose were incubated with antibody, followed by peroxidase -conjugated anti immunoglobulin and diaminobenzidine.

RESULTS

A Monoclonal Antibody Directed Against the Surface of P.vivax Female Gametes Both Blocks and Enhances Transmission

Two of a panel of monoclonal antibodies isolated against P.vivax gametes, Mab4 and Mab6, reacted with the surface of female gametes by IFT using live unfixed gametes in suspension. Both monoclonal antibodies reacted with a polypeptide doublet of approximate molecular weight 36 and 42 kDa, as shown by Western blot analysis of unreduced gamete extracts (Figure 1).

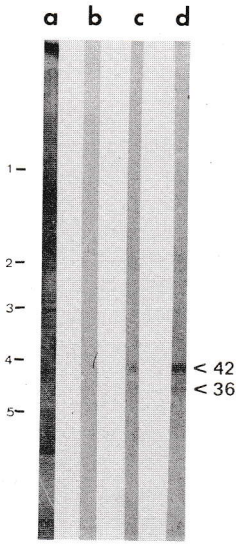

FIGURE 1. Western blot of non reduced P.vivax gametes a:human serum, b:myeloma cell supern., c: Mab4, d: Mab6

Transmission blocking effects. The effect of Mab4 and Mab6 on the infectivity of parasite isolates from patients was tested by membrane feeding (Table 1). Significant transsmission blocking effects were obtained with both Mab4 and Mab6 in 5 out of 7 experiments in which different parasite isolates were used. In 2 experiments Mab4 and Mab6 did not significantly affect infectivity, this possibly reflecting antigenic polymorphism on the surface of P.vivax gametes. (Control : Myeloma-cell supernatant)

TABLE 1
TRANSMISSION BLOCKING ACTIVITY OF ANTI GAMETE Mab 4 and Mab6

Experiment Number	Infectivity in Control (oocyst/gut)	%Block in Transmission Mab4	Mab6	Others
1	31.5	92	60	0
2	8.5	0	0	0
3	175	91	69	ND
4	86	12	54	12
5	59	98	91	ND
6	31	100	100	ND
7	22.5	78	88	0

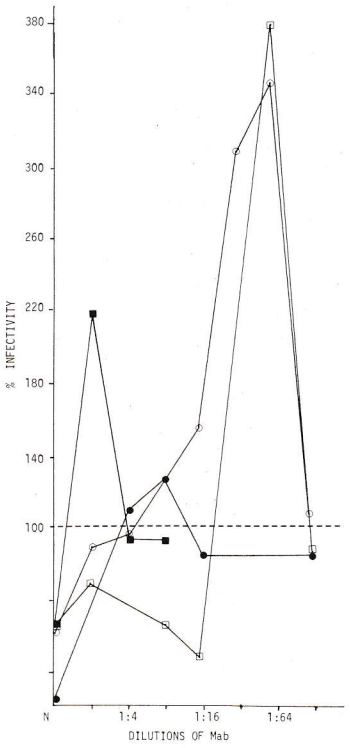

FIGURE 2. Enhancement of transmission of 4 different isolates through dilution of a transmission blocking monoclonal antibody.

Transmission enhancing effects. Monoclonal Mab6 was purified on ProteinA- Sepharose. Purified antibody was tested at increasing dilutions in membrane feeding experiments. As dilutions of antibody increased, blocking was replaced by a distinct enhancing effect of antibody on the infectivity of P.vivax gametes to mosquitoes (Figure2).

Antibodies Induced in Man by Natural P.vivax Infections Also both Block and Enhance Transmission.

Transmission blocking effects. Fifty acute vivax malaria patients presenting at the General Hospital in Colombo were investigated. In each case, the serum of the patient was tested for its effect on the infectivity of the

patient's parasite isolate in membrane feeding experiments, and compared with the intrinsic infectivity of that isolate estimated by membrane feeding in normal human serum. In 40 out of 50 patients the serum completely suppressed the infectivity of the parasite. Protein A - purified immunoglobulins had similar effects. Sera of all 50 patients reacted with the surface of gametes by IFT and the titres of anti-gamete antibodies correlated with the transmission blocking capacity of the sera. Sera with titers of 1/320 and beyond almost completely blocked transmission of parasite isolates. Monthly follow up of patients after drug cure revealed that blocking antibodies remained in the serum for about 4 months after treatment.

Results obtained with membrane feeding were confirmed by experiments in which laboratory-bred mosquitoes were fed directly on 25 acute vivax malaria patients. The transmission blocking activity of the sera correlated with the patients' past experience of malaria (Table 2). Patients in whom the infection was the primary attack (Group I), and those who had developed malaria previously but in whom the last infection occurred beyond 4 months previously (GroupII), had low levels of transmission blocking immunity and were highly infectious to mosquitoes. Patients who had experienced malaria previously, but in whom the previous infection occurred within the past 4 months (Group III) had high levels of transmission blocking immunity and were hardly infectious to mosquitoes. These findings indicate that naturally induced transmission blocking immunity is boosted by subsequent malaria infections, and that this boosting effect is seen only if an infection occurs within about 4 months of the previous attack.

