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PREVALENCE OF ANTI-P. FALCIPARUM SPOROZOITE ANTIBODIES IN ADULTS IN THE AMAPA REGION OF BRAZIL

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SUMMARY

17 of 20 adult sera from the Amapa region of Brazil were active in the inhibition of **P. falciparum** sporozoite invasion (ISI) assay which has been correlated with protective antibodies. In contrast 11 sera were positive in IFA tests and 6 were positive in CSP tests. These results suggest that the ISI assay will be useful for evaluating naturally acquired protective anti-sporozoite antibodies in endemic areas, particularly during vaccine efficacy studies using sporozoite-based vaccines.

KEY-WORDS: Anti-sporozoite antibody; inhibiton of sporozoite invasion (ISI) assay; circumsporozoite precipitation (CSP) assay; immunofluorescent antibody (IFA) assay.

INTRODUCTION

Candidate anti-malarial vaccines containing the **Plasmodium falciparum** CS protein repeat epitope region elicit antibodies in mice and rabbits that recognize intact sporozoites by the circumsporozoite precipitin (CSP) or immunofluorescent antibody (IFA) assay [1-10-11]. Such antibodies are likely to confer protection since they also block **in vitro** sporozoite invasion of cultured human hepatoma (HepG2-A16) cells or primary human hepatocytes [1-10-11]. This inhibition of sporozoite invasion (ISI assay has been correlated with protective antibodies [5], since sera from human voluteers immunized with irradiated sporozoites and immune to viable sporozoite challenge [2], also blocked invasion [5]. While the natural acquisition of anti-sporozoite antibodies has been demonstrated by immunofluorescent antibody (IFA) assays in the Gambia [6], only six adult sera were tested and only two were shown to possess protective antibodies using the ISI assay [5]. In anticipation of likely endemic area field trials we have examined sera from the Amapa region of Brazil for ISI activity and IFA with **P. falciparum** sporozoites, and compared these results with other serological assays of anti-sporozoite and anti-red blood cell stage.

MATERIALS AND METHODS

Sera: Sera were collected between the 2nd-4th July 1985 in Macapa, state of Amapa, Brazil from 20 male adults whose ages varied between 19 and 55 yrs old. Subjects [11,12,14,18] and 38 were under unspecified drug treatment when sample of blood for serum collection was obtained (Table 1).

Parasites: Anopheles stephensis mosquitoes were infected with **Plasmodium falciparum** sporozoites by feeding on **in vitro** cultures of **P. falciparum** NF54 gametocytes [7].

Inhibition of sporozoite invasion (ISI) assay: The technique of HOLLINGDALE et al. was used [5]. Human hepatoma (HepG2-A16) cells were grown on 1cm2 glass coverslips in MEM (Earle's) medium with 10% feta bovine serum, 50 U/ml penicillin and 50 µg/ml streptomycin

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TABLE I
Age, number of days with fever and medication of subjects studied

Serum No.	Age	Fever (days)	Medication
1	55	4	None
3	41	8	None
4	34	11	None
5	27	3	None
6	24	3	None
7	30	8	None
8	30	5	None
9	22	3	None
10	20	3	None
11	29	8	Yes
12	19	90	Yes
13	23	2	None
14	45	4	Yes
15	—	—	None
16	30	2	None
18	30	8	Yes
22	26	3	None
26	23	4	None
37	—	—	None
38	—	—	Yes

until confluent. Each human serum was diluted to 1:20 in culture medium and added to triplicate cultures. **P. falciparum** sporozoites were isolated from mosquito salivary glands and diluted in culture medium such that each culture received 30,000 sporozoites After incubation for 3hr, cultures were rinsed in phosphate buffered saline (PBS), fixed with methanol, and washed with PBS. Invaded sporozoites were visualized by an immunoperoxidase antibody (IPA) assay [4] using a monoclonal antibody directed to **P. falciparum** sporozoites. The percent inhibition was $1 - \frac{(I_c - I_t)}{I_c} \times 100$ where I_c = the number of sporozoites that invaded in the presence of control serum and I_t is the number of sporozoites that invaded in the presence of test serum. A positive ISI was arbitrarily chosen as 75% or greater [3].

Circumsporozoite precipitation (CSP) assay: This was performed as described by VANDERBERG et al. [9] 10µl containing 200 **P. falciparum** sporoozites suspended in culture medium were incubated with an equal volume of each undiluted serum sample at 37C for 60 minutes. Twenty-five randomly chosen sporozoites were evaluated by phase microscopy at 400 x magnification and graded as positive (distinct presence of granular precipitate on surface of sporozoite with or without visible trail) and negative (smooth sporozoites present).

Indirect fluorescent antibody test (IFA): Microscope slides containing 20,00 **P. falciparum** sporozoites were made and air dried. Slides were reacted with sera diluted 1:200, followed by fluorescein conjugated goat-anti-human antibodies.

