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Differential Cellular Recognition of Antigens During Acute *Plasmodium falciparum* and *Plasmodium vivax* Malaria

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Abstract

Background—*Plasmodium falciparum* and *Plasmodium vivax* are co-endemic in the Asia-Pacific region. Their capacity to induce and sustain diverse T-cell responses underpins protective immunity. We compared T-cell responses to the largely conserved merozoite surface protein-5 (*PfMSP5*) during acute and convalescent falciparum and vivax malaria.

Methods—Lymphoproliferation and IFN- γ secretion to *PfMSP5* and purified protein derivate were quantified in adults with falciparum ($n = 34$), and vivax malaria ($n = 12$) or asymptomatic residents ($n = 10$) of Papua, Indonesia. Responses were reassessed 7–28 days following treatment.

Results—The frequency of IFN- γ responders to *PfMSP5* was similar in acute falciparum (63%) or vivax (67%) malaria. However, significantly more IFN- γ -secreting cells were detectable during

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vivax compared with falciparum infection. Purified protein derivative responses showed a similarly enhanced pattern. While rapidly lost in vivax patients, *PfMSP5*-specific responses in falciparum malaria remained to day 28. By contrast, frequency and magnitude of lymphoproliferation to *PfMSP5* were similar for falciparum and vivax infections.

Conclusion—Cellular *PfMSP5*-specific responses are most frequent during either acute falciparum or vivax malaria, indicating functional T-cell responses to conserved antigens. Both effector and central memory T-cell functions are increased. Greater IFN- γ responses in acute *P. vivax*, suggest enhancement of pre-existing effector T-cells during acute vivax infection.

Half the world's population lives at risk of malaria. Of the malaria-causing parasites, *Plasmodium falciparum* is associated with the greatest mortality, responsible for approximately 550 million clinical cases and approximately 1 million deaths per year [1]. Although previously thought to be relatively benign, *Plasmodium vivax* malaria is increasingly recognized as causing major morbidity and has been associated with mortality [2, 3]. Affecting almost 40% of the world's population, *P. vivax* is estimated to cause up to 390 million clinical cases each year. Most vivax malaria cases occur in Southeast Asia [3], a region further burdened with 25% of the global falciparum malaria cases [1], as both parasite species are prevalent. In Southeast Asia, reductions in malaria incidence, and particularly falciparum malaria, following intensive vector control efforts and introduction of artemisinin-based combination therapy (ACT) is likely to increase the relative proportion of vivax malaria in the future. Vaccines will be needed to prevent infection with all *Plasmodium* species, not just *P. falciparum*, to achieve the goal of malaria elimination in the Asia-Pacific region.

The development and evaluation of vaccines effective against all *Plasmodium* species requires understanding of cellular immune responses to different *Plasmodium* species. T-cell responses are essential for the acquisition of long-lasting immunity to malaria, including B-cell help. Reportedly impaired during acute falciparum malaria [4], T-cell responsiveness is not well defined in acute vivax malaria despite the importance of *P. vivax*.

Merozoite surface protein 5 (MSP5) is expressed during the blood and liver stages of the *Plasmodium* life cycle [5] and is a potential target antigen for a combined liver- and blood-stage vaccine. *P. falciparum* MSP5 (*PfMSP5*) is relatively conserved between *P. falciparum* isolates [6, 7], and its EGF-like domain shows 72% homology with that of *P. vivax* MSP5 (*PvMSP5*) [8]. We previously reported frequent IgG₃, IgG₁ and IgM antibody recognition of *PfMSP5*, in *P. falciparum* and *P. vivax* infected residents of Papua, Indonesia [9]. Species-specific and *PfMSP5* and *PvMSP5* cross-reactive antibodies were identified. The extent of cellular responses to MSP5 following natural exposure and during acute malaria is currently unknown.

In the same region of Papua, Indonesia, we assessed the frequency and magnitude of lymphoproliferation and ex vivo IFN- γ secretion to *PfMSP5* and purified protein derivative (PPD) in 34 patients with acute uncomplicated falciparum malaria and 12 patients with acute vivax malaria. The data suggest that acute vivax but not falciparum malaria enhances pre-existing effector T cells specific for malarial and nonmalarial antigens.

