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*J Immunol* 2010; 184:2899-2907; Prepublished online 10 February 2010; doi: 10.4049/jimmunol.0901513

http://www.jimmunol.org/content/184/6/2899
Dendritic Cells Derived from Hemozoin-Loaded Monocytes Display a Partial Maturation Phenotype that Promotes HIV-1 Trans-Infection of CD4+ T Cells and Virus Replication

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Coinfection of HIV-1 patients with Plasmodium falciparum, the etiological agent of malaria, results in a raise of viral load and an acceleration of disease progression. The primary objective of this study was to investigate whether the malarial pigment hemozoin (HZ), a heme by-product of hemoglobin digestion by malaria parasites, can affect HIV-1 transmission by monocytes-derived dendritic cells (DCs) to CD4+ T cells when HZ is initially internalized in monocytes before their differentiation in DCs. We demonstrate in this study that HZ treatment during the differentiation process induces an intermediate maturation phenotype when compared with immature and fully mature DCs. Furthermore, the DC-mediated transfer of HIV-1 is enhanced in presence of HZ, a phenomenon that may be linked with the capacity of HZ-loaded cells to interact and activate CD4+ T cells. Altogether our findings suggest a new mechanism that could partially explain the increased HIV-1 virus production during a coinfection with P. falciparum. Understanding the multifaceted interactions between P. falciparum and HIV-1 is an important challenge that could lead to the development of new treatment strategies.


Together, malaria and AIDS cause more than 4 million deaths a year. By the end of 2007, an estimated 33.2 million people were living with HIV-1 that is considered as the most common cause of AIDS (1). Plasmodium falciparum, the infectious agent of malaria, is thought to be responsible for 1.5-2.7 million deaths and 350-500 million acute illnesses annually (2, 3).

P. falciparum proliferation within the host’s erythrocytes results in a variety of clinical manifestations like unarousable coma, severe anemia, renal failure, jaundice, and many more (4). All the symptoms cited in this study were indirectly or directly associated with persistent presence of malaria pigments within erythrocytes and leukocytes (e.g., monocytes and macrophages). Indeed, to properly infect, the parasite needs to grow by ingesting amino acids found in the host’s hemoglobin (5). This process leads to hemoglobin degradation and the formation of a by-product that is highly toxic to the parasite, namely, free heme. The principal and essential first step in detoxification of heme is its incorporation into an intracellular crystal called malarial pigment or hemozoin (HZ) (6). As the parasite proliferates and induces erythrocyte cell burst, HZ is ingested by phagocytes present in the blood circulation to finally accumulate in tissues (e.g., liver, bone marrow, and spleen). In fact, in a study made by Sullivan and coworkers, malaria pigments were found to weigh as much as 0.2% of the liver total weight after only 18 d of infection (7). At this point, HZ was suggested to be a maker for chronicity of malaria infection.

Monocytes and neutrophils being resilient phagocytes are recruited to the area of the parasitic infection. Consequently, monocytes will phagocyte free HZ and/or HZ-loaded RBCs. As much as 79% of HZ was found in monocytes, representing ~30% of the total cell weight (8). Monocytes are essential for replenishing resident macrophages and dendritic cells (DCs) under normal states, and generating an immune response. Actually, in response to inflammation signals, monocytes can move quickly to sites of infection and differentiate into macrophages and immature DCs (iDCs). On Ag uptake and inflammatory stimuli, iDCs start to migrate to the peripheral lymph nodes where they mature and acquire molecules rendering them potent APCs proficient to stimulate naïve T lymphocytes (9). In this regard, the best known maturation markers on the surface of mature DCs (mDCs) remain CD83 in addition to CD80 and CD86 that act as costimulatory molecules for complete T cell activation.