TABLE 2
EFFECT OF ANTI-GAMETE ANTIBODIES
ON THE INFECTIVITY OF PATIENTS TO MOSQUITOES

	Infectivity Oocysts/gut (Nb infected/total)		%Tx (Percentage transmission blockage)
	Membrane feeding in Nl.Human Serum	Direct feeding on patients	
Group I	42 (52/109)	48 (70/107)	34%
Group II	41 (69/99)	29 (61/90)	39%
Group III	40 (80/123)	10 (40/157)	84%

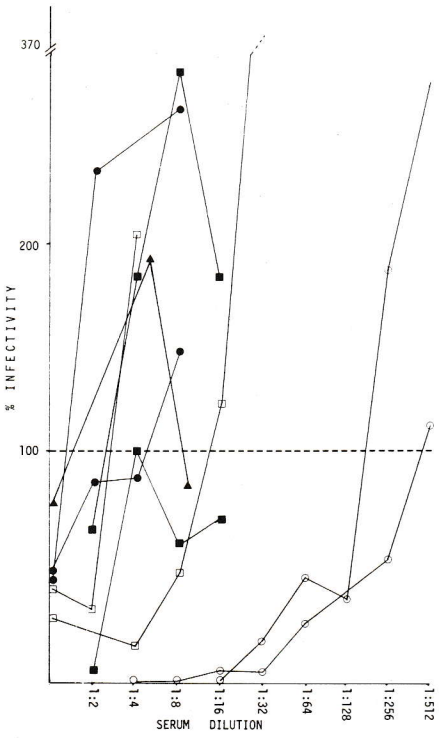

FIGURE 3. Effect of dilution of 7 sera on infectivity.

Transmission enhancing effects. Seven transmission blocking human immune sera were tested for their effects on infectivity at increasing dilutions (All dilutions were performed in normal human serum). In 6 cases, transmission blocking decreased with dilution of serum and activity switched to an enhancing effect on transmission. Up to a 4 fold increase of infectivity was observed (Fig 3). Enhancing effects (of upto 13-fold) were also seen with certain undiluted sera which had low anti gamete IFT titers.

Four patients were followed up after a P.vivax infection; serum was taken at various times after infection and membrane feeding experiments were performed in neat serum. Upto a 12-fold increase in transmission could be observed with serum taken several weeks after infection (Figure 4).

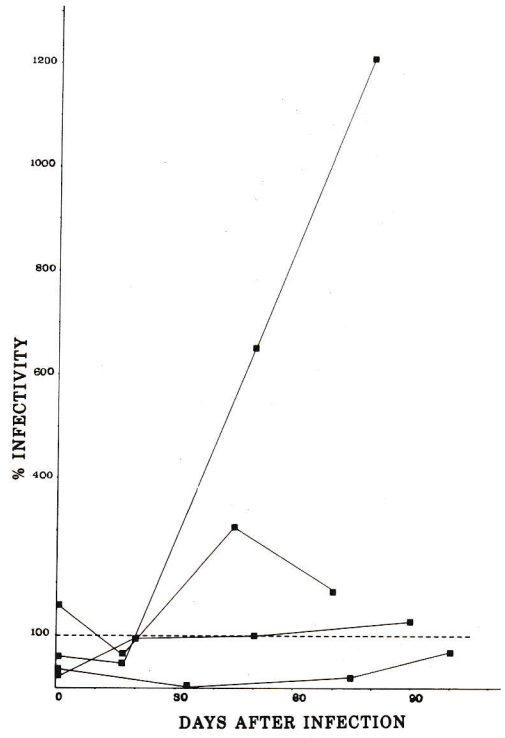

FIGURE 4. Effect of serum on infectivity during follow-up after curative treatment.

DISCUSSION

We have previously demonstrated that transmission of P.vivax to the mosquito can be blocked by antibodies directed against the surface of female gametes (7). In the present study, using monoclonal antibodies that block transmission, we demonstrate that two gamete-surface polypeptides of molecular weights 36 and 42 kDa constitute at least one set of target antigens involved in this immune reaction.

Immunity to the sexual stages of the parasite has not been previously demonstrated in human (12) or simian (13) malaria infections. Acquired immunity to malaria was thought to be directed mainly against the asexual blood stages and to some extent against sporozoites; it was thought to not

have any direct effect on the sexual stages of the parasite which transmit the infection from man to the mosquito. Our study demonstrates that P.vivax malaria infections in man readily induce an effective antibody-mediated transmission blocking immunity. Immunity is boosted by subsequent malaria infections, but only if an infection occurs within 4 months of the previous one; this indicates that the immune response is characterized by a relatively short memory.

It is clear from this study that antibodies directed against gametes can not only block but also enhance transmission. Transmission enhancing properties are found at sub-neutralizing concentrations of human immune sera and of monoclonal antibodies, both of which neutralize gametes and block transmission at high concentrations. After drug cure, decreasing activity in patients' sera can give way to enhancement of transmission. Enhancing activity is also present in sera from certain patients during the acute phase of P.vivax infections. Results obtained with purified immunoglobulin demonstrate that it is indeed antibody that mediates the modulation of transmission. The fact that a single monoclonal antibody can both block and enhance transmission indicates that the same gamete component(s) may be involved in both phenomena.

We have demonstrated that naturally acquired immunity can have a major influence on parasite transmission either by suppressing or enhancing infectivity. The extent to which these antibody-mediated effects influence malaria transmission in nature and the impact of a vaccine aimed at inducing such immunity needs careful evaluation.

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