MATERIAL AND METHODS

Study Participants and Samples

We recruited 56 participants in Timika, a lowland region of Papua, Indonesia, with perennial unstable malaria transmission of both *P. falciparum* and *P. vivax* at a ratio of 57:43. The entomological inoculation rate varies between 1 and 4 infectious bites per year [10]. Patients with malaria were enrolled in trials of chloroquine and sulphadoxine-pyrimethamine or artemisinin combination therapy after providing informed written consent [11]. Individuals with fever or a history of fever within 48 hours prior to enrollment, and with no alternative cause of fever identified, and microscope-identified *P. falciparum* or *P. vivax* were included in this study. Venous blood was collected at the time of presentation and approximately 7 and 28 days following antimalarial drug treatment. It was not possible to collect follow-up samples from all participants. Although collection of follow-up samples could be achieved in only 35% of patients (16 of 46), reassuringly there was no significant difference in the demographic breakdown between those patients with full and partial observations. Malaria-exposed asymptomatic participants (AC) were resident in the Timika district for at least 2 years, with no fever or symptoms of malaria within the 2 weeks preceding the study [12]. Malaria patients and controls were both resident in malaria-endemic areas within Timika, with comparable malaria exposure and ethnicity (predominantly highland Papuans). Peripheral blood mononuclear cells (PBMC) were separated and cryopreserved for analysis. The median cell viability following cryopreservation was 91% (interquartile range [IQR]: 82%–93%) for controls, 86% (IQR: 74%–90%) for falciparum, and 87% (IQR: 78%–91%) for vivax patients and did not differ significantly between groups ($P = .2$). The ethics committees of the National Institute of Health Research and Development, Ministry of Health, Jakarta, Indonesia, and Menzies School of Health Research, Darwin, Australia approved this study.

Antigens

We expressed and purified *P. falciparum* and *P. vivax* recombinant MSP5 as previously described [9, 13]. We used PPD from *Mycobacterium tuberculosis* (Statens Serum Institute) as a nonmalarial recall antigen and the mitogen phytohaemagglutinin (PHA; Sigma) as a positive control.

Ex Vivo Enzyme-linked Immunospot Assay

We cultured 0.4 million PBMC per well with or without antigen in 96-well flat-bottom nitrocellulose plates (MAIPS4510, Millipore) pretreated with 70% ethanol, coated with 5 $\mu\text{g}/\text{mL}$ of anti-IFN- γ monoclonal antibody (clone 1-D1K, Mabtech) and incubated at 37°C in 5% carbon dioxide (CO₂) overnight. We washed and developed and plated using 1 $\mu\text{g}/\text{mL}$ biotinylated anti-human IFN- γ mAb (clone 7-B6-1, Mabtech) followed by streptavidin-alkaline phosphatase (AP) (1:1000, Mabtech) and the colorimetric AP Kit (BioRad). We counted spots using an automated enzyme-linked immunospot assay reader (AID) and expressed them as spot-forming units (SFU) per million PBMC. A response was considered positive if the number of spots was significantly different ($P < .05$) from that of the control well, assuming a Poisson distribution [14].

Proliferation Assay

We plated 100,000 PBMC per well in RPMI-1640 (JRH) with 5% heat-inactivated human AB serum, 2 mM glutamine, 100 µg/mL streptomycin and 100 U/mL penicillin (all Sigma-Aldrich) in triplicate into 96-well round bottom plates (BD). Cells were incubated at 37°C in 5% CO₂ for 5 days in the presence of 10 µg/mL of antigen or 2–3 days in the presence of 5 µg/mL mitogen, pulsed with 1 µCi of ³[H]-thymidine (Amersham) and then incubated for a further 16 hours. The stimulation index (SI) was calculated as mean counts per minute (cpm) of stimulated cells divided by mean cpm of unstimulated cells. Responses with SI ≥ 2 were considered positive.

