DETECTION OF HUMAN ANTIBODIES AGAINST *PLASMODIUM FALCIPARUM* ANTIGENS IN BLOOD MEALS OF ANOPHELINE MOSQUITOES

CARMEN E. CONTRERAS AND JOHN C. BEIER

Department of Immunology and Infectious Diseases, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, MD 21205

ABSTRACT. Human IgG antibodies against *Plasmodium falciparum* asexual stages, gametocytes and sporozoites were detected by indirect fluorescent antibody (IFA) techniques in the blood meals of *Anopheles gambiae* s.l. from a malaria-endemic area of western Kenya. Field-collected mosquitoes, which had been stored dry for over 2 years, were screened first for human IgG by ELISA. In 141 blood meal samples from human-fed mosquitoes, the prevalence of stage-specific antibodies was 87.9% for asexual-stage parasites, 78.0% for gametocytes, and 87.9% for sporozoites. There were no differences in the prevalence of stage-specific antibodies for mosquitoes collected from 2 sites, before and after the long rainy season of 1988. The detection of specific human antibodies in mosquito blood meals by IFA, or by more efficient methods, may provide alternative approaches for large-scale, epidemiologic studies of malaria and other vector-borne diseases.

INTRODUCTION

Immunological tests for detecting antibodies against specific stages of malaria parasites are used to investigate various aspects of malaria epidemiology (Molineaux et al. 1988). Surveys to detect antibodies to asexual blood stages, an indicator of past infection, have been useful for mapping the large-scale distribution of malaria transmission (Sulzer et al. 1971). The detection of anti-sporozoite antibodies provides information on the intensity of recent exposure to transmission by vector populations (Nardin et al. 1979). In addition to the logistical difficulties in obtaining representative serum samples for analysis, there are also ethical issues associated with drawing blood from individuals who may not benefit directly from such studies.

New strategies and tools are needed to improve capabilities for malaria surveillance and for investigating malaria epidemiology (Institute of Medicine 1991). A possible alternative approach to serosurveys involves the detection of malaria antibodies in the blood meals of human-fed anopheline mosquitoes. Host antibodies remain detectable in blood meals for at least 24 h after feeding (Service et al. 1986, Beier et al. 1988). Even though each blood meal contains only 1–3 μl of blood, antibodies against specific stages of malaria parasites can be detected by simple immunoassays (Beier et al. 1989).

This study examines the potential for detecting human antibodies to *Plasmodium falciparum* asexual stages, gametocytes and sporozoites in dried blood meals of human-fed *Anopheles gambiae s.l.* Giles collected from 2 sites in western Kenya.

MATERIALS AND METHODS

Indirect fluorescent antibody (IFA) test: Methods to detect human antibodies against *P. falciparum* antigens in the blood meals of anopheline mosquitoes were simply modifications of standard procedures for detecting malaria antibodies (Contreras et al. 1988). Whole parasites used as antigens included mature schizonts, gametocytes and sporozoites. Schizonts were obtained from *in vitro* cultures with parasitemias of 6–8%. Stage IV and V gametocytes were from *in vitro* cultures with gametocytemias of 1–3%. Sporozoites were obtained by dissecting salivary glands of experimentally infected *An. gambiae* mosquitoes on days 14–16 post-infection. Five microliter aliquots of culture material containing either asexual stages or gametocytes, or Medium 199 (Gibco Laboratories, Grand Island, NY) containing 2,000 counted sporozoites were dispensed to 5 mm diam spots on standard 10-spot microslides. Slides were air-dried and then frozen at −70°C with desiccant.

Immediately before testing, slides were removed from the freezer and thawed at room temperature. Slides containing asexual stages and gametocytes were dehemoglobinized with 0.3 N HCl and slides with sporozoites were fixed with methanol. The procedure, which was the same for all 3 antigens, involved first adding to each spot a 5 μl aliquot of a 1:50 dilution of mosquito blood meal in phosphate buffered saline (pH 7.4). After incubating for 30 min at room temperature in a moist chamber, each spot was washed twice with PBS, and then incubated for another 30 min with 5 μl of a 1:10 dilution of FITC anti-human IgG (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD). After washing 3 times with PBS, a cover-slip mounted

with glycerol buffer was added. Slides were examined at 400× using fluorescent microscopy. The degree of fluorescence observed for each sample, for all parasite antigens, was evaluated against reactions for 1:20 to 1:80 dilutions of control and immune sera.

