MULTIPLE STRAINS OF *PLASMODIUM FALCIPARUM* ARE NECESSARY FOR THE GROWTH INHIBITION ASSAY

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INTRODUCTION

The growth inhibition test has been suggested as a method of assessing protective antibody against malaria (Cohen *et al.*, 1969). Antibodies against homologous strains of *Plasmodium falciparum* have higher inhibitory activity than those against the heterologous strains (Wilson and Phillips, 1976). Adult Gambian sera tested against 4 isolates of *P. falciparum* from infected children showed varying degree of inhibition (Wilson and Phillips, 1976) suggesting that for appropriate assessment of this test several strains of *P. falciparum* should be used in the test. In the present study, 3 strains of *P. falciparum* were used in the test against sera obtained from people living in a malaria endemic area in order to assess whether the use of multiple strains was necessary, and in addition, in a situation where there were only limited facilities permitting only one strain to be tested, what strain should be chosen as to provide the best information on the protective immune status.

MATERIALS AND METHODS

The three strains of *P. falciparum* chosen for the study were SO, G-112 and SN strains. The history and the isoenzyme pattern of the SO and G-112 strains have been reported previously (Tharavanij *et al.*, 1982). The SN strain was isolated on February 26, 1981 from a patient who contracted malaria from Chantaburi, Eastern Thailand. The patient admitted to the Hospital for Tropical Diseases, Bangkok had recrudescence 16 days after treatment with 1 gm of mefloquine, and subsequent treatment with 1250 mg of sulfadoxine pyrimethamine combination showed R III resistance.

Venous blood samples were obtained during March through April 1979, a non-transmission season, from 67 individuals living at Khao Kaeng Riang Village, Kanchanaburi Province, 190 Km west of Bangkok. The slide positive rate at the time of taking blood was 2%. These people were given drug treatment for malaria comprising sulfadoxine/pyrimethamine and primaquine after the blood had been taken. The sera were kept at −70°C until used. Sera from 60 blood donors who were Bangkok residents without past histories of malaria were used as the controls.

The growth inhibition assay used in this study was a modification from that originally described by Cohen *et al.*, (1969). The parasites were grown in group "O" human red blood cells for 3 days in isoleucine-free RPMI-1640 plus 10% AB serum from blood donors and gentamicin, (Lambros *et al.*, 1979), and placed in a candle jar for 30 hours so that most of the parasites developed to the late trophozoite stage. A smear was then made, stained with giemsa and the number of parasites counted. The cells were harvested, centrifuged, the supernatant removed and fresh medium added to give a haematocrit of

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8%. Further dilution was made with an 8% fresh red blood cell suspension so that the parasitaemia was 1%. Two hundred µl of the infected cell suspension was added to each well of a Falcon 3008 tissue culture plate. One µCi of ^{3}H-isoleucine (ICN, California) in 10 µl of RPMI-1640 was then added to each well followed by 90 µl of appropriately diluted serum in isoleucine-free RPMI-1640 plus 10% AB serum. The IgG content of serum had been previously determined by the technique of Mancini *et al.*, (1965). The final IgG content was 2 mg/ml and the haematocrit of the red blood cell suspension was 5.3%. Cultures were incubated in a candle jar at 37°C for 24 hours. Thereafter the cells were harvested on a glass fiber filter and washed with physiological saline using a multiple automated sample harvester (MASH-II). The filters were immersed in ice cold 10% trichloracetic acid for 30 minutes followed by brief dipping in absolute methanol and diethyl ether and dried under an infrared lamp. Each of the filter discs were detached from the whole filter strip and dropped into a counting vial containing a liquid scintillation cocktail, and then counted in a Packard liquid scintillation counter. The result was expressed as percent inhibition calculated according to the formula :-

$$\text{Percent inhibition} = 100 - \left(\frac{\text{CPM in the presence of tested serum}}{\text{CPM in the absence of tested serum}} \times 100\right)$$

The CPM of uninfected cells from the control wells were less than 300.

