

## Serological cross-reactivities between the retroviruses HIV and HTLV-1 and the malaria parasite *Plasmodium falciparum*

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### SUMMARY

Serum samples from three populations of Papua New Guinea, where *Plasmodium falciparum* malaria and human T-lymphotropic virus type 1 (HTLV-1) are coendemic at high prevalence rates, showed statistically significant ELISA co-seropositivity and co-seronegativity. Cross-reactivity was further indicated by the presence of 10 bands ranging from 134 kDa to 18 kDa on immunoblots of electrophoresed whole lysate *P. falciparum* antigen against serum of HTLV-1 seropositive patients from an area where malaria is not present. Similarly, sera from patients positive for human immunodeficiency virus (HIV) from a non-malarious region produced immunoblot bands ranging from 134 kDa to 33 kDa to the *P. falciparum* antigen. The HTLV-1 and HIV serum samples yielded a number of immunoblot bands when reacted to an electrophoresed human O type red cell membrane antigen, but those bands had no identity to the cross-reactive bands on the *P. falciparum* antigen immunoblots. Malaria-positive sera from Papua New Guinean subjects presumed to be uninfected with HIV produced a variety of bands, some of intense prominence, to HIV antigen on diagnostic Western blots.

### Introduction

There have been a number of conflicting observations on the antigenic and serological cross-reactivities between the malaria parasite *Plasmodium falciparum* and the retroviruses HIV and HTLV-1. Malaria and these retroviruses are coendemic in vast areas of the tropics and subtropics; the resolution of this question is therefore of importance in the interpretation of serological tests applied for both diagnostic and immunoepidemiological purposes. Moreover, the possibility also exists, as postulated by Butcher (1), that such cross-reactions may influence the immune response to both the parasite and virus and the consequent courses of infection.

Biggar et al. (2) found a strong correlation between the seropositivities of HTLV-1, HTLV-2 and HTLV-3 (HIV-1) and *Plasmodium falciparum* in Zairians. However,

they stated that this was not due to a true cross-reaction because of the absence of positive reactions when sera from Danish AIDS patients were tested against *P. falciparum* antigen or when the sera from American volunteers who had received *P. falciparum* malariotherapy for neurosyphilis were tested against HIV antigen. Nevertheless, their data show that some cross-reactions did, in fact, occur; of the 16 Americans who had received malariotherapy 1 was strongly and 9 borderline seropositive for HIV by enzyme-linked immunosorbent assay (ELISA). Moreover, of those 16 sera, 4 produced a moderately strong immunoblot band to the p24 HIV polypeptide and 1 sample precipitated bands at the p15 and p55 levels. Volsky et al. (3) reported that 3 of 12 Venezuelan patients (25%) with symptomatic *P. falciparum* malaria and 5 of 12 patients (42%) with *P. vivax* malaria were seropositive by ELISA and immunofluorescence (IFA) for HIV. The authors noted

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that the HIV positivity rate in healthy blood donors of the subject population was less than 1%. Parry et al. (4) reported that of a group of HIV-positive patients with no travel history to a malaria-endemic area, 70% of the homosexual men, 40% of those with haemophilia and 40% of injecting drug users gave a positive ELISA for malaria. In contrast, only 4-8% of the HIV-negative subjects in these groups were positive for malaria by ELISA. Similarly, Chrystie et al. (5) reported that of 174 HIV-positive caucasians without any travel history to malaria-endemic areas, 11% gave a strong positive reaction and 54% a weak positive reaction by ELISA using a malaria antigen (species not stated but presumably it was *P. falciparum*).