In all assays sera samples were not inactivated prior to use.

RESULTS

The results of testing each human serum in ISI, CSP and IFA anti-**P. falciparum**-sporozoite assays, are shown in Table 2. ISI activities ranged from 2% to 96%, and 17 of the 20 sera showed reactivity of 75% or greater. In CSP tests, 6 of the sera were positive, and in IFA tests 11 of the sera were positive.

TABLE II
Serological activities of sera Amapa in ISI, CSP and IFA and anti-blood stage assays

Serum No.	% ISI	CSP	IFA
1	9[a]	—	—
3	95	—	+
4	80	+	+
5	96	—	—
6	94	—	+
7	78	+	+
8	78	—	—
9	75	+	+
10	80	+	+
11*	93	—	—
12*	87	—	—
13	44	—	—
14*	83	—	—
15	2	—	+
16	91	—	+
18*	87	+	+
22	86	—	—
26	93	+	+
37	78	—	+
38*	81	—	—
Control	0	—	—

[a]Inhibition of sporozoite invasion expressed as per cent of control sera.
*Subjects under unspecified treatment at time of serum collection.

A comparison of anti-sporozoite activity by ISI, CSP or IFA is shown in Table 3. A total of 17 of 20 sera had anti-sporozoite antibodies detectable in one or more assay. Only 6 sera were positive in ISI, CSP and IFA tests.

TABLE III
Comparison of ISI, CSP and IFA Assays

Serum No.	ISI	CSP	IFA
4, 7, 9, 10, 18*, 26	+[a]	+	+
5, 8, 11*, 12*, 14*, 22, 38*	+	—	—
3, 6, 16, 17	+	—	+
15	—	—	+
1, 13	—	—	—

[a]Per cent inhibition of 75% or greater was scored as +
*subjects under unspecified treatment at time of serum collection

However, 7 sera positive in ISI had no detectable CSP or IFA activity and an additional 4 sera positive for ISI and IFA lacked CSP activity. It appears that ISI is the most sensitive, and CSP is the least sensitive assay for detecting anti-sporozoite activity. Generally, sera positive by IFA are also positive by ISI and less frequently by CSP, but only about 50% of sera positive by ISI were positive by IFA. Only 2 sera of the 20 sera tested lacked anti-sporozoite activity by all assays.

DISCUSSION

These results show that anti-**P. falciparum** sporozoite antibodies were detectable in 17 of 20 sera (85%) from adults in the Amapa region. The ISI assay was more sensitive than either IFA or CSP in detecting anti sporozoite antibodies. Whereas most sera positive in IFA tests were also positive by ISI, only about 50% of ISI positive sera reacted in IFA assays. It is likely that use of an ELISA assay may be more sensitive than IFA in detecting such antibodies. In a study in Flores, Indonesia an ELISA assay detected antibodies in 86% of adult sera examined [3], although ISI activity occured less frequently than in Amapa. The IFA or ELISA assays measure binding of antibodies to intact sporozoites, or selected sporozoite antigens. The ISI assay is a functional assay mediated by reaction of antibody with the CS repeat epitope region, and is thought to be predictive of protection [5]. Further studies will be required to define the relationship between IFA or ELISA with the ISI assay. There was no correlation between ISI activity and anti-blood stage activity (data not included). Thus, it is apparent that, in this region, antibody to the **P. falciparum** CS repeat epitope region may be a significant immunological factor in the prevalence of malaria.

Some subjects [11,12,14,18,38] mentioned unspecified drug treatment at the time of collection of serum samples. Therapuetic levels of chloroquine or primaquine have been shown to have no effect on **in vitro** sporozoite invasion of HepG2-A16 [8], although such activity of other drugs has not been evaluated.

Selection of populations from endemic areas for studying vaccine efficacy will require a detailed analysis of the factors affecting the immunological responses to **P. falciparum** sporozoites. It is likely that longitudinal studies will be conducted to measure both malaria transmission rates and the prevalence of antibodies to **P. falciparum** sporozoites. The sensitivity of the ISI assay suggests its role in these studies, at least to set a standard for comparison with other assays such as IFA and ELISA assays.

RESUMO

Prevalência de anticorpos anti-esporozoitos de P. falciparum em soros da região do Amapá, Brasil

17 de 20 soros humanos coletados na região de Amapá (Brasil) mostraram atividade nos testes de inibição de invasão de esporozoitos (ISI) in vitro que estão correlacionados com proteção por presença de anticorpos. Em contraste 11 soros foram positivos para IFA e 6 foram positivos para CSP. Estes resultados sugerem que ISI seja um ensaio mais adequado para medir respostas imunológicas em futuros estudos sobre eficácia de uma potencial vacina anti-malárica.

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