Antibodies to Pf155, a major antigen of *Plasmodium falciparum*: seroepidemiological studies in Haiti*

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The presence of malaria parasites and the serological antibody responses against whole *Plasmodium falciparum* and the Pf155 antigen were studied in the population of a small rural locality in Haiti in December 1985. Only 7% (1.5%) of the individuals were found to be infected with *P. falciparum*, the only species observed. Antibodies to *P. falciparum* were detected in an ELISA in 38.2% of the sera, the positivity rates being age-related. Anti-Pf155 antibodies were detected in 12.5% and 13.6% of individuals by two different techniques used. The anti-Pf155 positivity rates increased only after 25 years of age. No trends were detected for a clear-cut protective value of Pf155 antibodies against clinical malaria and further longitudinally conducted field surveys are needed to satisfactorily assess the potential protective effect of Pf155 antibodies.

In the context of limited resources, a sound knowledge of local epidemiological situations is necessary for the appropriate application of malaria control measures. A potential additional control measure, which might become available in the not too distant future, is immune intervention. To advance the progress towards development of a malaria vaccine and to help measure its potential impact, field studies are needed on the mechanisms involved in immune responses in persons exposed to malaria.

Malaria is a major public health problem in Haiti, as in many other developing countries. In December 1985 the extent of malaria endemicity and the immune experience of the population of a small locality in Haiti was measured. The studies concentrated on the immune responses of the population to Pf155, a *Plasmodium falciparum* antigen with a relative molecular mass (Mr) of 155,000, which is deposited in the membrane of ring-infected erythrocytes (1).

Pf155 is considered as a prime candidate for a vaccine against the asexual blood stages of *P. falciparum*, since antibodies against Pf155 have been reported to inhibit merozoite invasion (2) and to correlate with clinical immunity (3).

**SUBJECTS AND METHODS**

**Study area**

The studies were conducted in Laborde-1, about 24 km north-west of Les Cayes, the main city on the southern peninsula of Haiti. Situated at an elevation of 40 m, Laborde-1 is composed of 145 inhabited houses clustered on both sides of the road between Les Cayes and Jeremie. According to the records of the Service National des Endémies Majeures (SNEM) of Haiti, there is here a high incidence of *P. falciparum* malaria; perennial transmission occurs, with two peaks in June-August and November-January during the rainy periods.

Residual household insecticide spraying was conducted in Laborde-1 until 1983, but the principal control measure now consists of chloroquine distribution. The drug is prescribed mainly for presumptive treatment (single dose of 10 mg/kg body weight) of febrile cases by the primary health worker in the locality or by the local government outpatient clinic. Occasionally chloroquine has been given in mass drug administration; the last such administration in Laborde-1 was in November 1984.
Population investigated

Individuals were surveyed over a 7-day period in December 1985 during the reported transmission season. Information was obtained on the occupants in each house regarding their age, sex, geographic history, travel, fever or other illnesses and intake of antimalarial drugs. Axillary temperature was recorded and capillary blood specimens were obtained by fingerprick for examination of thick smears, determination of erythrocyte volume fraction (haematocrit), and serological studies. For the latter studies, the capillary blood collected in heparinized micro-haematocrit tubes was centrifuged; the tubes were then cut and the plasma/erythrocyte mixture collected (final volume varying from 30 to 200 µl) and stored at -20 °C for most of the remaining time.

Microscopic observations

Thick blood smears were stained with Giemsa and examined for parasites; smears where no parasites were seen in 100 oil-immersion fields (corresponding to 2000 leukocytes) were classified as negative.

Anti-P. falciparum enzyme-linked immunosorbent assay

The technique for the enzyme-linked immunosorbent assay (ELISA) described by Spencer et al. (4) was followed. The antigen preparation consisting of a whole parasite extract obtained by saponin lysis and sonication of in-vitro cultured P. falciparum (West African 1 strain) was dispensed in microtiter plates at a concentration of 1.5 µg protein per well. The wells were sequentially incubated with 200 µl of the following: (a) plasma diluted 1:100 in phosphate buffered saline (PBS), pH 7.2, containing 0.05% Tween 20 (30 minutes at 37 °C); (b) horseradish peroxidase (HPO)-conjugated rabbit IgG anti-human immunoglobulin G diluted at 1:2000 (same incubation conditions); and (c) o-phenylenediamine and 3% hydrogen peroxide as substrate (30 minutes at room temperature in the dark). The absorbance at λ = 492 nm was determined for each well on a multi-scan ELISA reader; samples with an absorbance of 0.3 or greater were considered as positive.