RESULTS

Result of the percent inhibition of sera from people in the endemic area and sera from blood donors is shown in Fig 1. The percentage of blood donors with sera showing $\geq$ 30 percent inhibition against strains SO, SN and G-112 were 3, 0 and 1.7 respectively. This level of inhibition was arbitrarily chosen to differentiate between positive and negative growth inhibition activity. Based on this criterion, 32 of 67 (47.8%) sera from people in the endemic area were positive when all 3 strains of *P. falciparum* were used in the test (Table 1). Amongst these 32 positive sera, 23 (71.9%) were directed against SO strain, whereas only 9 (28.1%) and 10 (31.3%) sera were directed against SN and G-112 strains respectively. When 2 strains of plasmodia were tested simultaneously the percent positivity against SO and SN, and SO and G-112 combination were 90.6 and 87.5 respectively whereas the percent positivity against SN and G-112 combination was only 50. Blood smears from microwells containing sera with high or low inhibitory activities showed that the parasites at the time of cell harvest were mostly in the ring stages with extremely few schizonts or trophozoites.

Table 1

Growth inhibition assay against single and multiple strains of *P. falciparum*.

Strains of *P. falciparum* to which positive sera react	Number of sera with growth inhibition activity (% total positive)	% positive of total sera tested
SO	16 (50)	23.9
SN	4 (12.5)	6.0
G-112	3 (9.4)	4.5
SO+SN	2 (6.3)	3.0
SO+G-112	4 (12.5)	6.0
SN+G-112	2 (6.3)	3.0
SO+SN G-112	1 (3.1)	1.5
Total	32 (100)	47.8

DISCUSSION

In the present study, it was clearly shown that sera from people living in a malaria endemic area could inhibit *in vitro* incorpo-

