Abstract

Co-infection with HIV and *P. falciparum* worsens the prognosis of both infections; however, the mechanisms driving this adverse interaction are not fully delineated. To evaluate this, we studied HIV-1 and *P. falciparum* interactions *in vitro* using peripheral blood mononuclear cells (PBMCs) from human malaria naive volunteers experimentally infected with *P. falciparum* in a malaria challenge trial. PBMCs collected before the malaria challenge and at several time points post-infection were infected with HIV-1 and co-cultured with either *P. falciparum* infected (iRBCs) or uninfected (uRBCs) red blood cells. HIV p24Ag and TNF-α, IFN-γ, IL-4, IL-6, IL-10, IL-17, and MIP-1α were quantified in the co-culture supernatants. In general, iRBCs stimulated more HIV p24Ag production by PBMCs than did uRBCs. HIV p24Ag production by PBMCs in the presence of iRBCs (but not uRBCs) further increased during convalescence (days 35, 56, and 90 post-challenge). In parallel, iRBCs induced higher secretion of pro-inflammatory cytokines (TNF-α, IFN-γ, and MIP-1α) than uRBCs, and production increased further during convalescence. Because the increase in p24Ag production occurred after parasitemia and generalized immune activation had resolved, our results suggest that enhanced HIV production is related to the development of anti-malaria immunity and may be mediated by pro-inflammatory cytokines.

Introduction

HIV-1 and *Plasmodium falciparum* malaria remain two of Sub-Saharan Africa’s major causes of morbidity and mortality. Together malaria and HIV caused nearly 2.5 million deaths in Africa during 2008. Although it was not initially fully appreciated [1,2], increasing evidence indicates that the two pathogens interact in individuals and in populations. During acute bouts of clinical malaria, plasma HIV-1 RNA levels rise [3–5], and CD4 cells decline by approximately 40 cells/µL/year with each malaria episode [6] compared to the rate of decline in individuals without clinical malaria episodes. Conversely, in regions of unstable malaria transmission, HIV infection is associated with increased malaria disease severity and death [7]. Recently, a mathematical model further explored the potential importance of the malaria/HIV interaction. Based on this model, in an area of Kenya, with an adult population of roughly 200,000 that has been exposed to both pathogens since 1980, the interaction of the two diseases may have caused 8,500 excess HIV infections and 980,000 excess malaria episodes [8]. Supporting this mathematical model, a study that examined geographical overlap of the two pathogens in East Africa found that those who live in areas of high *P. falciparum* incidence have about twice the risk of being HIV infected compared to individuals who live in areas of low incidence [9].

The host immune response to *Plasmodium* infection includes the rapid release of interferon-gamma (IFN-γ) by natural killer (NK) cells and tumor necrosis factor (TNF) by macrophages, while cells of the adaptive immune system release TNF, interleukin (IL)-12, and IFN-γ in response to parasitized erythrocytes [10]. Several cytokines that are elaborated during acute bouts of malaria can cause stimulation of HIV-1 replication. IL-1, IL-2, IL-3, IL-6, IL-12, granulocyte macrophage-colony stimulating factor (GM-CSF), and TNF-α/β have been identified as inducers of viral expression [11], prompting speculation that cytokines involved in the control and clearance of a *Plasmodium* infection, especially TNF-α, may cause the increase in HIV replication [5].

We developed a culture system to evaluate interactions between *P. falciparum* and HIV-1 *in vitro* using PBMCs collected from humans experimentally infected with *P. falciparum* under carefully controlled conditions in the context of a malaria human challenge trial (personal communication). Using this model system, we were able to further evaluate the mechanisms responsible for the deleterious interactions between HIV-1 and *P. falciparum*, and to
determine whether these interactions are affected by prior exposure to \(P. falciparum\).

**Results**

**P. falciparum-infected Red Blood Cells (iRBCs) Stimulate More HIV-1 p24 Ag Production than uRBCs**

Whole PBMCs isolated from HIV uninfected, malaria naïve donors produced significantly more HIV when co-cultured with iRBCs than when co-cultured with uRBCs (Figure 1B inset, \(p = 0.0045\), area under the curve comparisons). The increase in HIV-1 production was evident by day 8 in culture. By day 10, HIV production in the iRBC co-cultures was increased about 2.5-fold over parallel co-cultures with uRBCs (Figure 1B).

