Antigen Presenting Cells Ingest Malaria Parasites and Cause an Increase in HIV-1 Replication

Marika Orlov,[1,2] Florin Vaida,[1] Qianqian Deng,[1] David M Smith,[1] Kathryn Williamson,[2] Patrick E Duffy,[2,3] and Robert T Schooley[1].

[1]University of California San Diego, La Jolla, CA, [2]Seattle Biomedical Research Institute, Seattle, WA and [3]NIAID, NIH, Rockville, MD

Abstract

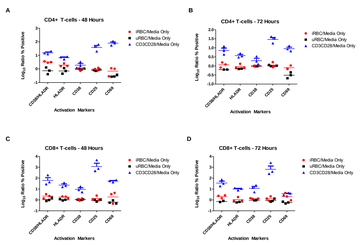
Background: An increasing body of evidence has demonstrated that bidirectional interactions between HIV-1 and *P. falciparum* enhance morbidity and mortality where both pathogens are endemic. Repeat bouts of malaria cause an increase in levels of HIV replication and accelerate CD4 cell decline. Increased levels of HIV-1 RNA persist well after parasites are cleared from peripheral blood following antimalarial therapy. The mechanisms contributing to this interaction have not been fully elucidated.

Methods: We developed a tissue culture system in which peripheral blood mononuclear cells (PBMCs) from healthy donors were infected with HIV *in vitro*, and co-cultured with *P. falciparum* infected (iRBCs) or uninfected (uRBCs) red blood cells. HIV-1 production was assessed by quantifying HIV-1 p24 antigen by ELISA in culture supernatants 4, 6, 8 and 10 days after initiation of the co-cultures. In certain experiments T-cell subsets were affinity purified by depletion with antibody-coated magnetic beads. T-cell surface activation markers were assessed by flow cytometry after 24 or 48 hours. Cytokine concentrations were quantified in the supernatants using a multiplex ELISA.

Results: HIV replication increased in the presence of iRBCs compared to uRBCs (area under the curve comparisons 20.6 vs 18.2 respectively, p=0.0045) and was associated with increased CD4 activation as assessed by CD4 HLA-DR/CD38 double positive cells at 48 hours (p=0.001). Supernatants from these co-cultures showed increased production of TNF-α, INF-γ, MIP-1α, but not IL-6. Neutralizing antibodies to TNF-α but not IFN-γ decreased HIV production when TNF-α was neutralized (p=0.04) but not IFN-γ (p=0.37). Depletion of macrophages and dendritic cells reduced HIV production (p=0.0074). In one experiment, macrophages and dendritic cells that had been pre-incubated with iRBCs for 48 hours stimulated HIV production from PBMCs without the addition of iRBCs to the co-cultures. Hemozoin crystals were observed within the dendritic cells after co-cultivation with iRBCs.

Conclusions: iRBCs stimulate replication of HIV-1 by-activated CD4 cells in a TNF-α dependent manner following processing of malarial antigens by macrophages and dendritic cells. These results suggest that the persistent elevation of HIV-1 replication following treatment for acute bouts of *P. falciparum* malaria may be due, at least in part, to ongoing stimulation of CD4+ T cells by hemozoin loaded macrophages within lymphoid tissues.

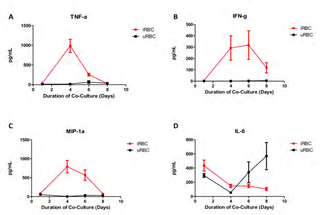
iRBCs activate CD4+ T-cells, but not CD8+ T-cells, in co-cultures



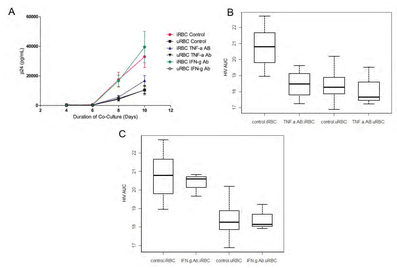
PBMCS were cultured with iRBCs or uRBCs (without HIV) for 48 or 72 hours. Cells were stained with either CD3-FITC, CD4-Pacific Blue, CD8-APC, HLA-DR-PerCP, and CD38-PE-Cy5.5 OR CD3-FITC, CD4-Pacific Blue, CD8-APC, CD69-PerCP-Cy7, and CD25-PE-Cy5.5 and acquired using an LSRII. A. iRBCs increased activation of CD4+ T-cells (CD38/HLA-DR double positive cells) at 48 hours compared to uRBCs, but less than that due to CD3/CD28 antibodies. B. iRBC induced activation decreased by 72 hours. C. and D. iRBCs did not significantly activate CD8+ T-cells when compared to uRBCs, but there was significant activation of CD8+ T-cells due to CD3/CD28 antibodies.

iRBCs Stimulate TNF-α, IFN-γ and MIP-1α production

Cytokine production in the iRBC (red lines) and uRBC (black lines) was measured at days 1, 4, 6, and 8 after initiation of co-cultures. Each point represents the average cytokine secretion from 5 donors; the error bars represent the standard error of the mean. iRBCs stimulated increased secretion of pro-inflammatory cytokines TNF-α (A), IFN-γ (B), and MIP-1α (C) and peak levels were reached at days 4-6. D. Levels of IL-6, another pro-inflammatory cytokine, were not increased in the iRBC co-cultures compared to uRBC co-cultures.