Flow Cytometry

PBMC were blocked with phosphate-buffered saline containing 10% fetal calf serum, stained with anti-CD3 antibody (Hit3a, Pharmingen), washed and fixed with 1% paraformaldehyde. Data were acquired on a FACSCalibur (BD) and analyzed using FlowJo (TreeStar) software.

Statistical Analysis

We used GraphPad Prism 5 (GraphPad Software) for all statistical analyses. In an a priori analytical plan, we compared responses in the *P. falciparum* group with *P. vivax* patients and separately with asymptomatic controls using the Mann–Whitney *U* test or Fisher exact test. We used the Spearman rank test for correlation analyses. We used the Wilcoxon signed rank test for analysis of longitudinal data.

RESULTS

Study Cohort

We included 34 adult patients with acute uncomplicated *P. falciparum* malaria (43% female; mean age 26; 60% Highland Papuan; median parasitemia 3068 [IQR 504–8084] parasites/µL), 12 adults with acute *P. vivax* malaria (54% female; mean age 25; 80% Highland Papuan; median parasitemia 638 [IQR 322–3195] parasites/µL) and 10 asymptomatic malaria-exposed adults (10% female; mean age 27; 100% Highland Papuan; median parasitemia 0 [IQR 0–0] parasites/µL) in this study. There was no significant difference in age, gender, or ethnicity between patients with acute *P. falciparum* and *P. vivax* malaria.

Frequent *P. falciparum* Merozoite Surface Protein 5–specific Proliferation During Acute Malaria

Proliferative responses to PfMSP5 were assessed in 28 patients with falciparum malaria and compared with responses in 10 patients with vivax malaria and 10 exposed asymptomatic persons. PfMSP5-specific proliferation following in vitro stimulation was detected in 13 of 28 patients with acute falciparum (46%) and in 3 of 10 patients with acute vivax malaria (30%; *P* = .47; Table 1), but only in 1 of 10 exposed asymptomatic individuals (10%; *P* = .06; Table 1). There was no significant difference in PfMSP5 reactivity between males (11 of 21) and females (5 of 17) either overall or after stratifying by species of infection (*P* = .35), although it should be noted that this study was not designed or powered to elucidate

differences between males and females. All samples proliferated following mitogenic stimulation with PHA, indicating that the cells were viable and capable of division. No significant difference in spontaneous proliferation was observed between falciparum malaria patients and either asymptomatic participants or patients with vivax malaria (Table 2) nor between vivax patients and asymptomatic controls ($P = .5$). Maximal proliferation in response to *PfMSP5* was observed on days 5 and 6 of culture with no significant difference in the stimulation index of responses between the groups (Table 2). The majority (60%–100%) of donors in all groups proliferated following stimulation with the nonmalarial antigen PPD (Table 1). There was no significant difference in the magnitude of PPD proliferative response between falciparum and vivax malaria (Table 2).

***P. falciparum* Merozoite Surface Protein 5–specific Interferon- γ Secretion During Acute *P. falciparum* and *P. vivax* Infection**

The presence of *PfMSP5*-specific effector T-cell responses was assessed by ex vivo IFN- γ ELISpot assay in 19 patients with acute *P. falciparum*, 12 patients with acute *P. vivax* malaria, and 7 malaria-exposed asymptomatic participants. IFN- γ responses were detected in most patients with falciparum (63%) or vivax (67%) malaria but in only 28% of asymptomatic individuals (Table 1). Similar to proliferative responses, IFN reactivity did not differ significantly when malaria patients were stratified by gender ($P = .1$). Interestingly, vivax but not falciparum malaria patients showed significantly higher background IFN- γ responses compared with asymptomatic controls ($P = .046$). There was no statistically significant difference in background IFN- γ responses between falciparum malaria patients and vivax patients (Table 2). In contrast, the magnitude of *PfMSP5*-specific IFN- γ responses (corrected for background) was significantly higher in patients with vivax malaria compared with those with falciparum malaria ($P = .01$; Table 2). This difference was not because of differences in the overall proportion of T cells in peripheral blood because both groups showed similar CD3+ T-cell frequencies (Table 2). Similar to *PfMSP5* responses, IFN- γ responses to PPD (corrected for background) were also significantly higher in patients with vivax malaria compared with those in patients with falciparum malaria ($P = .04$; Table 2).