**Repeat Exposure to \(P. falciparum\) Enhances Stimulation of HIV Production by iRBCs**

To study the effects of acute \(P. falciparum\) infection on HIV-1 p24 Ag production by PBMCs after in vitro infection with HIV-1, we used PBMCs isolated from the controlled human malaria challenge participants. As noted above, all samples from any given study participant were thawed and studied in the same experiment. Sufficient PBMCs were available from 5 of the 6 malaria challenge trial participants for the baseline, liver stage, and later post-exposure time points and from 4 of the 5 participants at the blood stage time point. Once thawed and cultured overnight, the study design was identical to that described above. Figure 1C depicts HIV production in the iRBC/uRBC co-cultures for the 5 participants at the 6 different visits. For all of the participants at the baseline, liver stage, and blood stage visits, the amount of HIV produced from the PBMCs co-cultured with iRBCs (pink lines) was about 2 fold higher than the amount of HIV produced from the PBMCs co-cultured with uRBCs (grey lines). For all participants at the convalescent visits (day 35, 56, and 90 post malaria exposure time points), HIV production from the iRBC co-cultures (burgundy lines) was about 3 fold higher than that from the respective PBMCs co-cultured with uRBCs (black lines).

In order to further explore the relationship between \(P. falciparum\) stimulation and HIV-1 production by cells collected from malaria-exposed volunteers, we performed an area-under-the-curve...
calculation of p24 Ag production in vitro at each time point under each condition and evaluated serial differences for each patient. There was a significantly greater amount of HIV produced at each time point in the iRBC co-cultures compared to the uRBC co-cultures (p<0.001, Figure 1D). The amount of HIV produced from the uRBC co-cultures (black line) was unchanged across all the visits. In contrast, we observed a significant increase in HIV production when iRBCs were co-cultured with PBMCs collected at the day 35 (p<0.001), day 56 (p = 0.004), and day 90 (p = 0.017) post-exposure visits, compared to iRBCs co-cultured with PBMCs collected at earlier time points. Because the enhanced HIV production occurred after chloroquine-mediated clearance of parasitemia in the study participants, our results suggest that development of malaria specific cellular immunity, rather than activated effector cells, are responsible for the heightened HIV production. In order to determine if CD4+ memory T-cells were...
and memory (CD45RO\(^+\) memory (CD45RO\(^+\) markers expressed by these cell types when obtained from malaria Day 56 post-malaria exposure compared to surface activation with Enhanced in iRBC Co-cultures Following in vivo Infection challenge trial participants to assess T-cell activation in cells

HIV Production in the Co-cultures

Systemic Inflammation does not Correlate to Increased HIV Production in the Co-cultures

In order to evaluate whether increases in HIV production at the later time points might be related to generalized immune activation following recovery from acute P. falciparum, we measured levels of C-Reactive Protein (CRP) in plasma obtained at the time of PBMC isolation. For all but one participant (011-3), levels of CRP appear to be higher during the early time points than at the later time points (Figure 3). Thus, CRP levels were generally higher during active infection compared to convalescent samples, where HIV-1 was seen to increase. In fact, a spike in the CRP levels was noted in two participants (018-4 and 016-6) at the blood stage time point of the active malaria infection, but there was no corresponding increase in HIV production at the same time point. This supports the concept that increased HIV production during the convalescent phase is not the result of systemic inflammation.

Discussion

Using PBMCs and plasma collected from donors before, during and after controlled infection with P. falciparum, we explored interactions between HIV-1 and P. falciparum. We confirm that iRBCs co-cultured with PBMCs from malaria naïve donors enhance replication of HIV-1, and show that HIV-1 production is further enhanced in PBMCs collected in the convalescent period after experimental infection with P. falciparum. HIV-1 replication after exposure to P. falciparum infected erythrocytes was accompanied by increased secretion of pro-inflammatory cytokines TNF-a, IFN-\(\gamma\), and MIP-1\(\alpha\), which was also enhanced after experimental P. falciparum infection. In addition, we noted a trend toward increased memory CD4+ T-cell activation in response to iRBCs during the convalescent period. Enhanced HIV production in vitro following controlled human infection with P. falciparum was not associated with systemic inflammation in vivo as assessed by plasma C-reactive protein levels.