AIDS has been described as a condition in which the immune system begins to fail, leading to life-threatening opportunistic infections. HIV-1 primarily infects vital cells in the human immune system, such as Th cells (better known as CD4+ T cells), macrophages and DCs. It is known that iDCs are part of the first cells encountered by the virus during sexual transmission. This cell subset plays a dominant role by transmitting HIV-1 to CD4+ T cells once the virus has been captured in the mucosa (10). Although both iDCs and mDCs can actively participate to HIV-1 transmission, the efficiency of virus propagation is augmented after maturation of DCs (11–13). Moreover, most studies have shown that iDCs are more susceptible to productive HIV-1 infection than mDCs (14, 15). HIV-1 is efficiently transferred from DCs to CD4+ T cells via a process involving two distinct phases (11–13, 16–19). Transport of viruses located on the cell surface and/or within endosomal compartments through a supramolecular structure termed a virological synapse is called the initial transfer phase (i.e., early...
transfer. This event is followed by a second kinetic phase (i.e., late transfer) dependent on productive virus infection of iDCs and eventual transfer of progeny virus to surrounding CD4⁺ T cells.

In endemic regions where *P. falciparum* and HIV-1 infections overlap, contamination with other opportunistic pathogens and chronic latent malaria were hypothesized to be responsible for the increased evolution of HIV-1 infection toward AIDS (20). In an attempt to clarify the putative modulatory effect of malaria with respect to HIV-1 dissemination, we assessed whether treatment of monocytes before their eventual maturation in DCs with the malarial pigment HZ can modulate the DC-mediated virus capture, infection, and transfer processes.

Materials and Methods

Reagents

Recombinant human IL-2 (rHL-2) was obtained from the AIDS Repository Reagent Program (Germantown, MD). LPS was purchased from Sigma-Aldrich (St. Louis, MO). IL-4 and IFN-γ were purchased from R&D Systems (Minneapolis, MN), whereas GM-CSF was a generous gift from Cangene (Winnipeg, Manitoba, Canada). The culture medium for monocyte-derived DCs consisted of RPMI 1640 supplemented with 10% FBS, penicillin G (100 U/ml), streptomycin (100 U/ml), primocine (Amaxa Biosystems, Gaithersburg, MD), and glutamine (2 mM).

Abs

FITC-tagged anti-DC-SIGN (clone eB-I290), R-PE-conjugated anti-HLA-DR (clone LN3), R-PE-tagged anti-CD1 (clone HIT3a), R-PE-tagged anti-CD19 (clone HB19), and FITC-tagged anti-CD83 (clone HB15e) were all purchased from eBioscience (San Diego, CA), whereas R-PE-tagged anti-CCR5 (clone 2D7) was obtained from BD Biosciences (Franklin Lakes, NJ). Anti-CD14 hybridomas (clones 183-H12-5C and 31-90-25) were obtained from the AIDS Repository Reagent Program and the American Type Culture Collection (Manassas, VA), respectively. These Abs were purified by using MAITrap protein affinity columns according to the manufacturer’s instructions (Pharmacia Technology, Uppsala, Sweden).

Cells

Human embryonic kidney 293T cells were obtained from the American Type Culture Collection and cultured in DMEM supplemented with 10% FBS. PBMCs from healthy donors were isolated by Ficol-Hypaque gradient and plated in 75 cm² flasks (1 x 10⁶ cells) for 2 h to separate, by adherence to plastic, monocytes (CD14⁺) from the other nonadherent cells. To generate iDCs, monocytes were cultured in complete culture medium that was supplemented every other day with a mixture made of GM-CSF (1000 U/ml) and IL-4 (200 U/ml) during 6 d. The percentage of cells expressing the surface markers CD3 and CD19 was evaluated to assess contamination with T and B cells, respectively. Experiments were performed with cell preparations that contained a minimal amount of contaminants (i.e., <2% CD4⁺ T cells and <1% B lymphocytes) (data not shown). Autologous CD4⁺ T cells were isolated using a negative selection kit according to the manufacturer’s instructions (StemCell Technologies, Vancouver, British Columbia, Canada) and the Auto-MACS technology (Miltenyi Biotec, Auburn, CA). These cells were activated with PHA-L (1 μg/ml) and maintained in complete culture medium supplemented with rHL-2 (30 U/ml) at a density of 2 x 10⁶ cells/ml.

