Evidence for the Transmission of \textit{Plasmodium vivax} in the Republic of the Congo, West Central Africa

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\textit{Plasmodium vivax} is not thought to be transmitted in western and central Africa, because of the very high prevalence of the red blood cell Duffy-negative phenotype in local populations, a condition which is thought to confer complete resistance against blood infection with \textit{P. vivax}. There are, however, persistent reports of travelers returning from this region with \textit{P. vivax} infections. To investigate whether transmission occurs in this region, the presence of antibodies specific to \textit{P. vivax} preerythrocytic-stage antigens was assessed in individuals from the Republic of the Congo. A total of 55 (13%) of 409 samples tested by enzyme-linked immunosorbent assay had antibodies to \textit{P. vivax}-specific antigens.

Transmission of \textit{Plasmodium vivax} is not generally thought to occur in western or central continental Africa, where 95%–99% of the human population is refractory to \textit{P. vivax} blood infection because of the protective effect of the red blood cell (RBC) Duffy-negative condition [1, 2]. Despite this, reports of Duffy-positive nonimmune travelers returning from these areas with infections diagnosed as being due to \textit{P. vivax} are common and have persisted over many years of surveillance [3]. Furthermore, a recent report has implied that \textit{P. vivax} transmission may occur in a population consisting of very high percentages of Duffy-negative individuals, with the presence of \textit{P. vivax}–specific proteins reported in 0.65% of mosquitoes from an area of western Kenya [4]. An additional study reported evidence of \textit{P. vivax} infections in 2 Duffy-negative individuals in Brazil [5]. Some investigators have interpreted such findings as implying that the parasite may be in the process of evolving the ability to infect Duffy-negative individuals [6]. However, we have argued elsewhere [3] that \textit{P. vivax} transmission can be expected in populations with high levels of RBC Duffy negativity and in which malaria transmission intensities are sufficiently high, as is the case in many areas of western and central Africa. Notwithstanding this expectation, a recent polymerase chain reaction (PCR)–based parasite species–typing survey of 2588 blood samples obtained from patients in 9 western and central African countries failed to find any \textit{P. vivax} parasites, except on the island of Sao Tome, where \textit{P. vivax} transmission is known to occur [3].

In the present study, we used serological testing to search for evidence of \textit{P. vivax} transmission in Pointe-Noire, a city on the west coast of the Republic of the Congo, where >95% of the population is expected to be RBC Duffy negative and, thus, refractory to \textit{P. vivax} blood infection. In September 2007, we collected blood samples from 415 Pointe-Noire residents and searched for the presence of antibodies to the \textit{P. vivax}–specific antigens \textit{P. vivax} circumsporozoite protein (PvCSP) and \textit{P. vivax} merozoite surface protein 1 (PvMSP1). Both antigens are expressed in liver-stage parasites and induce antibodies even in the absence of \textit{P. vivax} blood infection [7]. Detection of antibodies to these \textit{P. vivax}–specific antigens in a largely Duffy-negative human population could be evidence of its transmission there.

Materials and methods. By means of passive case detection, 415 samples were collected from the Mbota health center in Pointe-Noire, located on the west coast of the Republic of Congo. Reprints or correspondence: Dr. Richard Culleton, Dept. of Protozoology, Institute of Tropical Medicine, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852–8523, Japan (richard@nagasaki-u.ac.jp). DOI: 10.1086/644510

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Figure 1. Adjusted absorbances against *Plasmodium vivax*-specific antigens *P. vivax* circumsporozoite protein (PvCSP) (A), *P. vivax* merozoite surface protein 1 (PvMSP1) (C), and the *Plasmodium falciparum* antigen *P. falciparum* merozoite surface protein 1 (PfMSP1) (E) for 409 individuals from Pointe-Noire, Republic of the Congo. B, D, and F, Corresponding adjusted absorbances for nonexposed individuals (from Japan and the United Kingdom). Cutoff values are denoted by horizontal dashed lines, and positive individuals are denoted by the areas shaded dark gray. The cutoff value was calculated as the mean value (+3 standard deviations) of the adjusted absorbances of 30 nonexposed individuals. All absorbances were measured at 405 nm.

the Congo, during September 2007. No age restrictions were applied to individuals from whom samples were obtained. The samples were collected on Whatman 31ETCHR filter paper. Travel histories were obtained from individuals before sample collection, and those who had traveled outside of the Republic of the Congo were excluded from the study (n = 6). Approval of the sample collection was obtained from the ethics committee at the Research Institute of Microbial Diseases, Osaka University (Osaka, Japan), and sampling was authorized by the administrative authority of the Ministry for Research and the Ministry for Health in the Republic of the Congo. Written informed consent was obtained from individual patients, and antimalarial treatment was provided when appropriate. An additional 10 blood samples were collected from *P. vivax*-infected patients from Siverek-Sanliurfa in the southeast of Turkey, for use as positive controls, and from 30 individuals from Japan and the United Kingdom with no previous exposure to *P. vivax* (ie, nonexposed individuals), for use as negative control samples (for collection details, see the description of supplementary methods in the Appendix, which appears only in the electronic version of the Journal).