The assertion that HTLV-1 and *Plasmodium falciparum* antibodies are cross-reactive is also currently in dispute. The case for a relationship between HTLV-1 and *P. falciparum* has been made by Hayes et al. (6). In their study carried out in a coendemic area of the Philippines they found a strong statistical correlation between IFA antibody titre to *P. falciparum* and ELISA immunoreactivity to HTLV-1. Further evidence of cross-reactivity was the great reduction in HTLV-1 antibody titre following absorption with a *P. falciparum* lysate antigen. Paradoxically, HTLV-1 antigen did not absorb out malaria antibody. The difficulty in interpreting the results obtained by Hayes et al. is compounded by the facts that, firstly, the *P. falciparum* antigen did not absorb HTLV-1 antibody in sera from every population group studied and, secondly, all the sera positive for HTLV-1 by immunoblot lacked antibodies to Env proteins.

In contrast to the above findings, neither Anthony et al. (7) in their sample of sera from an isolated area of Irian Jaya nor Lal et al. (8) in their collections of sera from Indonesia, Brazil, Papua New Guinea and India were able to demonstrate any serological cross-reactivity between HTLV-1 and malaria. Furthermore, Lal et al. (8) were unable to block a serological response to HTLV-1 by absorption with a *P. falciparum* antigen. Subsequent investigation of these sera, however, suggested that there was immunological cross-reactivity between structural proteins of HTLV-1 and blood-stage

antigens of *P. falciparum* (9).

In this paper we analyze, by ELISA, the seroepidemiological co-positivity between *P. falciparum* and HTLV-1 in serum samples from three populations of Papua New Guinea. All three groups have been shown to be subject to endemic malaria and to HTLV-1 (10-13). Antigenic cross-reactivity of parasite and viruses was further demonstrated by immunoblotting the sera from HTLV-1 patients with tropical spastic paraparesis (TSP) and HIV-positive patients from non-malarious regions against electrophoresed *P. falciparum* antigens. In addition, sera from presumably HIV-negative Papua New Guineans with high titre (ELISA and Western blot) anti-*P. falciparum* antibody were tested using Western blotting against electrophoresed HIV antigens.

## Materials and Methods

### Samples and patient populations

Serum samples for serology and thick/thin blood films for the parasitological diagnosis of malaria were obtained in 1992 from three Papua New Guinean populations. The first was an inland village group consisting of approximately 1000 individuals living in and around Salata (Maprik District, East Sepik Province) which is situated at 150-180 metres above sea level in the foothills of the Prince Alexander Range; serum and blood films were obtained from 195 individuals aged 4 years and above. The second population was a coastal group from Wewak (East Sepik Province) and included 83 women giving birth at the Boram (Wewak) General Hospital. The third population was the Hagahai, a population of approximately 300 hunter-gatherer shifting horticulturists who occupy a territory on the north side of the Yuat River, in the far western corner of Madang Province, ranging from 350 to 2400 metres above sea level. Although considered to be an isolated group first contacted in 1982 by missionaries and medical-social scientists, the Hagahai have undoubtedly had contact with neighbouring groups as they moved throughout their wide area via an extensive system of bush tracks. A detailed account of Hagahai history, culture and health status has been given by Jenkins (14) and Jenkins et al. (15). Finger-stick blood for malaria films and serum samples were obtained from 102 Hagahai of various ages, the

youngest being 6 years old.

Serum samples were also collected from anonymous donors resident in an area where malaria does not occur who were serologically positive for HTLV-1 by ELISA and/or Western blot. 7 serum samples came from Hawaiian HTLV-1-positive adults, 4 with tropical spastic paraparesis (TSP) and 3 who were asymptomatic. 4 serum samples were obtained from anonymous Hawaiian residents who were HIV positive by ELISA and Western blot; 2 sera were from patients who had AIDS and 2 were from asymptomatic individuals.

### Serological methods

The ELISA for the detection of IgG antibody to *P. falciparum* antigen was performed according to the modified method (16) of Quakyi (17). The immunoblot analyses followed the method described by Desowitz et al. (16) using a whole-parasite (FUP strain maintained in culture) saponin-extracted antigen mixed with Laemmli's reduced buffer and electrophoresed in 10% polyacrylamide gels containing 0.1% sodium dodecyl sulphate.