***P. falciparum* Merozoite Surface Protein 5 Responses are Short-lived in *P. vivax* but Not in *P. falciparum* Malaria**

Whereas on the day of admission, *PfMSP5*-specific IFN- γ responses were higher in patients with vivax malaria, these responses were short-lived and dropped significantly within 7 days post treatment (Figure 1A, right panel). In contrast, the magnitude of *PfMSP5*-specific IFN- γ responses in patients with falciparum malaria remained unchanged up to at least 28 days post treatment (Figure 1A, left panel). This observation was further supported by longitudinal samples from individual patients with falciparum ($n = 3$) and vivax ($n = 4$) malaria showing that *PfMSP5*-specific IFN- γ responses were short lived in vivax malaria (Figure 1B, right panel) but were maintained during convalescence following falciparum malaria treatment (Figure 1B, left panel).

Similar to IFN- γ responses, *PfMSP5*-specific proliferation was still observed in 60% of individuals 28 days following treatment of falciparum malaria (Figure 1C, left panel)

compared with only 1/4 following vivax malaria (Figure 1C, right panel). We observed no correlation between proliferative responses and IFN- γ responses in patients with falciparum ($r_s = .19$; $P = .5$) or vivax malaria ($r_s = .32$; $P = .4$). Similarly, there was no significant relationship between proliferative or IFN- γ responses and parasitemia in either *P. falciparum* or *P. vivax* infection. We observed no correlation between T-cell responses and previously reported antibody responses [9] (data not shown).

The Majority of *P. falciparum* Merozoite Surface Protein 5 Responders also Recognize *P. vivax* Merozoite Surface Protein 5

The high effector T-cell reactivity to *PfMSP5* in patients with vivax malaria led us to further test a subgroup of 11 individuals with acute *P. falciparum*, 7 with acute *P. vivax* malaria and the 10 asymptomatic individuals for concordant reactivity to recombinant *P. vivax* MSP5 (*PvMSP5*). Figure 2 shows the reactivity pattern of IFN- γ and proliferative responses for each individual. Reactivity to *PvMSP5* was observed in all groups. Overall, during acute malaria, 71% of *PfMSP5* responders also responded to *PvMSP5*.

DISCUSSION

We report frequent lymphoproliferation and IFN- γ secretion to *PfMSP5* and PPD in adults with acute *P. falciparum* or *P. vivax* malaria in Papua, Indonesia. IFN- γ -secretion, but not proliferative responses, was significantly greater during acute vivax compared with falciparum malaria but was rapidly lost following anti-malarial treatment. Cellular responses to *PfMSP5* were detectable in *P. falciparum* malaria patients until at least day 28 post treatment. The higher magnitude of IFN- γ responses to *PfMSP5* and PPD during acute vivax malaria suggests both specific and non-specific effector T-cell activation by *P. vivax*, which may contribute to the stronger host inflammatory responses per infected erythrocyte known to occur for vivax malaria [15, 16].

Proliferative capacity is a fundamental prerequisite for the maintenance of long-term protective immunity. The low proliferative reactivity in residents without clinical malaria suggests that long-lasting *PfMSP5*-specific memory responses did not develop in these donors despite at least 2 years of *P. falciparum* exposure and high frequencies of antibodies to *PfMSP5* [9], albeit in a region of unstable rather than hyperendemic transmission [10]. Similarly low responsiveness has been reported to other malarial antigens [17–20], whereas studies from hyperendemic areas, especially those with seasonal transmission, show higher proliferation reactivity to malarial antigens [21–23] in individuals with life-long exposure. Together these studies highlight the broad diversity of T-cell responsiveness amongst different geographic areas with different malaria endemicity.