Under the controlled conditions of the standard human challenge model [12-14], subjects are diagnosed and treated at low parasite density, often prior to the onset of clinical symptoms and likely prior to the development of the cytokine storm observed in severe bouts of clinical malaria in non-immune individuals [15]. Despite the limited duration of parasitemia in the experimental malaria infection, production of HIV-1 by P. falciparum-exposed PBMCs was substantially enhanced following treatment and resolution of the blood stage of infection. Although not examined in these initial experiments, the changes we observed might have been more pronounced had study participants progressed to clinical disease. Our data suggests that T-cell activation in these participants was modest at the time parasitemia was first demonstrated on peripheral blood smears, although flow cytometry was not performed longitudinally to assess levels of activation of unstimulated T-cells before and after experimental infection. It has previously been shown that chloroquine reduces the number of activated CD8+ memory T-cells and, to a lesser extent, CD4+ memory T-cells in the peripheral blood and inhibits the production and secretion of TNF-a [16,17]. Since the participants in the malaria challenge trial received chloroquine after the blood stage blood draw, PBMCs isolated at the first three time points were free of the immunomodulatory effects of chloroquine. PBMCs collected at the later post-exposure time points (days 35, 56, and 90) might have been exposed to varying levels of chloroquine (in view of its biological half-life of 1–2 months). Thus, although chloroquine may have immunomodulatory effects, the time points at which we noted enhancement of HIV-1 production were those when it might have been present and we would have expected immunomodulatory effects of chloroquine to exert an effect opposite to that which we observed.

The role of cytokines during malaria has been extensively studied. In controlled Plasmodium infections of malaria-naïve subjects, serum levels of pro-inflammatory cytokines, including TNF-\(\alpha\), IL-6, IFN-\(\gamma\), and IL-12p40, increase at the time that parasites emerge from the liver and at the first appearance of parasitized erythrocytes [10]. McCall et al. reported that PBMCs isolated from participants who were infected with P. falciparum in a controlled human malaria infection and exposed to cryopreserved iRBCs secreted higher levels of IFN-\(\gamma\) by NK cells than naïve
In semi-immune individuals living in malaria endemic areas, the amount of IFN-γ secreted is lower upon repeat exposure to malaria antigens than in malaria naive individuals, and malaria episodes are less severe [19]. Separately, several TNF-α alleles have been correlated to increased plasma TNF levels, increased susceptibility to severe malaria [20], and increased susceptibility to cerebral malaria [21]. Higher levels of circulating TNF-α were found in adults and children with severe malaria compared to both uncomplicated malaria cases and healthy individuals [20,22]. While IFN-γ is a pro-inflammatory cytokine, it inhibits HIV infection in vitro and has been used in patients with advanced AIDS to reduce the number of opportunistic infections [23,24]. Thus, even though both TNF-α and IFN-γ are classified as pro-inflammatory cytokines and even though both increase during acute bouts of clinical malaria, TNF-α is a better candidate cytokine than IFN-γ to stimulate HIV production during P. falciparum infection. Furthermore, peak plasma levels of TNF-α do not decrease with repeat P. falciparum infections while those of IFN-γ decline with repeated infection.

Our data extend those of Xiao et al., who employed a similar in vitro system to examine the effects of P. falciparum on HIV-1 production by CD8+ T-cell depleted PBMCs from malaria-naive individuals [25]. In their hands exposure to P. falciparum merozoites and hemozoin increased HIV-1 replication [25]. In our studies, in an effort to more closely mimic a natural malarial infection, we stimulated unfractionated PBMCs with P. falciparum-infected red blood cells rather than with merozoites or hemozoin. In the model system reported by Xiao, HIV-1 replication was the result of increased TNF-α production, but not the result of IL-6 production, demonstrated by the use of TNF-α and IL-6 blocking antibodies. We also observed that TNF-α increased in iRBC co-cultures compared to uRBC co-cultures and in post-exposure iRBC cultures compared to the early cultures, while IL-6 production did not change significantly in any of these conditions. Although there are clear-cut differences between the experimental systems and although neither system fully captures all aspects of natural infection, HIV-1 production was enhanced by P. falciparum exposure under both sets of experimental conditions. It would be of significant interest to further examine the cell types and mechanisms driving the increase in HIV production in response to P. falciparum stimulation.