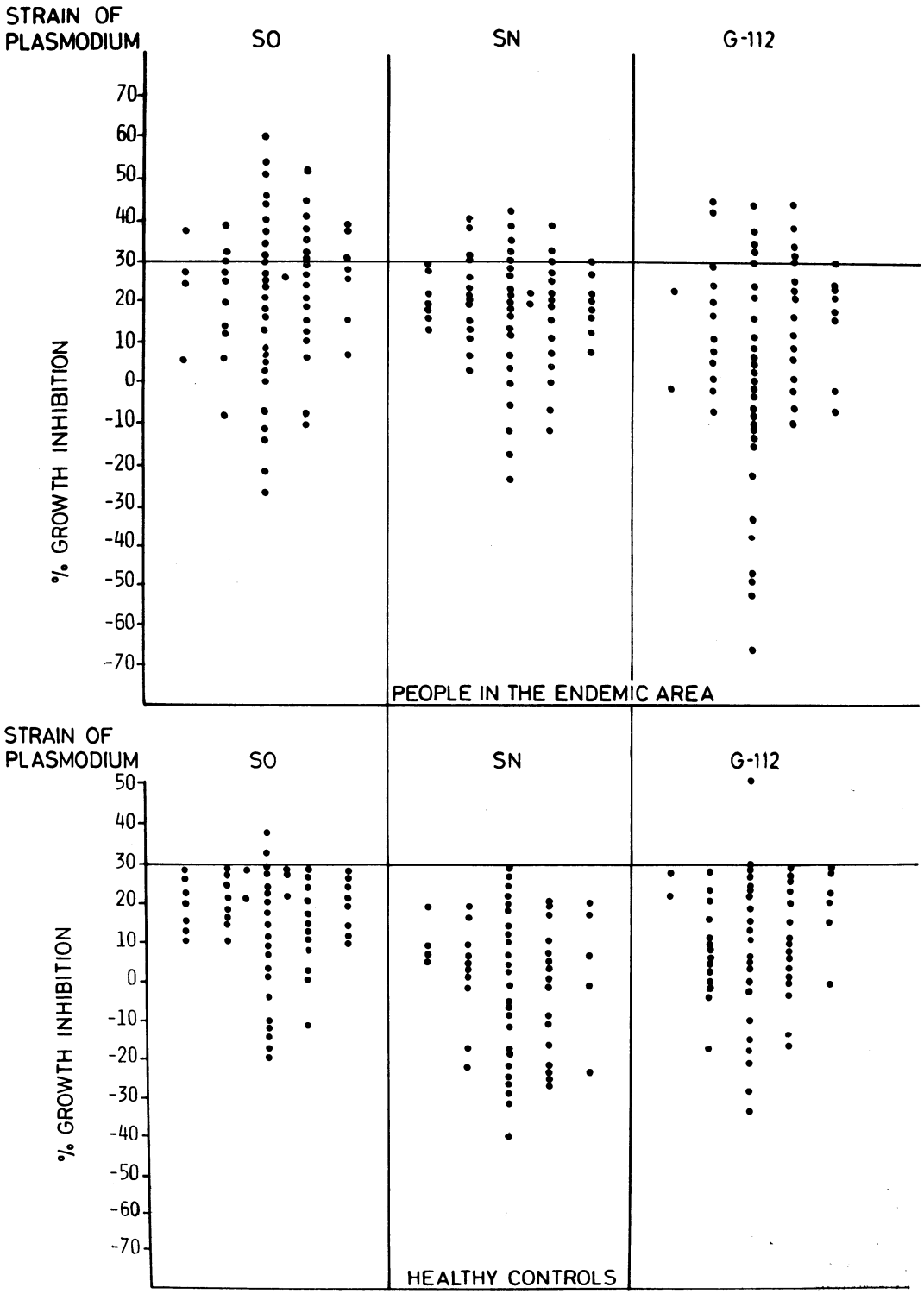

Fig. 1—Growth inhibition assay of sera from people at Khao Khaeng Riang, a malaria endemic area and sera from Bangkok healthy blood donors tested against strains SO, SN and G-112 of *P. falciparum*. Percent inhibition of $\geq$ 30 was considered positive.

ration of tritiated isoleucine by malarial parasites. This apparent inhibition was mostly due to antibody and not the anti-malarial drugs, since serum samples were collected from these people before drug administration was carried out for the control of malaria in that village. The stage of malarial parasite affected by the serum factor is not known. It is most likely that these sera exerted their inhibitory effect at the time when merozoites were released and reinfected red blood cells in the culture similar to the situation documented in *P. knowlesi* (Cohen *et al.*, 1969) and *P. falciparum* (Mitchell *et al.*, 1976). Our findings that at the time of cell harvest the parasites were mostly at ring stage indicated that the inhibitory effect was directed against reinvasion of merozoites.

The parasite strains used in this study were maintained in culture for a period of longer than 6 months before testing during which the loss of the antigens in some isolates (Langreth *et al.*, 1979) cannot be excluded. Yet it was constantly observed at least in the SO strain, that trophozoites and schizonts could be recovered substantially after physiogel floatation, and since ability to float in physiogel has been reported to be associated with the knobs (Reese *et al.*, 1979) it could be interpreted to mean that the SO strain is knobby and this characteristics could be retained for at least 6 months.

It was observed that in the presence of some sera, enhancement of incorporation of the tritiated isoleucine by the parasites occurred. The factor(s) responsible for this enhancement is not clear. Such sera might provide additional nutrients to promote better growth of the parasites. Alternatively these sera might contain antibody which enhances parasite growth especially when they were tested against the heterologous strain. Growth enhancement by antisera had been observed when some monoclonal antibodies against the Papua-New Guinea strains were tested against strains from Thailand and the Netherlands (Schofield *et al.*, 1982).

Our results clearly show that only when all 3 strains were used that the highest percentage of growth inhibition was obtained. Most interesting was the finding that the use of the SO strain alone could detect as many as 71.9% of positive sera, and additional testing on either the SN strain or the G-112 strain brought the level of sensitivity up to 90.6% and 87.5% respectively. Our results indicate that for an optimum rate of detection of growth inhibition positive sera in Thailand at least 2 falciparum strains should be used one of which should be the SO strain. This recommendation is in accord with the finding of Wilson and Phillips (1976) who showed that 10 of 11 adult Gambian sera exerted more than 20% inhibition against isolate 2 but only 3, zero and 6 of these 11 sera were inhibitory for isolates 1, 3 and 4 respectively. In terms of suitability for use in the growth inhibition assay the SO strain used in our study would be comparable to the isolate 2 used by Wilson and Phillips (1976).

The overall percent positivity in our study (47.9%) was lower than that (61%) reported from Gambia (Wilson and Phillips, 1976). This difference would be attributable to the different degree of malaria endemicity, being lower in our study area than that in the Gambia.

SUMMARY

Sixty-seven serum samples from individuals living in a malaria endemic area and sixty sera from healthy blood donors living in Bangkok (non-malarious area) were tested for growth inhibition activity against 3 strains (SO, SN and G-112) of *Plasmodium falciparum*. Forty-eight percent of the sera from the endemic area were positive when all 3 strains were tested. Among the positive

sera, positive rates of 90.6% were observed for the SO and SN strain combination, 87.5% for the SO and G-112 combination, and 50% for the SN and G-112 combination. It is therefore recommended that multiple parasite strains should be tested in the growth inhibition assay. If facilities are limited, a minimum of two strains should be used, one of which is the SO strain or its equivalent.

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