The low frequency of *PfMSP5*-specific IFN- γ responses detected in asymptomatic persons was anticipated because ex vivo ELISpot assays detect circulating effector T cells, and overnight culture is too short to expand cells. In acute malaria, however, such expansion occurs in vivo as evidenced by the presence of proliferating Ki-67 positive T cells in patients with acute falciparum or vivax malaria [24]. *PfMSP5*-specific IFN- γ responses detected by ex vivo ELISpot in asymptomatic participants likely reflect expansion of circulating *PfMSP5*-specific effector T cells due to a recent *P. falciparum* infection [25]. IFN- γ -

secreting effector memory T cells directly contribute to antibody independent protective immunity to malaria blood-stages as evidenced by rodent [26, 27] and human experimental malaria challenge data [28]. IFN- γ responses similar to the *PfMSP5*-specific reactivity reported here have also been observed to other malarial antigens [14, 20, 29].

Frequent detection of IFN- γ and proliferative responses to *PfMSP5* during both acute falciparum and vivax malaria suggests that *PfMSP5* responses are boosted by the current *Plasmodium* infection. Interestingly, significantly more *PfMSP5*- and PPD-specific IFN- γ -secreting cells were detected in vivax malaria patients compared with falciparum malaria patients. *PfMSP5*-specific responses following acute *P. vivax* infection were only short-lasting and declined by day 7 post infection. Thus, observed responses may represent *MSP5*-specific cross-reactive memory T cells directly activated by the current infection or indirectly activated as bystanders by the inflammatory *P. vivax* infection [15, 30]. We previously reported cross-reactive IgG responses in a minority of responders [9]; hence cross-reactive T-cell epitopes may also exist. However, greater IFN- γ responses to the nonmalarial antigen PPD in vivax malaria compared with falciparum malaria and the high background IFN- γ responses suggest either bystander activation and enhanced T-cell mobilization or less immune suppression in vivax malaria. Regardless of the mechanism by which reactivity to *P. falciparum* antigen is started during *P. vivax* infection, such reactivity is important because cellular immune responses to blood-stage antigens are shown herein to have potential to be highly interdependent of the other parasite.

The T-cell frequency was similar among falciparum and vivax malaria patients and asymptomatic controls, suggesting that the fewer IFN- γ -secreting effector T cells seen in falciparum malaria were not attributable to a proportional loss of T cells from the periphery. *P. falciparum* antigen specific T cells may transiently relocate from circulation [31, 32], particularly because IFN- γ responses increased following falciparum malaria treatment, potentially reflecting the return of sequestered T cells into the periphery [33]. However, such relocation would not account for the fewer PPD-specific T cells detected in falciparum compared with vivax malaria patients. The increase in IFN- γ secreting cells during convalescence of falciparum malaria is in line with a previously reported increase of CD4 and CD8 T cells capable of IFN- γ production (following phorbol myristate acetate-ionomycin stimulation) during early convalescence [34]. We show here that these contain antigen-specific circulating effector T cells, both to *P. falciparum* malarial antigen and PPD, which are detectable in the periphery until at least day 28 of convalescence.

During acute *P. falciparum* malaria, impairment of T-cell responsiveness is manifest by reduced IFN- γ responses [35], lymphoproliferation [4, 36], delayed type hypersensitivity reaction to recall antigens [37] and virus-specific T-cell responses [38, 39]. However, the magnitude of lymphoproliferation or IFN- γ response to *PfMSP5* or PPD was no higher in falciparum malaria patients than in asymptomatic exposed individuals, with stimulation indices in both groups being low. We previously observed a transient increase in regulatory T cells [40] in adults with acute falciparum malaria from the same area, which may contribute to impaired T-cell responsiveness during acute disease. T-cell responses during acute vivax malaria are not well described. Patients with acute clinical *P. vivax* malaria have been reported to show reduced delayed type hypersensitivity reaction to recall antigens [37]

and low lymphoproliferative responsiveness to *P. vivax* schizont lysate and recombinant *P. vivax* proteins has been observed in convalescent vivax malaria patients from malaria-endemic Sri Lanka [41]. We found no immediate evidence of immune suppression in patients with acute *P. vivax* malaria, indeed responses were greater during acute malaria compared with convalescence and background IFN- γ responses were greater than those of controls. The lack of suppression of IFN- γ responses during acute *P. vivax* malaria suggests elementary differences in immune regulation between infections with these 2 *Plasmodium* species that are yet to be explained. A recent comparative study reports that T cells from adult falciparum patients [42, 43] but not vivax patients [43] show elevated expression of the T-cell inhibitory antigen CTLA-4. Such inhibition may occur independent of regulatory T cells because, like falciparum malaria [40], vivax malaria is accompanied by a relative increase in CD4⁺CD25⁺Foxp3⁺ regulatory T cells [44, 45]. Further comparative studies are required for a better understanding of immune regulatory differences in falciparum and vivax malaria.