Memory CD4+ T-cells have been an active area of inquiry in the malaria field. Some have proposed that memory responses to malaria are very short lived and that malaria specific CD4+ T-cells are deleted, resulting in very little memory against future infection [26,27]. Others have proposed that the apoptosis of antigen-specific CD4 cells would be expected following resolution of acute infection [28]. Animal models of malaria have shown that fully functional memory CD4+ T-cells are maintained for prolonged periods of time [29]. Recently, Wipasa et al. showed that malaria specific CD4+ effector memory responses decay with a half-life of about 3 years and that malaria specific CD4+ central memory responses are maintained for at least six years after the last documented clinical episode of malaria in humans [30]. If the enhanced production of HIV-1 observed in our system is truly attributable to the development of adaptive immune responses to P. falciparum antigens, our results suggest that memory CD4+ T-cells also develop in association with subclinical malarial infection and that they persist for at least 90 days post-exposure.

Our study examined for the first time HIV-1 replication (in vitro) induced by exposure to P. falciparum using samples derived from human volunteers experimentally infected with malaria. As others have demonstrated, malarial antigens increase HIV-1 replication in vitro prior to experience with the parasite in vivo. We have demonstrated that this increase is further enhanced following an experimental infection with P. falciparum. These findings provide experimental support to the clinical observations that plasma levels of HIV-1 RNA rise during clinical bouts of malaria [3–5]. Although our study examined an initial exposure to P. falciparum, persons living in regions endemic for P. falciparum undergo repeated exposures to the parasite and serial bouts of clinical illness. In the course of these repeated infections, P. falciparum-specific CD4+ T-cells develop and play a critical role in ameliorating morbidity and mortality [30–32]. In the setting of HIV-1 infection it might be expected that pathogen specific CD4+ cells would be activated by malarial infection and, thus, would be likely to be especially vulnerable to HIV-1 infection. Such an
interaction would be expected both to erode malaria-specific immunity and to contribute to enhanced replication of HIV-1 as has been demonstrated both in the case of HIV-1 and Mycobacterium tuberculosis-specific immunity [33]. This unfortunate immunopathogenic interaction would then form, at least in part, the basis for a progressively more deleterious bidirectional clinical interaction between these two pathogens with advancing HIV-1-associated immunodeficiency.

Further studies are needed to more fully examine the interactions between HIV-1 and *P. falciparum* infection both in the clinic and in *in vitro* model systems. We believe that our studies lend further support to the emerging evidence that these pathogens are not indifferent to each other in areas where both pathogens are endemic. This experimental culture system provides a convenient platform in which to more carefully examine the bidirectional interactions between these pathogens both in patients with recurrent bouts of clinical malaria and in those with a subclinical infection.

**Materials and Methods**

**PBM C Collection and Isolation**

PBM Cs were isolated from human subjects enrolled in an experimental malaria challenge trial (protocol MC-001) at the Malaria Clinical Trials Center at Seattle Biomedical Research Institute (personal communication). The experimental infection of human subjects was conducted according to standard procedures as previously described [34]. Briefly, healthy malaria-naïve adult volunteers were infected with *Plasmodium falciparum* sporozoites from bites of five *P. falciparum* (strain NF54)-infected *A. stephensi* mosquitoes under controlled conditions. Volunteers were closely monitored in the post-challenge period and treated with standard doses of chloroquine phosphate (CQ) upon diagnosis of parasitemia by positive thick blood films. Blood sampling for isolation of PBM Cs occurred prior to challenge (baseline) and at regular intervals after challenge (Figure 1A) including day 5 (corresponding to the liver stage of parasite development), the day of the first positive blood smear (corresponding to the blood stage), and in the post-treatment period approximately 35, 56, and 90 days following mosquito bites. Cryopreserved PBM Cs were thus available prior to the challenge, during the liver and blood stage parasitemia, and at three time points during convalescence.