Anti-Pf155 assays

Monolayers of glutaraldehyde-fixed and air-dried P. falciparum-infected erythrocytes from cultures (clone B3 of strain Honduras-1/CDC) were used as antigen to detect the presence of anti-Pf155 antibodies. Two previously described techniques were used: modified immunofluorescence assay (MIFA) and cell-ELISA (CELISA) (1).

Modified indirect immunofluorescence assay.

Multitest microscope slides previously coated with infected erythrocytes (1-3% parasitaemia) were layered sequentially for 30 min with the following: (a) plasma diluted in Tris Hanks buffer (THB): 10 ml of 0.15 mol/lTris, pH 7.2, 90 ml of 0.9% NaCl, 100 ml of Hanks' balanced salt solution (1); (b) goat antibodies against human immunoglobulins labelled with biotin (30 µg/ml); and (c) fluorescein isothiocyanate-conjugated avidin (50 µg/ml). Parasite nuclei were stained by ethidium bromide (10 µg/ml). Plasmas were studied at 1:4 dilution; samples giving a positive reaction were titrated further with 4-fold stepwise dilutions.

Cell-ELISA. Wells of microtiter plates were coated with either uninfected or infected erythrocytes (7% parasitaemia), and unbound sites were then blocked by overnight incubation with 0.5% casein in PBS at room temperature. Plasmas were reacted in three wells (two wells coated with infected erythrocytes and one well with uninfected erythrocytes). Each plasma was tested at 1:5000 dilution in PBS containing 0.05% bovine serum albumin, conditions which had previously been determined as optimal (P. Deloron et al., unpublished data). Each well was filled sequentially with 100 µl of the following: (a) diluted plasma (two hours at room temperature); (b) HPO-conjugated goat IgGs against human immunoglobulins diluted 1:1000 in PBS (same conditions); and (c) o-phenylenediamine and 3% hydrogen peroxide as substrate (20 minutes at room temperature in the dark). The absorbance at 492 nm was determined as described. Samples were considered as positive when a difference in absorbance of at least 0.4 OD was observed between the mean value of the two wells containing infected erythrocytes and the value of the well containing uninfected erythrocytes. All positive reactions were confirmed by repeating the assay with negligible variations of the results.

Samples giving discordant results between MIFA and CELISA were further studied by repeating both assays and, in selected samples, by Western immunoblotting.

Western immunoblotting

Western blots were performed essentially as described by Tsang et al. (5). Plasmas were diluted 1:100 in PBS containing 0.3% Tween 20 and in-

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1 Immune II, Dynatech Laboratories Inc., Alexandria, VA, USA. Use of trade names is for identification only and does not imply endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

2 Miles Laboratories Inc., Elkhart, IN, USA.

3 TiterTech Multiscan from Flow Laboratories Inc., McLean, VA, USA.

4 Vector Laboratories Inc., Burlingame, CA, USA.

5 Cappell Laboratories, Cochranville, PA, USA.
cubated overnight at 4 °C with nitrocellulose strips containing electrophoretically transferred polypeptides from sodium dodecyl sulfate polyacrylamide gel (3.3–20% gradient) electrophoresis (SDS-PAGE) separation of a merozoite-enriched parasite preparation (I) (isolate Indochina-1/CDC). Bound antibodies were detected by incubation with HPO-conjugated goat antibodies to human IgG in PBS containing 0.3% Tween 20 (1 hour at room temperature), followed by staining with diaminobenzidine and hydrogen peroxide.