Erythrocyte membrane antigen was prepared from human O type cells obtained from the Hawaii Blood Bank. The blood sample was centrifuged for 15 minutes at 2500 rpm after which the overlying plasma and buffy coat were removed and discarded. The packed erythrocytes were haemolyzed by adding (1:7 v/v) PBS that had been diluted 1:14 with sterile glass-distilled water, and the suspension stirred for 15 minutes. The suspension was centrifuged for 10 minutes at 12,000 rpm followed by three washes in PBS and centrifugation at 18,000 rpm for 20 minutes. After the final wash the overlying PBS was removed and the pellet consisting of erythrocyte membranes was ready for use as an antigen for the Western blot in a manner similar to that of the *P. falciparum* antigen described above.

Commercially prepared kits were used to test for antibody to HTLV-1 by ELISA (Organon Technika, Durham, North Carolina) and Western blot (Cambridge Biotech Corporation, Worcester, Massachusetts). HIV serologies were performed with an ELISA kit supplied by Genetic Systems Corporation (Redmond, Washington) and a Western blot kit supplied by Biotech Research Laboratories, Du Pont de

Nemours (Wilmington, Delaware).

### Results

#### Malaria and HTLV-1: seroepidemiological relationships (Papua New Guinea)

Table 1 shows the age-stratified malaria parasitaemia rates and species ratios for the three Papua New Guinean populations studied. In all three groups *P. falciparum* is the predominant parasite, although *P. malariae* is, as it has been for many years, highly prevalent in the Salata population (11, 18).

Table 2 summarizes the malaria and HTLV-1 ELISA seropositivity rates and relationships. Although the risk of malaria is high for all population groups, as evidenced in the parasitological data, the malaria seropositivity rates are relatively moderate in Maprik and Wewak compared to that of the Hagahai. A possible reason for this difference is that antimalarial chemotherapy is readily available, and availed of, in Maprik and Wewak whereas the isolated Hagahai hunter-gathers have little access to health care. Moreover, the ELISA positivity threshold excludes those with lower concentrations of antibody, levels that would be revealed by more sensitive techniques such as Western blotting (16). There is, however, a significant overall correlation between *P. falciparum* and HTLV-1 seropositivity, particularly for the Maprik group ( $p=0.003$ ). The very small number of malaria seronegatives amongst the Hagahai and a sample limited to parturient women for Wewak may have obscured any observable seroepidemiological relationships for these two groups. Nevertheless, even in these populations there is some suggestion, albeit not of high statistical significance, of serological associations between the retrovirus and the malaria parasite.

#### Cross-reactivity between *P. falciparum* antigen and HTLV-1 antibody

The observed serological co-positivity rates described above may not be due to any antigenic homologies between virus and malaria parasite but merely reflective of similar epidemiological factors associated with risk to infection. To provide further insight into this question we tested, by immunoblotting against *P. falciparum* antigen, the sera of symptomatic

**TABLE 1**

AGE-STRATIFIED POINT PREVALENCE MALARIA PARASITAEMIA RATES AND PARASITE SPECIES RATIOS IN THE THREE PAPUA NEW GUINEAN POPULATIONS OF THIS STUDY

Age (years)	Percent parasitaemia		
	Salata (Maprik) (n=195)	Yilu (Hagahai) (n=102)	Wewak (n=83)
4-6	87.5	-	-
7-15	67.5	26.1	-
>15	30.0	17.1	19.3/7.2*
All ages	49.8	19.2	-
<b>Species ratios (all ages)</b>			
<i>P. falciparum</i>	45.4	73.7	93.8**
<i>P. vivax</i>	15.5	26.3	6.2
<i>P. malariae</i>	39.1	0	0