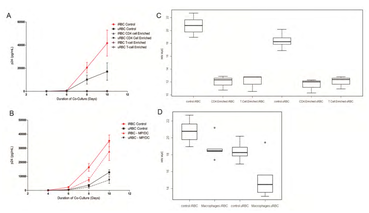


TNF-α, but not IFN-γ, neutralizing antibodies inhibit HIV production in co-cultures



Co-cultures were set up in the presence of either a TNF-α neutralizing antibody (500ng/mL) or an IFN-γ neutralizing antibody (300ng/mL). p24 was measured in the supernatants at days 4, 6, 8, and 10 post-initiation of co-cultures. A. HIV p24 levels increase more rapidly in the presence of iRBC, and this is reversed by TNF-α- but not IFN-γ-neutralizing antibody. The points are the average of all donors and the error bars represent the standard error of the mean. B. HIV production decreases in the presence of the TNF-α neutralizing antibody (p<0.001). C. HIV production is unchanged in the presence of the IFN-γ neutralizing antibody (p=0.53).

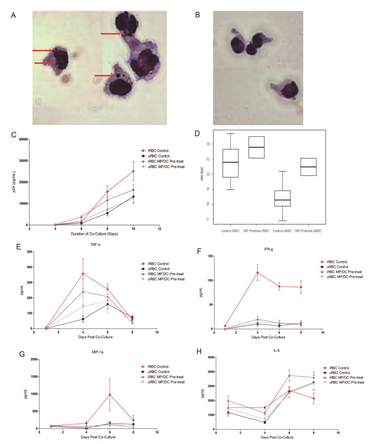
Phagocytic, antigen presenting cells are necessary for increased HIV production in co-cultures



Enrichment kits were used to isolate either only CD3+ T-cells (99.42% CD3 cells) or only CD4+ T-cells (98.47% CD4 cells). For macrophage and dendritic cell depletions, cells that did not attach to the plastic were collected and used for experiment (36-44% reduction in CD11c+/CD14+ double positive cells). A. There was minimal HIV production in co-cultures that were set up with only CD4+ T-cells (blue: iRBC; black triangle: uRBC) or only CD3+ T-cells (purple: iRBC; black circle: uRBC) compared to whole, unfractionated PBMCs (red: iRBC; black square: uRBC). B. There was reduced HIV production in macrophage/dendritic cell depleted co-cultures (dashed red line: iRBC; dashed black line: uRBC) compared to whole, unfractionated PBMCs (solid red line: iRBC; solid black line: uRBC). C-D. For maximal HIV production in the co-cultures, macrophages and dendritic cells need to be present (p<0.001), and neither CD4+ T-cells nor CD3+ T-cells were sufficient on their own to induce much HIV production.

Macrophages and dendritic cells pre-treated with iRBCs are sufficient for inducing HIV production

A. Giemsa smears of macrophages and dendritic cells that have been co-cultured with iRBCs for 48 hours. Arrows point to hemozoin deposits in the cytoplasm of the leukocytes. B. Giemsa smears of macrophages and dendritic cells that have been in co-culture with uRBCs for 48 hours. No evidence of hemozoin deposits in all cells visualized. C-D. HIV production was similar in control co-cultures (PBMCs co-cultured with iRBC, red; or uRBCs, black) and MP/DC pre-treated co-cultures (PBMCs co-cultured with iRBC pre-treated MP/DC, purple; or uRBC pre-treated MP/DC, grey). E-H. Cytokine secretion in the control (iRBC, red lines; and uRBC, black lines) and MP/DC pre-treated (iRBC, purple lines; and uRBC grey lines) co-culture supernatants. TNF-α is the only cytokine whose secretion increased in the presence of iRBC pre-treated macrophages and dendritic cells. Hemozoin loaded macrophages and dendritic cells are sufficient to cause an increase in HIV production, likely via TNF-α, in the absence of iRBCs.