RESULTS

During the study, 136 houses were visited and 642 persons were interviewed, from whom 628 usable blood samples were obtained; 51% of the individuals were male and 49% were female. The age distribution of the population was as follows: <1 year of age (3.1%), 1–4 years (11.7%), 5–14 years (26.6%), 15–24 years (20.4%), and ≥25 years (38.2%).

Parasitological findings

A total of 618 thick blood smears were examined. Nine subjects (1.5%) were found to be infected with malaria parasites, *P. falciparum* being the only species identified. Two of the 9 infected subjects had only gametocytes in their peripheral blood and were afebrile (axillary temperature <37.5 °C). The 7 others were found to have asexual parasites; 5 were afebrile (one 13-year-old girl and 4 males, aged 3, 14, 16 and 21 years) and 2 were febrile (a 7-year-old girl and a 54-year-old man). Fever was noted in 18 of the non-parasitaemic individuals.

*Anti-*P. falciparum ELISA

Six hundred and five samples were available for testing. A positive reaction was obtained in 38.2% of the plasmas. The positivity rate was 5.9%, 16%, 29.8%, 34.2% and 55.6% in the age groups <1, 1–4, 5–14, 15–24 and ≥25 years, respectively; the difference between each group was significant (*P*<0.001) (Fig. 1). The mean OD titre increased with age from 0.15±0.04 in the youngest group to 0.38±0.11 in the oldest group.

*Anti*-PF155 assays

Six hundred and sixteen samples were available for MIFA studies. Antibodies against parasite antigens in the membrane of ring-infected erythrocytes were detected by immunofluorescence in 12.5% of the samples. The positivity rate was 5.9%, 4%, 5.5% and 9.8%, respectively, in the four younger age groups, and 21.4% in the oldest one. A significant difference was observed between the four younger groups and the oldest one (*P*<0.001) but not among the younger groups.

Six hundred and nine samples were available for CELISA studies. Antibodies were detected in 13.6% of the plasmas. The positivity rate was 5.9%, 9.3%, 7.9% and 8.2%, respectively, in the four younger age groups and 22.3% in the oldest one (*P*<0.001).

Although the anti-PF155 antibody positivity rates detected by MIFA and CELISA were similar within

![Antimalarial antibodies detected by whole-parasite ELISA (□) and anti-PF155 antibodies detected by MIFA (□) and by CELISA (□) in the population of Laborde-1.](image)

**Table 1.** Anti-PF155 antibodies in 609 subjects in Laborde-1, Haiti: correlation between the results of MIFA (reciprocal titres) and CELISA (OD/492nm). Performance as described in the text.

<table>
<thead>
<tr>
<th>MIFA titres</th>
<th>CELISA (OD/492nm)</th>
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<tr>
<td>&lt;0.4</td>
<td>&lt;0.4</td>
</tr>
<tr>
<td>0.4–0.5</td>
<td>0.4–0.5</td>
</tr>
<tr>
<td>0.5–0.7</td>
<td>0.5–0.7</td>
</tr>
<tr>
<td>&gt;0.7</td>
<td>&gt;0.7</td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>4–64</td>
<td>4–64</td>
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<tr>
<td>256–1024</td>
<td>256–1024</td>
</tr>
<tr>
<td>&gt;4096</td>
<td>&gt;4096</td>
</tr>
<tr>
<td>Total</td>
<td>Total</td>
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<tr>
<td>528</td>
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<td>47</td>
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<td>12</td>
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</tr>
<tr>
<td>609</td>
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Table 2. Anti-PF155 antibodies in 609 subjects in Laborde-1, Haiti: results of MIFA and CELISA by age group