All samples were screened by enzyme-linked immunosorbent assay (ELISA) for the detection of immunoglobulin G antibodies to 3 *Plasmodium*-specific proteins. The first of these proteins was PvCSP recombinant protein. This *Escherichia coli*-expressed recombinant protein encompasses the N-terminal and C-terminal regions of PvCSP flanking a chimeric repeat region [8]. The second protein, PvMSP1 recombinant protein, was expressed using a wheat germ cell-free protein translation system [9] that encompasses N-terminal blocks 1 and 2 of
Plasmodium vivax antigens. P. vivax merozoite surface protein 1 (PvMSP1) and P. vivax circumsporozoite protein (PvCSP) (A), the Plasmodium falciparum antigen P. falciparum merozoite surface protein 1 (PfMSP1) and PvCSP (B), and PfMSP1 and PvMSP1 (C) for 409 individuals from Pointe-Noire, Republic of the Congo. Coefficient of determination values (r²) for each antigen pair are shown on the graph, and linear regression lines are denoted by dashed gray lines. The solid horizontal and vertical lines denote the positive cutoff values for each antigen.

**Results.** Figure 1 shows the results of ELISAs performed on the 409 samples collected from patients presenting to Mbota health center in Pointe-Noire, Republic of the Congo. For 25 (6%) of these samples, adjusted anti-PvCSP absorbance readings were greater than the mean value (+3 standard deviations) for 30 serum samples obtained from nonexposed individuals and were therefore considered to be positive for antibodies to this protein. For 39 (10%) of the samples, adjusted absorbance readings were greater than the cutoff value noted for PvMSP1. A total of 197 individuals (48%) were found to be positive for antibodies to PfMSP1, a P. falciparum antigen. All P. vivax–positive samples were independently tested twice more in duplicate, and the same positive results were obtained.

Of the 25 samples that were positive for PvCSP antibodies, 9 (36%) were also positive for antibodies to PvMSP1, and 16 (64%) were positive for antibodies to PfMSP1. Of the 39 samples that were positive for PvMSP1, 31 (79%) were also positive for PfMSP1. To investigate the possibility that there was cross-reactivity between antibodies to P. falciparum and P. vivax antigens, correlation and linear regression analyses were performed for the antigen pairs PvCSP/PvMSP1, PfMSP1/PvMSP1, and PfCSP/PfMSP1. Adjusted absorbance values were log transformed to meet the normality and homoscedasticity assumptions of the analysis, and coefficient of determination (r²) values and linear regression lines were generated (Figure 2). There was a highly significant medium-strength positive correlation between antibody responses against PvCSP and PfMSP1 (r² = 0.38; 409 df; P < .001) but a much weaker, although still significant, low correlation between PvCSP and PfMSP1 (r² = 0.09; 409 df; P < .001). There was a stronger correlation between PfMSP1 and PfMSP1 (r² = 0.27; 409 df; P < .001), but this was also much weaker than the correlation between the 2 P. vivax antigens. Furthermore, serum antibody absorbance ELISA experiments performed with known positive serum samples incubated separately with P. vivax and P. falciparum MSP1 antigens showed no evidence of cross-reactivity between the respective antibodies (figure 3, which appears only in the electronic version of the Journal).