\* Placental/peripheral blood parasitaemia rates

\*\* All placental parasitaemias were of *P. falciparum*; there was one woman with a peripheral blood *P. vivax* parasitaemia without any accompanying placental infection

**TABLE 2**

SEROPOSITIVITY (ELISA) RELATIONSHIPS BETWEEN *PLASMODIUM FALCIPARUM* AND HTLV-1 IN THE THREE PAPUA NEW GUINEAN POPULATION GROUPS STUDIED

	Maprik n=70	Hagahai n=90	Wewak n=46	All n=206
Malaria+	42 (60%)	82 (91%)	25 (54%)	149 (72%)
HTLV-1+	35 (50%)	25 (28%)	17 (37%)	77 (37%)
Malaria+/HTLV-1+	27	24	11	62
Malaria+/HTLV-1-	15	58	14	87
Malaria-/HTLV-1+	8	1	6	15
Malaria-/HTLV-1-	20	7	15	42
Probability	0.003*	0.28**	0.28*	0.04*

\*  $\chi^2$

\*\* Fisher's Exact Test

TABLE 3

COMPARISON OF THE *PLASMODIUM FALCIPARUM* ANTIGENS RECOGNIZED BY SERUM SAMPLES POSITIVE FOR HTLV-1 AND HIV SHOWN IN THE IMMUNOBLOTS OF FIGURE 1

<i>P. falciparum</i> antigen kDa	Lane number in Figure 1					
	3	4	5	6	8	9
	HIV	HIV	HIV	HIV	HTLV-1	HTLV-1
134	±	±	±	±	+	+
75	-	+++	-	++	+	+
65	+	+	-	-	±	±
62	-	+	±	±	±	±
47	+	+	-	-	±	±
43	-	-	+	+	++	++
39	±	-	++	++	+	+
33	±	-	+	+	-	-
31	-	-	-	-	+	+
20	-	-	-	-	+	+
18	-	-	-	-	+	+

and nonsymptomatic HTLV-1-positive patients from areas where malaria is not present. Lanes 8 and 9 of Figure 1 show two representative HTLV-1 seropositive samples, one from an Okinawan patient with tropical spastic paraparesis (TSP) and the other from an asymptomatic Hawaiian resident of Japanese descent, tested by Western blotting against the electrophoresed *P. falciparum* antigen. Table 3 shows the molecular weights of the *P. falciparum* antigens to which the two HTLV-1 sera cross-reacted in the Western blot. All serum samples, from both symptomatic and asymptomatic individuals, gave a similar immunoblot pattern of 10 bands, of varying intensity, ranging from 134 kDa to 18 kDa, the most prominent being at the 43 kDa level.

#### Cross-reactivity between *P. falciparum* antigen and HIV antibody

Figure 1 (lanes 3-6) and Table 3 show the immunoblot patterns produced by HIV-positive sera from donors not exposed to malaria, when reacted against the electrophoresed *P. falciparum* antigen. Amongst the four sera tested, 8 bands were evident (134, 75, 65, 62,

47, 43, 39 and 33 kDa). Compared to the nearly coincident immunoblot band patterns of the HTLV-1 sera, the four HIV immunoblots were heterogeneous in pattern and intensity (Table 3). Of the 8 HIV bands, all but the band at the 33 kDa level were also present on the HTLV-1 immunoblots. The main difference between the HIV and HTLV-1 immunoblots was the absence in all four HIV immunoblots of the three low molecular weight bands at the 31 kDa, 20 kDa and 18 kDa levels that were relatively prominent in the HTLV-1 immunoblots.