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>No. examined</th>
<th>MIFA + CELISA−</th>
<th>MIFA − CELISA+</th>
<th>MIFA + CELISA+</th>
<th>MIFA − CELISA−</th>
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<tr>
<td>&lt;1</td>
<td>17</td>
<td>0</td>
<td>0</td>
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<td>1−4</td>
<td>75</td>
<td>2.7</td>
<td>8</td>
<td>1.3</td>
<td>98</td>
</tr>
<tr>
<td>5−14</td>
<td>163</td>
<td>3.1</td>
<td>5.6</td>
<td>2.5</td>
<td>99</td>
</tr>
<tr>
<td>15−24</td>
<td>121</td>
<td>9.1</td>
<td>6.6</td>
<td>0.8</td>
<td>83.5</td>
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<tr>
<td>≥25</td>
<td>238</td>
<td>7.7</td>
<td>8.2</td>
<td>14.6</td>
<td>69.5</td>
</tr>
<tr>
<td>Total</td>
<td>609</td>
<td>6.1</td>
<td>7.1</td>
<td>6.2</td>
<td>80.6</td>
</tr>
</tbody>
</table>

Each age group, discrepancies between the results obtained by the two techniques were observed and confirmed by repeated assays in 13.2% of the samples: 6.1% were MIFA+/CELISA−; 7.1% were MIFA−/CELISA+. High MIFA reciprocal titres were associated overall with positive reactions by CELISA, and high CELISA OD values were associated with a positive MIFA (Table 1). Further analysis showed no detectable difference between the age groups in terms of the correlation between MIFA and CELISA results (Table 2).

Of the samples positive by both MIFA and CELISA, 92.1% were also positive by standard ELISA. Of the samples negative by both MIFA and CELISA, 30.3% were positive by standard ELISA.

Fig. 2. Western blots of 37 plasma samples collected in Laborde-1. Plasma samples from subjects with anti-PF155 antibodies detectable by both MIFA and CELISA (group A), by MIFA only (group B), or by CELISA only (group C) were reacted with nitrocellulose strips containing electrophoretically separated *Plasmodium falciparum* antigens. Arrow indicates the location of the PF155 band.
Western blot

Discrepancies between the MIFA and CELISA results were investigated by performing Western blots on 37 plasmas that gave positive results by either or both assays. The plasmas reacted with several polypeptides electroblotted from parasite preparations. A 155 × 10^3 M_r polypeptide was recognized by all the 11 plasmas positive by both MIFA and CELISA. In the case of 16 plasmas positive by MIFA only, this polypeptide was recognized strongly by 8 plasmas, more weakly by 6 plasmas, and was not detected by 2 plasmas. In the case of 10 plasmas positive by CELISA only, the polypeptide was recognized strongly by 2 plasmas, weakly by 3 plasmas, and was not detected by the remaining 5 plasmas (Fig. 2).

DISCUSSION

The overall slide and serology positivity rates confirm the indication by the SNEM of active malaria transmission in Laborde-1, and can be compared to those found (J. G. Breman, personal communication) in rural northern Haiti in September 1983. These authors observed by indirect immunofluorescence a seropositivity rate (40.7%) similar to that of Laborde-1 (38.2%), but the slide positivity rate found in northern rural Haiti (6.1%) was higher than that noted in Laborde-1 (1.5%). This latter finding might be due to the fact that transmission in Laborde-1 had decreased just before the survey was conducted. Alternatively, antimalarial drugs might be more readily available in Laborde-1 than in northern rural Haiti, with the Laborde-1 inhabitants seeking treatment earlier for symptoms suggestive of malaria.

Antibodies against P. falciparum antigens found in the membrane of ring-infected erythrocytes were detected in 12.5–13.6% of the plasmas, depending on the technique used. Most of the plasmas containing these antibodies also had antibodies against whole P. falciparum antigens, as detected by ELISA. This relationship was not reciprocal, since a substantial proportion of plasmas positive by whole-parasite ELISA did not contain detectable anti-Pf155 antibodies.

Although the serological profiles obtained by MIFA and CELISA were similar, discrepancies between the results of these two tests for anti-Pf155 antibodies were not exceptional. Spencer et al. (6) found a similar phenomenon for whole-parasite malaria antibodies in comparative ELISA/IFA studies. Out of 261 sera from individuals in Viet Nam, 5.4% were IFA+/ELISA--; and 17.6% were IFA-/ELISA+. Out of 351 sera from individuals in Honduras, 6.6% were IFA+/ELISA--; and 22.8% IFA-/ELISA+. The authors attributed these discrepancies to the fact that different antibodies were measured by the two serological tests, an explanation that might also apply to MIFA and CELISA.