Plasmids and virus production

The pNL4-3Balenv is a full-length infectious molecular clone of HIV-1. This virus is a R5 (macrophage)-tropic strain where the NLA-3 env gene has been replaced with env gene from the R5-tropic Bal strain (kindly provided by R. Pomerantz, Thomas Jefferson University, Philadelphia, PA) (21). Viruses were produced by the calcium phosphate coprecipitation method in 293T cells as described previously (22). The virus-containing supernatants were harvested at day 2 after transfection, filtered through a 0.22-μm cellulose acetate syringe filter, ultracentrifugated, and normalized for virion content by using an inhouse enzymatic assay specific for the major viral p24 protein. In this test, 183-H12-5C and 31-90-25 are used in combination to quantify p24 levels (23).

HZ production

Synthetic HZ (shZ) was produced using a previously described procedure with slight modifications (24). In brief, 90 mg heme chloride (Sigma-Aldrich) was solubilized in DMSO (polymerization solvent) and added to a 4-M acetate solution at pH 5.0. The suspension was stirred with a magnet for 6 h at 65°C. After adding a volume of 10% SDS, the suspension was centrifuged at 12,500 g for 25 min. The pellet was then sonicated twice at the lowest setting in 100 mM sodium bicarbonate (pH 9.0) supplemented with 0.5% SDS and centrifuged again. The pellet was then washed three times in 2% SDS, at least five times in sterile H₂O, and once in endotoxin-free PBS to wash out residual SDS. The final pellet was resuspended in endotoxin-free PBS and aliquotated to obtain a solution of 652 μg/ml shZ. The total amount of HZ was determined as described by Sullivan and coworkers (7). In brief, polymerized heme was incubated for 1 h in 2% SDS/20 mM NaOH to solubilize polymer into monomeric heme, which has a molar extinction coefficient at 400 nm of 1×10⁵.

Acute HIV-1 infection

Monocytes were plated at a concentration of 1 x 10⁶ cells in a final volume of 100 μl in 96-well plates. Cells were either treated with GM-CSF/IL-4 (called Control) or both GM-CSF/IL-4 and shZ (called shZ). Next, cells were infected with a fixed amount of virus (i.e., 5 ng p24 per 5 x 10⁵ cells). Every 3 d and for a period lasting 12 d, half of the medium was removed and kept frozen at −20°C until assayed. Virus production was estimated by measuring p24 levels in culture supernatants by ELISA.

HIV-1 transfer assay

Monocytes were treated with a mixture consisting of GM-CSF and IL-4 and either left untreated or treated with shZ or a mixture made of LPS (10 μg/ml) and IFN-γ (1000 U/ml) for 6 d. Treatment of iDCs (i.e., monocytes treated with GM-CSF and IL-4) with LPS and IFN-γ is a well-known method to induce full maturation of DCs (16, 25) and was used in this study as a positive control. Next, cells were pulsed for 1 h with NL4-3Balenv (10 ng p24). The virus-cell mixture was washed three times with PBS to remove unadsorbed virions and cocultured with autologous CD4⁺ T cells at a 1:3 ratio in complete RPMI 1640 medium supplemented with rHL-2 (30 U/ml) in 96-well plates in a final volume of 200 μl. Virus production was estimated by measuring p24 levels in cell-free culture supernatants every 2 d for a period of 6 d. In some studies, monocytes differentiated in the same conditions mentioned previously, were cocultured with activated autologous CD4⁺ T cells that have been infected first for 2 h with NL4-3Balenv and extensively washed.

Flow cytometry analysis

Before staining, cells were incubated for 15 min at 4°C with 10% pooled human sera to block nonspecific binding sites and washed once with PBS supplemented with 0.5% BSA. To monitor cell surface expression of CD83, DC-SIGN, HLA-DR, and CCR7 on monocytes and DCs, samples from various healthy donors were prepared and incubated with specific Abs or appropriate isotype-matched irrelevant control Abs (for nonspecific staining) for 30 min at 4°C. Cells were then washed with PBS/0.5% BSA and incubated with an appropriate R-PE- or FITC-conjugated goat anti-mouse IgG for 30 min at 4°C. After two washes with PBS/0.5% BSA, cells were fixed in 2% paraformaldehyde and analyzed by FACS (Epics ELITE ESP, Coulter Electronics, Burlington, Ontario, Canada).