#### Cross-reactivity between erythrocyte membrane antigen and antibodies to HTLV-1 and HIV

Since the *P. falciparum* antigen used in the Western blots was prepared from parasites extracted from the host red blood cells it was possible that the observed cross-reactivities between HTLV-1 and HIV antibodies and *P. falciparum* antigens were artifacts in which the bands were formed by antibody binding to contaminating erythrocyte membrane, rather than parasite, antigens. To control for this,

Western blotting was performed by electrophoresing and blotting a solubilized membrane antigen prepared from non-infected human erythrocytes and reacted against the HTLV-1 and HIV serum samples described above. Bands were present in all the erythrocyte membrane antigen immunoblots but in no instance were they homologous to the cross-reactive bands of the parasite antigens. Two examples of paired parasite antigen-erythrocyte membrane antigen immunoblots are illustrated in Figure 1. Lanes 6 and 7 are of an HIV-positive serum reacted against the parasite and erythrocyte antigen respectively, while lanes 9 and 10 are the Western blots of an HTLV-1-positive serum against the two antigens. The HTLV-1 sample cross-reacted with erythrocyte antigens at the 158, 157 and 103 kDa levels while the HIV sample showed a strong band at 86 kDa. Bands at these kDa levels did not develop to the parasite antigens (Table 3).

**Cross-reactivity between HIV antigen and *P. falciparum* antibody**

Figure 2 shows the HIV antigen Western blots of six serum samples from parturient Wewak women, all with high levels of antibody to *P. falciparum* as evidenced by parasite-specific Western blot and ELISA. Compared to the HIV-positive serum control in lane 9 none of the Wewak sera would be considered as being HIV seropositive. Nevertheless, all the serum samples tested produced immunoblot bands of varying number, pattern and intensity against HIV antigens. The strong 17 kDa band in the blots of samples 1, 3 and 8 are sera from multiparous women while the blots of samples 6 and 7 in which the 17 kDa band is faint are serum samples from a primiparous woman and a woman of parity 2 respectively. The 24 kDa band present in the blots of samples 6, 7 and 8 was the other antigen that had identity to the corresponding band in the HIV seropositive control. However, there were distinct bands produced by the anti-*P. falciparum* antibody that had no homology in the HIV control immunoblot. These were bands at the 75 kDa, 70 kDa, 57 kDa, 52 kDa, 50 kDa and 47 kDa antigen levels.

**Discussion**

That there are associations, possibly due to

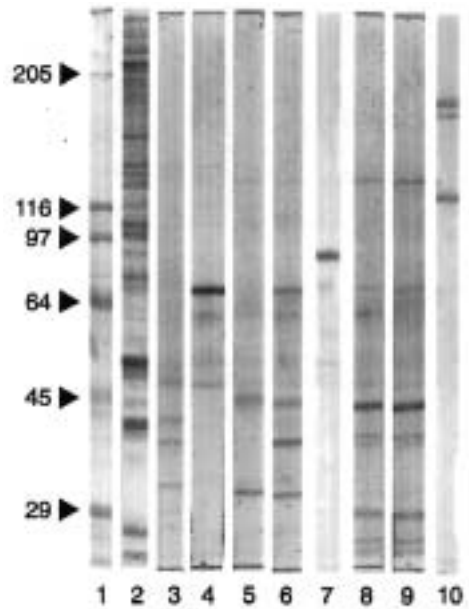


Figure 1. Reactivity to *Plasmodium falciparum* antigen of sera from patients resident in a non-malarious area who are positive for HIV (lanes 3-6) or HTLV-1 (lanes 8-9). Lane 1 is a blot of molecular weight standards. Lane 2 is a positive malaria control serum from an inhabitant of an area of Papua New Guinea where falciparum malaria is hyperendemic. Lane 7 is the serum of the HIV-positive patient of lane 6 reacted to human erythrocyte membrane antigen and lane 10 shows the reaction of the HTLV-1-positive serum of lane 9 to the erythrocyte membrane antigen.