The overall results of MIFA and CELISA suggest that, in addition to Pf155, antibodies to other malaria antigens might be detected by MIFA only or by CELISA only. The exact nature and cellular location of such antigens remain to be determined. With Western blot detection techniques as a reference standard, MIFA appears to be more specific for anti-Pf155 antibodies than CELISA (P < 0.05).

Although a progressive age-related increase in antibody titres can be detected by whole-parasite ELISA, a significant increase in prevalence of anti-Pf155 antibodies is detectable only after 25 years of age. In addition, no trends were detected for a clear-cut protective value against clinical malaria of anti-Pf155 antibodies, which is different from the findings reported from a holoendemic area in Liberia (3). Of the 7 parasitaemic subjects, two individuals, one febrile and one afebrile, had antibodies detectable by both MIFA and CELISA (MIFA reciprocal titres, 1/64 and 1/4096; CELISA OD, 0.72 and 0.75, respectively), a rate similar to that found in the non-parasitaemic population. Further field investigations, conducted longitudinally, will be required for satisfactorily ascertaining the potential protective effect of anti-Pf155 antibodies.

ACKNOWLEDGEMENTS

We thank the personnel of the Service National des Endémies Majeures (SNEM) of Haiti, and particularly Marc M. Day, Gerard M. Jeanty, Ilem Aristil and Remus Brionne for their excellent technical assistance. We gratefully acknowledge the enthusiastic support of Dr Mario Alvarez, Director of the SNEM, in the conduct of this investigation. This project was supported by the U.S. Agency for International Development and the World Health Organization.
En décembre 1985, on a évalué l’ampleur de l’endémicité palustre et l’état immunitaire de la population d’une petite localité de Haïti, Laborde-1. Lors de visites à domicile étalées sur une semaine, 628 habitants ont été interrogés et des prises de sang effectuées en vue de l’examen de frottis épais, de la détermination du volume globulaire hémato-crite et d’études sérologiques. Sept sujets (soit 1,5%) étaient infectés par Plasmodium falciparum, la seule espèce de plasmodies observée. La méthode immuno-enzymatique en phase solide (ELISA) destinée à mettre en évidence les anticorps dirigés contre *P. falciparum* a donné lieu à une réaction positive dans 38,2% des échantillons de sérum. On a observé une augmentation, en rapport avec l’âge, des taux de positivité anticorps. En outre, on a évalué la réponse immunitaire au P155, un antigène de *P. falciparum* (M. 155 000), déposé dans la membrane d’hématies infectées par des schizontes, au moyen d’une réaction d’immunofluorescence modifiée (MIFA) et d’une méthode immuno-enzymatique à médiation cellulaire (CELSA), toutes deux fondées sur l’utilisation d’une monocouche d’hématies infectées par *P. falciparum*, fixées par le glutaraldéhyde et séchées à l’air. Une réaction positive a été obtenue dans 12,5% des échantillons avec l’épreuve d’immunofluorescence (MIFA) et 12,6% avec la technique immunoenzymatique (CELSA). De même qu’avec la méthode ELISA, le pourcentage des séropositifs en MIFA et en CELISA augmentait avec l’âge, mais cette augmentation survenait plus tard, après l’âge de 25 ans. Dans 13,2% des échantillons, les résultats obtenus avec les deux épreuves MIFA et CELISA convergeaient et, pour certains échantillons, on a poussé les investigations en recourant à la technique d’immunotransfert. Cette dernière technique, utilisée comme épreuve de référence, a montré que les sérum qui ont présenté une réaction positive avec l’épreuve d’immunofluorescence (MIFA) contenaient plus souvent des anticorps anti-P155 que les sérums réagissant avec la technique CELISA. On n’a cependant observé aucune tendance des anticorps anti-P155 à assurer une protection franche contre la maladie clinique. Il sera nécessaire de faire d’autres études longitudinales sur le terrain pour évaluer l’éventuelle action protectrice de ces anticorps.

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6. SPENCER, H. C. ET AL. The enzyme-linked immuno-