We assessed whether various factors (patient age or sex; pres-
ence or absence of parasites; parasite species, if infected; and
district of residence) were associated with an increased prob-
ability of seropositivity against *P. vivax* antigens. There was no
association between the district of residence and the presence
of antibodies to *P. vivax* or *P. falciparum* (Table 1, which appears
only in the electronic version of the *Journal*). Similarly, there
was no strong correlation between age and the presence of
antibodies to PvCSP (\( r^2 = 0.09, 409 \text{ df}; P < .01 \)), PvMSP1
(\( r^2 = 0.13; 409 \text{ df}; P < .01 \)), and PfMSP1 (\( r^2 = 0.16; 409 \text{ df};
P < .01 \), as determined by Spearman rank correlation tests. The
presence or absence of parasites in blood, as detected by mi-
croscopy, as well as whether those parasites were *P. falciparum*,
*Plasmodium malariae*, or *Plasmodium ovale*, was not correlated
with the presence of antibodies to either *P. vivax* or *P. falciparum*;
however, the numbers of *P. malariae*– and *P. ovale*–infected individuals were low (\( n = 5 \text{ and } n = 7 \), respectively),
precluding statistical analysis (Table 1). Interestingly, females
were significantly more likely than males to be seropositive for
*P. vivax* antibodies, with 45 (17%) of 269 females positive for
antibodies to PvCSP or PvMSP1, or both, compared with 10
(7%) of 145 males (6.51, by test; 1 \( \text{df}; \ P = .01 \)). There was,
however, no difference in seropositivity for *P. falciparum
antibodies between the sexes, with 137 (51%) of 269 females and
60 (41%) of 145 males having positive responses against
PfMSP1 (1.81, by \( \chi^2 \) test; 1 \( \text{df}; \ P = .18 \)).

DNA was extracted from the 55 samples for which positive
antibody responses against either of the 2 *P. vivax*–specific an-
tigens were demonstrated by ELISA. *Plasmodium* species iden-
tification was performed by polymerase chain reaction (PCR),
and *P. vivax* DNA was not detected in any samples. The Duffy
genotype status of the 55 individuals was determined by PCR
[11], and all these individuals were found to be homozygous carriers of the *FY*Bnull allele and, thus, of the RBC Duffy-
negative phenotype.

**Discussion.** We have shown that the serum samples from
55 (13%) of 409 individuals from Pointe Noire in the Republic
of the Congo contained antibodies to the *P. vivax*–specific an-
tigens PvCSP (25 samples [6%]), PvMSP1 (39 samples [9.5%]),
or both (9 samples [2.2%]). These results suggest that *P. vivax*
is transmitted in an area of west central Africa where the fre-
quency of the Duffy-negative genotype is 95%–99% [1]. This
finding goes against the current orthodoxy that *P. vivax* is not
transmitted in western Africa and offers an explanation for the
many cases of *P. vivax* contracted by Duffy-positive travelers
in this region.

It has been established elsewhere [7] that Duffy-negative in-
dividuals who are refractory to the blood stages of *P. vivax* may
develop antibodies to such antigens as CSP and MSP1, which
are expressed in the preerythrocytic stages of this parasite in
areas of endemicity. This finding is supported by evidence of
the establishment of preerythrocytic immunity in individuals
undergoing anti–blood-stage chemoprophylaxis for *P. falcipa-
rum* [12] and in mice with *Plasmodium yoelii* [13].

Although initial experiments indicated that there was no
cross-reactivity between antibodies to the PvMSP1 and PfMSP1
antigens used in the present study, we did find a weak corre-
lation between the antibody responses to the 2 species-specific
versions of this antigen. There was also a very weak correlation
between antibody responses to the PvCSP and PfMSP1 anti-
gens. We do not consider, however, that these correlations are,
in themselves, evidence for antigenic cross-reactivity between
*P. vivax* and *P. falciparum* antigens. Indeed, if 2 species of ma-
laria parasites are coendemic, this result is predicted from the
fact that exposure to infection by one species of malaria parasite
will be highly correlated with the risk of exposure to infection
by other species.

Our data indicate that, in the region of study in western and
central Africa, there is an endemic entity present that is in-
ducing antibodies specific to the preerythrocytic stages of *P.
vivax* in the RBC Duffy-negative human populations of the
region. We suggest that this entity is most likely sporozoites of
*P. vivax* itself, delivered by the local malaria vector mosquitoes.
In conjunction with the frequent reports of travelers returning
from western and central Africa with diagnosed *P. vivax
infections, these findings make a strong argument for the presence
and continued transmission of *P. vivax* in this region. Given
the very high malaria transmission intensity in this area, it is
possible that the transmission of *P. vivax* is maintained within
the local population by the ∼1%–5% of Duffy-positive individ-
uals who are presumed to be present in the local population.

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**References**

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**Table 1. Descriptive Statistics for Adjusted Absorbencies against 3 Antigens for 409 Individuals from Pointe-Noire, Republic of the Congo**

(This table is available in its entirety in the online edition of *The Journal of Infectious Diseases*.)