PBM Cs were isolated and cryopreserved according to standard methods and frozen within 8 hours of venipuncture to ensure optimal viability [33] under Good Clinical Laboratory Practices (GCLP). Fresh PBM Cs were isolated by Histopaque 1077 (Sigma) centrifugation and cultured overnight at 37°C in 5% CO2/90% N2 and incubated at 37°C. Parasite cultures were maintained continuously and split 1–2 days prior to setting up co-cultures, iRBCs were also placed into culture on the same day as rRBCs were split. Unwashed iRBCs were used once 6–7% of the RBCs in the culture were parasitized as assessed by light microscopy, *P. falciparum* stage was not synchronized. Cultures were routinely monitored for mycoplasma contamination by PCR (Takara) and shown to be mycoplasma-free.

**Cytokine Quantification**

Cytokine levels were measured in culture supernatants using a 6-plex BioPlex platform (BioRad). A 6-plex kit containing IL-10, IL-17, IL-6, TNF-α, IFN-γ, and MIP-1α was used according to the manufacturer’s protocol. Briefly, the antibody coupled beads were mixed with 50 μL of the collected supernatants, which were diluted 1:2 with media and incubated on a shaker for 30 minutes. After three washes, the detection antibodies were added and the plate was incubated on the shaker for 30 minutes. After another three washes, Streptavidin-PE was added to each well and plates were incubated on the shaker for 10 minutes. The beads were resuspended in 1% formaldehyde in assay buffer and after a 30 second shake the plate was read on a BioPlex200 (BioRad). An 8-point standard curve was used to determine cytokine concentrations using a 5 parameter logistic regression curve. Detection limits for cytokines are as follows: IL-4, 0.7 pg/mL; IL-6, 2.6 pg/mL; IL-10, 0.3 pg/mL; IL-17, 3.3 pg/mL; IFN-γ, 6.4 pg/mL; TNF-α, 6.0 pg/mL; MIP-1α, 1.6 pg/mL.

**CRP ELISA**

CRP Quantikine Kit (R&D) was used to quantify the CRP in the plasma of the participants. Briefly, plasma (diluted 1:100) was added to a 96 well plate pre-coated with anti-human CRP antibodies and incubated at room temperature for 2 hours. The plate was washed 4 times with wash buffer. A horseradish peroxidase-conjugated CRP antibody was added and incubated at room temperature for 2 hours. Followed by another 4 washes with wash buffer, color solutions A and B were added in equal amounts and were allowed to incubate at room temperature for 30 minutes at which point a stop solution was added. The plate was read at 450 nm and a plate correction was read at 540 nm. The log10 of the concentration for the standards was plotted against the log10 of the respective OD values and a regression line was obtained that
was used to calculate the CRP concentrations for the obtained OD values for the unknowns.

Flow Cytometry
PBMCs were collected, frozen, and then thawed as described above. PBMCs from day 56 malaria challenge and from malaria naïve donors were plated (2 × 10^5 PBMCs/well) in a 96 well plate and co-cultured with either iRBCs or uRBCs (2 × 10^6 RBCs/well) in triplicate. Cultures were incubated at 37°C, 95% O2 for 48, 72, or 96 hours. At the indicated time point, all three wells for a given condition were combined into a single FACS tube. Cells were washed once in FACS buffer (PBS + 2% FBS), resuspended in 50 μL of Live/dead – Aqua stain (Invitrogen) and stained with either CD3-FTTC (eBioscience), CD4-Pacific Blue (eBioscience), CD8-APC (BD Pharmingen), CD45RO-PE (BD Pharmingen), HLA-DR-PerCP (Biolegend), and CD35-PE-Cy5.5 (BD Pharmingen) or CD3-FTTC, CD4-Pacific Blue, CD8-APC, CD45RO-PE, CD69-PerCP-Cy7 (eBioscience), and CD25-PE-Cy5.5 (eBioscience). Cells were stained at room temperature for 20 minutes and washed twice with FACS buffer. The red blood cells were then lysed using 120 μL of BD FacsLyse at room temperature for 15 minutes, washed once with PBS and resuspended in 130 μL of 2% formaldehyde in distilled H_2O. Samples were subjected to flow cytometric analysis within 18 hours of fixing on the BD LSRII. All data were analyzed using FlowJo (Treestar).