Control tests with experimentally fed mosquitoes: Two groups of 3–5 day old *Anopheles gambiae* (G-3 strain) mosquitoes were fed by membrane feeding on 1:1 mixtures of washed human erythrocytes and human sera. One group was fed pooled sera from 3 individuals with no prior exposure to malaria and the other group was fed pooled sera from 3 life-long residents of a malaria-endemic area of western Kenya. Prior to mosquito feeding, IFA tests confirmed that the sera from residents of Kenya reacted with all 3 *P. falciparum* antigens while the pooled control sera was negative. Mosquitoes were held after feeding in an insectary at 27°C. At 4 and 24 h after feeding, 20 immune-fed and 20 control-fed mosquitoes were killed by freezing. Half of these mosquitoes were frozen at −70°C and half were stored dry in a desiccator. After one week, mosquitoes were prepared individually for IFA testing by trituration in 1.5 ml microtubes containing 50 μl PBS. Samples were centrifuged at 16,000 *g* for 5 min and the supernatants were stored at −20°C. The 80 mosquito samples were tested by IFA against each of the 3 antigens as described above.

Testing of blood meals from field-collected anopheline mosquitoes: Anopheline mosquitoes were collected from February to July 1988 during malaria field studies at Kisian and Saradidi, two sites in western Kenya which are endemic for *P. falciparum*, *P. malariae* and *P. ovale* (Beier et al. 1990). Each week, over 50 houses per site were sampled for indoor resting mosquitoes by standard aspiration collections made by teams of 2 collectors. Mosquitoes from each house were identified, and stored dry at room temperature for over 2 years in 1.5 ml microtubes which were labeled according to species, site, house number and collection date.

Tests were performed on individual, blood fed *Anopheles gambiae s.l.*; *Anopheles funestus* Giles, collected at the same time, were not tested. The selection process involved choosing, at random, up to 5 vials (i.e., representing up to 5 houses) from Kisian and 5 vials from Saradidi for each of 8 wk before the rainy season (February and March 1988) and the same for after the rainy season (June and July 1988). For each of the vials, up to 3 blood-fed *An. gambiae s.l.* per vial were selected at random for testing.

Individual *An. gambiae s.l.* were triturated in 50 μl PBS, samples were centrifuged at 16,000 *g* for 5 min, and supernatants were stored at −20°C. Samples diluted to 1:50 with PBS were screened first for the presence of human or cow IgG using a direct ELISA for blood meal identification (Beier et al. 1988). Samples testing positive for human IgG were then tested by IFA against the 3 *P. falciparum* antigens as described above.

RESULTS

Blood meals from *An. gambiae* fed by membrane feeding on either control or immune sera were tested by IFA to detect human IgG antibodies against *P. falciparum* asexual stages, gametocytes and sporozoites. Positive reactions against all 3 antigens were detected for all mosquitoes fed on immune sera, killed after 4 h, and held either frozen or dry. For mosquitoes fed on immune sera and killed after 24 h, only asexual-stage antibodies were detected in 30 and 20% of the blood meals from mosquitoes stored frozen and dry, respectively. Antibody reactions were not detected for any of the mosquitoes fed on normal, control sera.

Blood meals from field-collected anophelines were first tested by ELISA to identify those mosquitoes that had fed on humans. Of 189 blood meal samples, human IgG was detected in 74.6% (141/189), cow IgG was present in 5.3% (10/189), and 20.1% (38/189) were not identified.

The 141 blood meals positive for human IgG by ELISA were then tested by IFA for *P. falciparum* stage-specific antibodies (Table 1). Positive reactions were detected in 87.9% of the samples tested against asexual stages, in 78.0% of the samples tested against gametocytes, and in 87.9% of those tested against sporozoites. Overall, there were no differences in the prevalence of antibodies in blood meals before (n = 57) or after (n = 84) the rainy season, for samples tested against asexual stages ($\chi^2 = 2.72$, df = 1, $P = 0.10$), gametocytes ($\chi^2 = 0.37$, df = 1, $P = 0.54$) and sporozoites ($\chi^2 = 0.21$, df = 1, $P = 0.65$) (data not shown). Similarly, there

Table 1. Human IgG antibodies against *Plasmodium falciparum* antigens detected by IFA in 141 blood meals of human-fed *Anopheles gambiae s.l.* collected from 2 sites in western Kenya.

Site	n	Percent positive by IFA		
		Asexual	Gametocytes	Sporozoites
Kisian	52	86.5	71.2	82.7
Saradidi	89	88.8	82.0	91.0
Total	141	87.9	78.0	87.9

were no differences between sites in the proportions of positive reactions against asexual stages ($\chi^2 = 0.15$, df = 1, $P = 0.70$), gametocytes ($\chi^2 = 2.26$, df = 1, $P = 0.13$) and sporozoites ($\chi^2 = 2.14$, df = 1, $P = 0.14$).

