ABSTRACT

P. falciparum was cultivated *in vitro* using the Trager-Jensen's technique. The parasites were grown in RPMI-1640 medium supplemented with 25 mM HEPES buffer and 10% serum under low oxygen tension (17%). Under this cultivation technique the parasites could be grown to high parasitemia of 10%.

The effect of innate resistance and humoral factors on growth of *P. falciparum* was studied using the *in vitro* cultures. For studies on innate resistance, HbE - containing red cells were used since previous epidemiological data had indicated a protective role of HbE against falciparum malaria infection. Using four isolates of *P. falciparum* from different geographical regions (FCR-1 from Vietnam, FCM-1 from Chonburi, K-1 from Kanchanaburi and G-112 from Gambia), no differences in both invasion rate and intracellular development of *P. falciparum* in normal red cells (AA) (12 cases), heterozygous AE (7 cases) and homozygous EE (15 cases) were observed. In addition, there were also no differences in the abilities of AA, AE and EE cells to support parasite growth under low oxygen tension (3% O_2) as well as high oxygen tension (30% O_2). Therefore HbE-containing red cells did not inhibit development of *P. falciparum* under the cultivation technique. Thus any protective effect of HbE-containing red cells must occur within infected individuals.

Studies on the effect of humoral factors on growth of *P. falciparum* involved identification of sera that inhibited growth of malaria parasites as well as identification of antigens characterized by IgG which were able to inhibit parasite invasion.

Merozoite invasion inhibition test was used to identify immune sera. Forty-three sera collected from patients or people living in endemic areas in Kanchanaburi, Saraburi and Chantaburi were added to the culture medium and degree of parasite growth inhibition was determined. Sera which gave more than 50% inhibition were considered as positive or immune sera. It was noted that higher positive merozoite invasion inhibition was obtained with sera collected during the dry season of the year (October, November and January) in which malaria transmission was low. Results with three isolates of parasites (K-1 from Kanchanaburi, CC from Prachinburi and G-112 from Gambia) tested demonstrated that 50% (21/43) of sera collected during dry season were positive while only 13% (4/32) of those collected during rainy season (June and July) were inhibitory. There seemed to be some correlation between immune status and the presence of gametocytes in blood from which sera were obtained. Results with all isolates of parasites tested showed that as much as 60% (6/10) of sera obtained from blood with gametocytes could inhibit merozoite invasion while only 20% (5/24) of sera from ring-containing blood and 31% (14/45) of sera from blood with no apparent parasites were inhibitory. In addition, some correlation between parasites and positive sera in relation to geographical origin were observed since 15, 25 and 75% of sera obtained from Kanchanaburi gave positive merozoite invasion inhibition when tested against an isolate from Prachinburi (CC), a Gambian isolate (G-112), and an isolate from Kanchanaburi (K-1), respectively.

Upon metabolic labelling with ^{35}S-methionine cultures of *P. falciparum* from different origins (K-1, CC and G-112) showed more than 30 protein bands (size ranging from 16-155 kd) on SDS-polyacrylamide gel electrophoresis which were similar in all isolates. IgG from inhibitory sera specifically precipitated parasite proteins of 85 and 200 kd indicating their possible role in protective immunity. Antigen of 85 kd was synthesized throughout the developmental cycle whereas that of 200 kd was schizont-specific. These two antigens were not located on the infected red cell membrane since their presence could not be observed when gelatin-or Percoll-concentrated infected red cells were surface labelled with Iodogen and ^{125}I and, furthermore, no surface-labelled proteins were specifically precipateted by inhibitory IgG.

BIOGRAPHY

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