Statistical Methods
Total HIV-1 p24Ag and cytokine production were calculated over the 10 days in culture using a trapezoidal method (area under the curve). Log-transformed HIV p24Ag and cytokine production were compared between the iRBC and uRBC groups at each time point following HIV infection of co-cultures to which iRBCs or uRBCs had been added, and between earlier and later time points following exposure, using a subject-specific random intercepts ANOVA model including time (as a factor), group (iRBC versus uRBC), their interaction, and a random subject effect. Therefore, different mean values were allowed at each time point and for each of the two groups for the log_{10} p24 AUC values, while correlation of data points measured for the same subject was modeled by a normally-distributed random intercept. The main comparisons of interest between the iRBC and uRBC groups were of the change normally-distributed random intercept. The main comparisons of data points measured for the same subject was modeled by a

Ethics Statement
Human studies undertaken at the Seattle Biomedical Research Institute were reviewed and approved by the Western Institutional Review Board. Studies undertaken at UCSD were reviewed and approved by the UCSD Human Research Protections Program.

References
1. Chandramohan D, Greenwood BM (1998) Is there an interaction between human immunodeficiency virus and Plasmodium falciparum? Int J Epidemiol 27: 296–301.
2. Kalyesubula I, Musoke-Mudido P, Marum L, Bagenda D, Aceng E, et al. (1997) Effects of malaria infection in human immunodeficiency virus type-1-infected Ugandan children. Pediatr Infect Dis J 16: 676–811.
3. Kapiga SH, Sam NE, Shao JF, Requien B, Musenga EJ, et al. (2002) HIV-1 epidemic among female bar and hotel workers in northern Tanzania: risk factors and opportunities for prevention. J Acquir Immune Defic Syndr 29: 409–417.
4. Mwapasa V, Rogersson SJ, Molyneux ME, Abrams ET, Kamwendo DD, et al. (2004) The effect of Plasmodium falciparum malaria on peripheral and placental HIV-1 RNA concentrations in pregnant Malawian women. AIDS 18: 1051–1059.
5. Kühlin JG, Pataik P, Jere CS, Miller WC, Hoffman IF, et al. (2005) Effect of Plasmodium falciparum malaria on concentration of HIV-1-RNA in the blood of adults in rural Malawi: a prospective cohort study. Lancet 365: 233–240.
6. Mermin J, Lule JR, Ekwaru JP (2006) Association between malaria and CD4 cell count decline among persons with HIV. J Acquir Immune Defic Syndr 41: 129–130.
7. Grimwade K, French N, Mbatha DD, Zungu DD, Dedicoat M, et al. (2004) P. falciparum Enhances HIV Replication Study participants provided written informed consent prior to their participation in the study.