Of the 141 blood meal samples, 75.9% were positive for the 3 antigens compared with only 3.5% of the samples which were negative for the 3 antigens (Table 2). From 2.1 to 8.5% of the samples were positive for 4 of the remaining 6 possible antibody combinations but none of the samples contained antibodies that reacted with either gametocytes alone, or with sporozoites and gametocytes but not asexual stages.

DISCUSSION

The high prevalence of human antibodies to *P. falciparum* asexual stages (87.9%), gametocytes (78.0%) and sporozoites (87.9%) in blood meals of human-fed *An. gambiae s.l.* is consistent with the prevalence and intensity of malaria parasite transmission. In the Kisian and Saradidi sites in western Kenya, residents receive annually about 300 sporozoite inoculations from infective anophelines (Beier et al. 1990). In the Saradidi site, *P. falciparum* prevalence rates normally exceed 80% (Spencer et al. 1987), 95% of residents have antibodies to *P. falciparum* asexual stages (Spencer et al. 1987), and 96% of adults (Hoffman et al. 1987) and 60% of children (J. C. Beier, unpublished data) have circumsporozoite antibodies. Antibodies against gametocytes have not previously been examined.

Profiles of antibodies from blood meals, for all 3 plasmodial antigens, did not differ between the 2 sites or between 2 periods of transmission. This is not surprising because of the similarities in transmission between the sites; daily entomological inoculation rates exceeded 0.5 infective bites per person during both collection periods (Beier et al. 1990). Antibody levels, representing cumulative exposure to the plasmodial antigens, probably did not vary significantly over the short period of observation. The probability of detecting differences between sites or between periods of transmission could have been increased by testing more samples.

Table 2. *Plasmodium falciparum* stage-specific antibody combinations detected by IFA in 141 blood meals of human-fed *Anopheles gambiae s.l.* collected in western Kenya.

Human-fed mosquitoes		Antibody combination (IFA)		
n	%	Asexual	Gametocytes	Sporozoites
5	3.5	−	−	−
9	6.4	+	−	−
0	0.0	−	+	−
12	8.5	−	−	+
3	2.1	+	+	−
5	3.6	+	−	+
0	0.0	−	+	+
107	75.9	+	+	+

The detection of antibodies against 3 plasmodial antigens provided information on the potential sensitivity and specificity of the assays for testing blood meals. In this situation of high antibody levels in the human population, human IgG from all but 3.5% of the blood meals reacted with at least one antigen and most (75.9%) reacted with all 3 antigens. The antibodies reacted with whole parasites on IFA slides but the degree of antibody specificity was not determined. Reasonably, no samples reacted with only gametocytes, or with gametocytes and sporozoites but not asexual stages. Frequencies of antibody combinations may differ under conditions of epidemic or seasonal transmission. The immediate need is to determine how precisely antibodies in blood meals can be used to predict corresponding antibody prevalence in human populations.

Potentially, assays more efficient than the IFA could be used to detect stage-specific malaria antibodies in anopheline blood meals. For example, we have already used an ELISA to demonstrate circumsporozoite antibodies in the blood meals of 45% of over 1,500 anophelines from the same 2 sites (Beier et al. 1989). There is a need to develop further appropriate and sensitive methods for detecting antibodies in blood meals. There is also a good potential that a battery of tests could be used eventually to analyze individual blood meals simultaneously for antibodies, antimalarial drugs, malaria parasites, and even drug-resistant parasites, to address specific epidemiologic questions.

There is a growing concern that national efforts in the area of malaria surveillance must be re-oriented from the mass collection and screening of blood slides toward the collection and analysis of epidemiologically relevant information (Institute of Medicine 1991). In addition to large-scale surveillance for disease in humans and patterns of drug resistance, efforts need to focus on the intensity of transmission by vector populations to identify high-risk groups and to predict potential epidemics. Effective approaches will depend upon proper vector sampling and the efficient detection of sporozoites by immunological methods (Wirtz et al. 1987). In areas such as Africa where vectors are highly anthropophilic and endophilic, there is a tremendous but unexplored potential for developing new approaches for evaluating epidemiologic

situations by analyzing blood meals from anopheline mosquitoes. In the future, it may be feasible in some areas to evaluate epidemiologic conditions of malaria on a large-scale without even drawing blood from a single individual.

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