The lack of correlation of lymphoproliferation with antibody responses or IFN- γ responses is in accordance with previous reports [46–48] as is the lack of correlation between lymphoproliferation and parasitemia [49, 50], highlighting our still limited understanding of the complex network of immune responses in malaria infection.

This study presents the first data on cellular responsiveness to PfMSP5 and demonstrates that unstable *Plasmodium* exposure generates PfMSP5-specific cellular and antibody responses [9] that are boosted by a current *Plasmodium* infection. The contribution of MSP5-specific cellular and humoral responses to protection against clinical disease remains to be determined. Greater T-cell reactivity during acute vivax malaria suggests that acute vivax but not falciparum malaria enhances or mobilises pre-existing effector T cells specific for malarial and nonmalarial antigens. Greater understanding of interspecies differences in cellular responses is needed for the development of vaccines against all species causing malaria.

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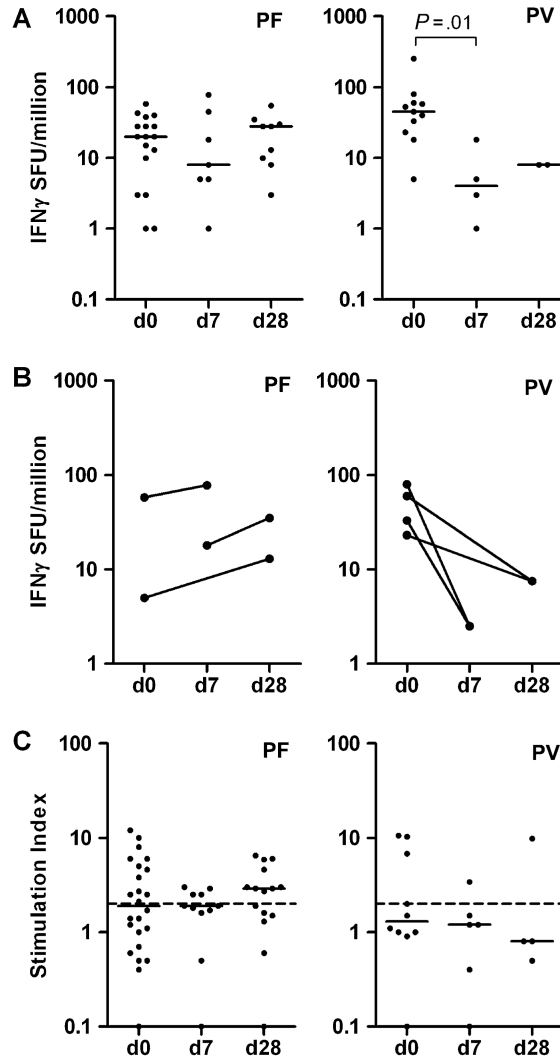


Figure 1.

Longitudinal responses to merozoite surface protein 5. (A) Longitudinal interferon- γ (IFN- γ) responses to merozoite surface protein 5 (MSP5) in samples from patients with acute *P. falciparum* malaria (Pf, left panel) and acute *P. vivax* malaria (Pv, right panel). (B) Longitudinal IFN- γ response to MSP5 in paired samples from 3 patients with acute *P. falciparum* malaria (Pf, left panel) and 4 patients with acute *P. vivax* malaria (Pv, right panel). All graphs show number of spot forming units (SFU) per million PBMC with background SFU subtracted. Horizontal lines depict the median. (C) Longitudinal proliferation response to PfMSP5 in cross-sectional samples from patients with acute *P. falciparum* malaria (Pf, left panel) and acute *P. vivax* malaria (Pv, right panel). Positive response defined as stimulation index (SI) > 2. Horizontal lines depict the median. Dashed lines represent cut-off value for positive response (SI = 2).