Supporting Information
Figure S1 Activation of memory and total CD4/8 T-cells by iRBCs in D56 post malaria challenge PBMCs. PBMCs were cultured with iRBCs or uRBCs (without HIV) for 48, 72 or 96 hours. Cells were stained with either CD3-FTTC, CD4-Pacific Blue, CD8-APC, CD45RO-PE, HLA-DR-PerCP, and CD35-PE-Cy5.5 or CD3-FTTC, CD4-Pacific Blue, CD8-APC, CD45RO-PE, CD69-PerCP-Cy7, and CD25-PE-Cy5.5 and acquired using an LSRII. The percent of cells activated by iRBCs was normalized to the amount of activation due to uRBCs; this value was then logged and plotted. Line and error bars represent the mean and standard error of the mean. All values above the dotted line represent stimulation due to iRBCs. p-values were determined using an unpaired, two-tailed T-test. A. CD4 cells from the D56 post-malaria exposure PBMCs only show increased expression of CD25 (p = 0.018) and CD69 (p = 0.027) at 72 hours and there are no obvious trends toward increased activation in the malaria exposed PBMCs. B. While expression of CD69 at 48 hours (p = 0.008), CD38 (p = 0.011), CD25 (p = 0.046), and CD69 (p = 0.034) at 72 hours and CD38 (p = 0.003) at 96 hours are significantly increased in CD4+CD45RO+ D56 post malaria challenge PBMCs compared to naïve controls at 72 hours, there is also a trend toward increased activation in the HLA-DR/CD38 double positive cells in the PBMCs from malaria exposed donors compared to naïve controls at both 72 and 96 hours post co-culture in the memory CD4 compartment. C – D. For the total and memory CD8 T-cells, there is increased activation in the malaria exposed Day 56 PBMCs at 72 and 96 hours compared to naïve controls. For total CD8 cells at 72 hours, there is increased expression of CD38 (p = 0.016) and CD69 (p = 0.001); at 96 hours, there is increased expression of HLA-DR/CD38 double positives (p = 0.025) and CD38 alone (p = 0.015). For memory CD8 cells at 72 hours, there is increased expression of CD38 (0.036) and CD69 (0.016); at 96 hours, there is increased expression of CD38 alone (p = 0.0003); (TIF)

Acknowledgments
We would like to thank all the participants of the malaria challenge trial, the clinical staff at the MCTC, and the malaria naïve blood donors. We would also like to thank Kathryn Williamson for culturing all parasites used for the in vitro studies.

Author Contributions
Conceived and designed the experiments: MO FV OCF DMS AKT RW SHK RTS PED. Performed the experiments: MO FV QD RTS PED. Analyzed the data: MO QD RTS PED. Contributed reagents/materials/analysis tools: DMS RW SHK RTS PED. Wrote the paper: MO RTS PED. Critical edits: FV OCF DMS AKT RW SHK RTS PED.
8. Abu-Raddad LJ, Pannai P, Kublin JG (2006) Dual infection with HIV and malaria fuels the spread of both diseases in sub-Saharan Africa. Science 314: 1603–1606.

9. Cuadros DF, Bramcum AJ, Crowley PH (2011) HIV-malaria co-infection: effects on malaria on the prevalence of HIV in East sub-Saharan Africa. Int J Epidemiol 40: 931–9.

10. Walther M, Woodruff J, Edele J, Jeffries D, Tongren JE, et al. (2006) Innate immune responses to human malaria: heterogeneous cytokine responses to blood-stage Plasmodium falciparum correlate with parasitological and clinical outcomes. J Immunol 177: 5736–5745.

11. Pantaleo G, Fauci AS (1996) Immunopathogenesis of HIV infection. Annu Rev Microbiol 50: 825–854.

12. Chulay JD, Schneider I, Couglin TM, Hoffman SL, Ballou WR, et al. (1986) Malaria transmitted to humans by mosquitoes infected from cultured Plasmodium falciparum. Am J Trop Med Hyg 35: 66–68.

13. Hoffman SL (1997) Experimental challenge of volunteers with malaria. Ann Intern Med 127: 233-235.

14. Epstein JE, Rao S, Williams F, Freilich D, Luke T, et al. (2007) Safety and clinical outcome of experimental challenge of human volunteers with Plasmodium falciparum-infected mosquitoes: an update. J Infect Dis 196: 145–154.

15. Angulo I, Frenos M (2002) Cytokines in the pathogenesis of and protection against malaria. Clin Diagn Lab Immunol 9: 1145–1152.

16. Murray SM, Down CM, Boulware DR, Stauffer WM, Cavert WP, et al. (2010) Reduction of immune activation with chloroquine therapy during chronic HIV infection. J Virol 84: 12062–12066.

17. Savartino A, Bochart JR, Cassone A, Majari G, Cauda R (2003) Effects of chloroquine on viral infections: an old drug against today’s diseases? Lancet Infect Dis 3: 722–727.

18. McCall MB, Roestenberg M, Ploemen I, Teirlinck A, Hopman J, et al. (2010) Memory-like IFN-gamma response by NK cells following malaria infection reveals the crucial role of T cells in NK cell activation by P. falciparum. Eur J Immunol 40: 3472–3477.