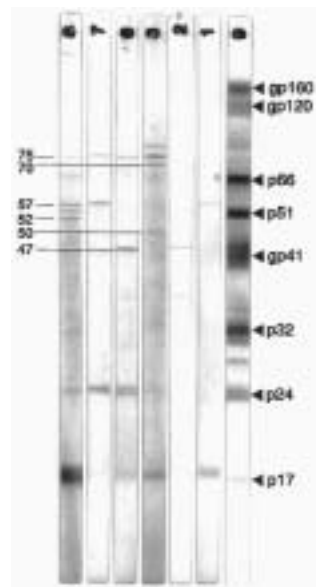


Figure 2. Reactivity to HIV antigen of sera from Papua New Guinean patients who are hyperimmune to falciparum malaria. Blot 9 is an HIV-positive control.

true antigen-antibody cross-reactivities, between the retroviruses HTLV-1 and HIV and the malaria parasite *P. falciparum* is supported by the findings described in this paper. Our application of the Western blot technique has yielded results suggestive of the presence of shared peptide epitopes between the malaria parasite and the retroviruses. Nevertheless, the possibility exists that this is an artifact, and that the immunoblot bands produced by anti-retrovirus antibody to the malaria antigen and the anti-malaria antibody to the retrovirus antigen are reactions to common host cell antigens. This is somewhat mitigated by the observation that although antibody to the viruses produced immunoblot bands to both the electrophoresed *P. falciparum* and erythrocyte membrane antigens there were no bands of identity between the blots of those two antigens. There are viruses of parasitic protozoa and therefore there is the possibility, albeit remote, that the cross-reaction is due to an unidentified virus of the malaria parasite. Viruses of *Trichomonas vaginalis*, *Giardia lamblia*, *Entamoeba histolytica*, *Leishmania brasiliensis*, *Eimeria* spp. and *Babesia* spp. have all been described. Furthermore, all viruses of the parasitic protozoa identified so far have been, like the retroviruses, RNA viruses (19). We have no explanation as to why the cross-reactive antibodies mainly reacted with epitopes that were either not present or weak in the antigen-specific immunoblots. The exceptions to this were the prominent bands at the 17 kDa and 24 kDa levels of the HIV antigen immunoblots developed with sera containing antibody to *P. falciparum*. Both HTLV-1 and HIV have a gp24 antigen that may be a conserved core protein shared by the two viruses. There may also be some homology between the virus gp24 peptide and a *P. falciparum* peptide since Hayes et al. (6) found, by competitive immunoblot serological assay, that a whole parasite *P. falciparum* extract blocked development of the gp24 HTLV-1 band. That malaria antigens may share epitopes with other microorganisms, including viruses, has been noted by Butcher and Clark (20). Alternatively, gp24 may be a shared epitope of both virus and parasite that mimics a human antigen. This is suggested by the recent observation of Hohmann et al. (21) that there is a common p24 epitope of HIV and human

platelets.

While the question of shared epitopes is intriguing, the problem of immediate concern is whether retrovirus serodiagnosis, particularly for HIV, is affected by malaria in coendemic regions. Does antibody to falciparum malaria cause false positive HIV and HTLV serologies? We believe, from our observations, that this is improbable when the Western blot is used as the diagnostic method. However, that technique is usually too expensive for most third world health facilities and the ELISA is the favoured serological method. Those facilities often do not have an ELISA reader and the plates are judged by visual inspection. By this subjective judgement of colour intensity, samples are deemed positive that otherwise would be called borderline positive by photometry. It is these borderline values that may represent the most common type of false seropositivity elicited by malaria. In a study in Southeast Africa one of us found that of the serum samples judged to be ELISA HIV-positive by visual inspection, about 15% were shown to be of borderline positivity, according to the manufacturer's criteria, when processed by an ELISA reader. Moreover, it was found that all the true positives were also positive by Western blot whereas none of the ELISA borderline sera were HIV blot-positive (Mutambu and Desowitz, unpublished data). In view of these uncertainties we believe that it is highly important to determine to what extent, if any, malaria may be contributing to the false serodiagnosis of HIV and HTLV-1 in tropical, coendemic regions.

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