	Donor	IFN γ		Proliferation	
		<i>Pf</i> MSP5	<i>Pv</i> MSP5	<i>Pf</i> MSP5	<i>Pv</i> MSP5
<i>Pf</i>	21				ND
	69				
	268			ND	ND
	231			ND	ND
	264			ND	ND
	362			ND	ND
	229			ND	ND
	19	ND	ND		
	27	ND	ND		
	75	ND	ND		
	91	ND	ND		
<i>Pv</i>	61				
	122				
	168			ND	ND
	47				
	49				
	1001		ND		
	25	ND	ND		
AC	129				
	131				
	135				
	147				
	149	ND	ND		
	153	ND	ND		
	159				
	187	ND	ND		
	133				
	143				

Figure 2.

Reactivity pattern to *P. falciparum* merozoite surface protein 5 and *P. vivax* merozoite surface protein 5. Eleven patients with falciparum malaria (*Pf*), 7 with vivax malaria (*Pv*) and 10 asymptomatic individuals (AC) were tested for IFN-gamma and proliferation responses to *Pf*MSP5 and *Pv*MSP5. Positive responses are shown as black squares. ND, not determined.

Table 1
Reactivity to Merozoite Surface Protein 5 and Nonmalarial Antigen

		AC	<i>Pf</i>	<i>Pv</i>	<i>P</i> value [#]	<i>P</i> value [*]
MSP5	Proliferation	1/10 (10%)	13/28 (46%)	3/10 (30%)	.06	.47
	IFN- γ	2/7 (28%)	12/19 (63%)	8/12 (67%)	.19	1.0
PPD	Proliferation	6/10 (60%)	17/20 (85%)	8/8 (100%)	.18	.54
	IFN- γ	3/7 (43%)	4/9 (44%)	7/8 (88%)	1.0	.13

NOTE. MSP5, merozoite surface protein 5; AC, asymptomatic malaria-exposed; *Pf*, acute *P. falciparum* malaria; *Pv*, acute *P. vivax* malaria.

[#] Fisher exact test AC vs *Pf*;

^{*} Fisher exact test *Pf* vs *Pv*.

Table 2
Cellular Immune Responses to Merozoite Surface Protein 5 and Purified Protein Derivative

		AC	<i>Pf</i>	<i>Pv</i>	<i>P</i> value [#]	<i>P</i> value [*]
Proliferation ^a	<i>n</i>	10	28	10		
	Media [cpm]	394 [129–2371]	381 [189–828]	685 [375–1535]	.87	.08
	MSP5 [cpm]	488 [144–1616]	691 [230–1134]	1292 [653–2857]		
	MSP5 [SI]	1 [0.8–1.5]	1.9 [0.8–4.9]	1.3 [1–7.7]	.14	.92
	PPD [cpm]	5274 [219–11,485]	6770 [1427–19,062]	22,124 [7128–49,498]		
	PPD [SI]	5 [1–28]	13 [5–25]	16 [10–68]	.18	.36
IFN- γ ^b	<i>n</i>	7	19	12		
	Media	0 [0–10]	5 [0–20]	9 [3–39]	.09	.4
	MSP5	0 [0–45]	20 [10–38]	49 [26–75]	.16	.01
	PPD	38 [0–200]	10 [3–33]	136 [77–271]	.77	.04
T-cell frequency ^c	<i>n</i>	10	22	12		
	CD3+	54 [29–70]	57 [44–60]	57 [54–64]	0.8	0.3

NOTE. AC, asymptomatic malaria-exposed; *Pf*, acute *P. falciparum* malaria; *Pv*, acute *P. vivax* malaria; IFN, interferon; MSP5, merozoite surface protein 5; PPD, purified protein derivative; SI, stimulation index.

^aData expressed as median cpm or SI [interquartile range (IQR)]

^bData expressed as median SFU/million [IQR]

^cexpressed as % of peripheral blood mononuclear cells [IQR];

[#]Mann-Whitney U test AC vs *Pf*;

^{*}Mann-Whitney U test *Pf* vs *Pv*.