19. Artavanis-Tsakonas K, Riley EM (2002) Innate immune response to malaria: rapid induction of IFN-gamma from iNKT cells by live Plasmodium falciparum-infected erythrocytes. J Immunol 169: 2956–2963.

20. Sinha S, Mishra SK, Sharma S, Patibandla PK, Mallick PK, et al. (2008) Significant association between TNF-alpha (TNF) promoter allele (−1051C, −863C, and −857G) and cerebral malaria in Thailand. Tissue Antigens 69: 277–290.

21. Hananantachai H, Patapatikul J, Ohashi J, Naka I, Kudsood S, et al. (2007) Protection against a malaria challenge by sporozoite inoculation. N Engl J Med 361: 468–477.

22. Stephens R, Albano FR, Quin S, Pascal BJ, Harrison V, et al. (2005) Malaria-specific transgenic CD4+ T cells protect immunodeficient mice from lethal infection and demonstrate requirement for a protective threshold of antibody production for parasite clearance. Blood 106: 1676–1684.

23. Riddell LA, Pinching AJ, Hill S, Ng TT, Arbe E, et al. (2001) A phase III study of recombinant human interferon gamma to prevent opportunistic infections in advanced HIV disease. AIDS Res Hum Retroviruses 17: 789–797.

24. Xiao L, Owen SM, Rudolph DL, Lal RB, Lal AA (1998) Plasmodium falciparum antigen-induced human immunodeficiency virus type 1 replication is mediated through induction of tumor necrosis factor-alpha. J Infect Dis 177: 447–445.

25. Urban BC, Roberts DJ (2005) Inhibition of T cell function during malaria: implications for immunology and vaccineology. J Exp Med 197: 137–141.

26. Xu H, Wipasa J, Yan H, Zeng M, Makenyong MO, et al. (2002) The mechanism and significance of deletion of parasite-specific CD4+ T cells in malaria infection. J Exp Med 195: 881–892.

27. Struijks SS, Riley EM (2004) Does malaria suffer from lack of memory? Immunol Rev 201: 268–290.

28. Stephers R, Albano FR, Quin S, Basil CJ, Harrison V, et al. (2005) Malaria-specific transgenic CD4+ T cells protect immunodeficient mice from lethal infection and demonstrate requirement for a protective threshold of antibody production for parasite clearance. Blood 106: 1676–1684.

29. Xu H, Wipasa J, Yan H, Zeng M, Makenyong MO, et al. (2002) The mechanism and significance of deletion of parasite-specific CD4+ T cells in malaria infection. J Exp Med 195: 881–892.

30. Wipasa J, Okell L, Sakkhachornphop S, Suphavilai C, Chawansuntati K, et al. (2011) Short-lived IFN-gamma effector responses, but long-lived IL-10 memory responses, to malaria in an area of low malaria endemicity. PLoS Pathog 7: e1001281.

31. Roestenberg M, McCall M, Hopman J, Wiersma J, Luty AJ, et al. (2009) Protection against a malaria challenge by sporozoite inoculation. N Engl J Med 361: 468–477.

32. Pombo DJ, Lawrence G, Hirunpetchcharit C, Razepczyk G, Bryden M, et al. (2002) Immunity to malaria after administration of ultra-low doses of red cells infected with Plasmodium falciparum. Lancet 360: 610–617.

33. Douek DC, Brenchley JM, Betts MR, Ambrozak DR, Hill BJ, et al. (2002) HIV preferentially infects HIV-specific CD4+ T cells. Nature 417: 95–98.

34. Cummings JF, Spring MD, Schwark RJ, Ockenhouse CF, Kester KE, et al. (2010) Recombinant Liver Stage Antigen-1 (LSA-1) formulated with AS01 or AS02 is safe, elicits high titer antibody and induces IFN-gamma/IL-2 CD4+ T cells but does not protect against experimental Plasmodium falciparum infection. Vaccine 28: 5135–5144.

35. Bull M, Lee D, Stucky J, Chiu YL, Rubin A, et al. (2007) Defining blood processing parameters for optimal detection of cryopreserved antigen-specific responses for HIV vaccine trials. J Immunol Methods 322: 57–69.