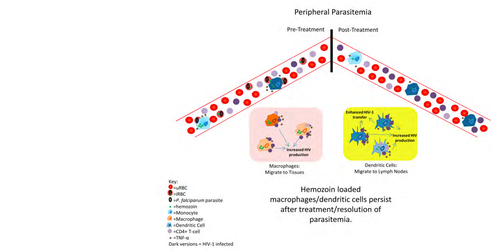


HIV/*P. falciparum* Interactions – Summary

	Control			Culture Condition			Interaction
Condition	p24 Ratio (CI)	ES (CI)	P	p24 Ratio (CI)	ES (CI)	P	P
CD3+ T-cells Only	1.7 (1.5, 1.9)	2.4 (1.8, 2.9)	<0.001	1.0 (0.7, 1.4)	0 (-1.3, 1.3)	1	0.0028
CD4+ T-cells Only	1.7 (1.5, 1.9)	2.4 (1.8, 2.9)	<0.001	1.1 (0.8, 1.5)	0.4 (-0.8, 1.7)	0.47	0.0092
Macrophage Depleted	1.7 (1.5, 2.0)	1.8 (1.3, 2.4)	<0.001	2.3 (1.7, 3.0)	2.9 (1.9, 3.8)	<0.001	0.067
Neutralized TNF-α	1.7 (1.5, 1.9)	2.3 (1.9, 2.8)	<0.001	1.1 (0.9, 1.4)	0.4 (-0.5, 1.4)	0.34	0.0013
Neutralized IFN-γ	1.7 (1.5, 1.9)	2.4 (1.9, 2.9)	<0.001	1.6 (1.2, 2.0)	2 (0.8, 3.2)	0.002	0.53
Macrophage Pre-treat	1.7 (1.5, 1.9)	2.3 (1.9, 2.8)	<0.001	1.4 (1.0, 1.8)	1.3 (0.0, 3.2)	0.046	0.14

	Malaria Parasites Present (iRBC)			Malaria Parasites Absent (uRBC)			Interaction
Condition	p24 Ratio (CI)	ES (CI)	p	p24 Ratio (CI)	ES (CI)	p	P
CD3+ T-cells Only	0.1 (0.1, 0.2)	-8.7 (-9.8, -7.8)	<0.001	0.2 (0.2, 0.3)	-6.4 (-7.5, -5.3)	<0.001	0.0028
CD4+ T-cells Only	0.1 (0.1, 0.2)	-8.9 (-10.0, -7.6)	<0.001	0.2 (0.2, 0.3)	-6.9 (-8, -5.8)	<0.001	0.0092
Macrophage Depleted	0.6 (0.5, 0.8)	-1.5 (-2.4, -0.7)	0.001	0.5 (0.4, 0.6)	-2.6 (-3.4, -1.7)	<0.001	0.067
Neutralized TNF-α	0.6 (0.5, 0.8)	-2 (-2.8, -1.2)	<0.001	1.0 (0.8, 1.2)	-0.1 (-0.9, 0.7)	0.79	0.0013
Neutralized IFN-γ	1.0 (0.8, 1.3)	0 (-1, 1)	0.98	1.1 (0.9, 1.4)	0.4 (-0.6, 1.4)	0.44	0.53
Macrophage Pre-treat	1.1 (0.9, 1.5)	0.5 (-0.6, 1.6)	0.33	1.4 (1.1, 1.8)	1.5 (0.4, 2.7)	0.0095	0.14

Proposed mechanism for HIV-1/*Plasmodium falciparum* interactions



Hemozoin loaded macrophages/dendritic cells persist after treatment/resolution of parasitemia.

Whole iRBCs and debris from ruptured iRBCs is ingested by monocytes in the peripheral blood. Monocytes are activated as hemozoin crystals accumulate in their cytoplasm and as malaria GPI fragments and malaria DNA fragments containing CpG motifs are ingested, causing a release of TNF-α. Once monocytes leave the blood stream, they differentiate into either macrophages or dendritic cells and migrate either to tissues or lymph nodes, respectively. HIV-infected dendritic cells aid in the transfer of HIV to uninfected CD4+ T-cells and both macrophages and dendritic cells cause an increase in CD4+ T-cell activation, which causes an increase in HIV production. Even after the malaria episode has resolved, hemozoin crystals persist in the macrophages and presumably dendritic cells and continue to drive the increase in HIV production.

Conclusions and Acknowledgments

Conclusions:

- *Plasmodium falciparum* infected RBCs increase CD4+ T-cell activation and stimulate HIV production in PBMCs from malaria naïve donors.
- Neutralization of TNF-α, but not IFN-γ, abrogates malaria's effects on HIV replication.
- Macrophages and dendritic cells need to be present in the co-cultures for iRBCs to have maximal effect on enhancement of HIV production
- Macrophages and dendritic cells that have been pre-treated with iRBCs are alone sufficient to drive increased HIV production.
- We hypothesize that iRBCs stimulate replication of HIV-1 by activated CD4 cells in a TNF-α dependent manner following processing of malarial antigens by macrophages and dendritic cells.
- Persistent elevation of HIV-1 replication following acute bouts of *P. falciparum* malaria may be due, at least in part, to ongoing stimulation of CD4+ T cells by hemozoin loaded macrophages within lymphoid tissues.

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