Real-time RT-PCR

RNA was purified using standard Trizol extraction and isopropanol precipitation. cDNA was synthesized from RNA (1 μg) using random primers and superscript reverse transcriptase. Thereafter, 8 μl 10- to 100-fold diluted cDNA was used for real-time PCR analysis using SYBRgreen mastermix (Applied Biosystems, Foster City, CA) and 0.5 μM specific primers in a Rotor-Gene RG-3000 apparatus (Corbett Research, Sydney, Australia). Primers used were as follows: 5'-GGTGCCTCCTGGTCAAGTTA-3' and 5'-TGTGTTTCG-3 for CD83, 5'-TCCAGGTATGCCGGCTTTAAAGTTCCGCACGTC-3' and 5'-TCCAGGTATGCCGGCTTTAAAGTTCCGCACGTC-3' for CCR7, and 5'-TAGAAGGACAAGTGGCCGTCT-3' and 5'-CGCTGACCGACGTACGGTACGTC-3' for 18S. Standard curves for relative quantification consisted of a mix of all analyzed cDNA, submitted to consecutive 2-fold dilutions. Each PCR reaction was performed in triplicate, and 18S levels were used to standardize cDNA quantities.

Statistical analysis

Results presented are expressed as means ± SEM of at least triplicate samples. All experiments were repeated at least three times and each figure combines the results obtained with all the different donors unless otherwise mentioned. Because CCR5 expression levels vary largely among various DC preparations, a repeated measures ANOVA test had to be used. Statistical significance between groups was determined by ANOVA.
Calculations were made with Prism version 3.03. The p values <0.05 were considered statistically significant. Mostly, statistical significance of the results was defined by performing a one-way ANOVA with Bonferroni post tests to compare treated and control samples or with Bonferroni post tests to compare all pairs of columns.

**Results**

DCs acquire an intermediate maturation profile on treatment with shZ and GM-CSF/IL-4

In *P. falciparum*-infected individuals, an important level of circulating monocytes is loaded with HZ. On infection or inflammation, these cells migrate to peripheral tissues to differentiate into macrophages or DCs. To mimic these events, we designed an experimental model system that is illustrated in Fig. 1. Because circulating DCs represent <1% of the total cell population in human peripheral blood, we used monocytes known to be a usual progenitor for the in vitro generation of iDCs when cultured with a cytokine mixture made of GM-CSF and IL-4 (26–29). For the purpose of our study, monocytes were separated from PBMCs by cell adherence as described previously (30, 31). As expected, when cultured in vitro in the presence of GM-CSF and IL-4, monocytes acquire some specific surface markers and features of immature myeloid DCs (e.g., DC-SIGN and CD1a), while losing markers typical of monocytes and macrophages, such as CD14 (data not shown) (27, 32, 33). The first set of experiments was aimed at defining whether exposure to shZ can modulate monocyte differentiation into iDCs. To this end, all cell samples were treated with GM-CSF and IL-4 at day 0 to induce differentiation of monocytes into iDCs. In some samples, monocytes were treated also with a chosen amount of shZ (i.e., 10 μg/ml) at day 0. In addition, in some instances, monocytes that were left untreated with shZ but exposed to the GM-CSF/IL-4 combination were also treated at day 4 with LPS and IFN-γ to generate mDCs. We initially made comparative morphologic and phenotypic analyses for monocytes subjected to the various treatment groups [i.e., GM-CSF/IL-4 (used as positive control for iDCs) (called control), GM-CSF/IL-4 and LPS/IFN-γ (used as a positive control for mDCs) (called LPS/IFN-γ), and shZ and GM-CSF/IL-4 (called shZ)]. Microscopic studies revealed that monocytes cultured in the presence of shZ and GM-CSF/IL-4 exhibit an intermediate state of maturation (i.e., semimature DCs) when compared with both iDCs and mDCs (Fig. 2A). It is important to notice that most DCs derived from HZ-loaded monocytes contain the malarial pigment but at different levels. Furthermore, as expected, mDCs derived in presence of LPS/IFN-γ remained attached to the bottom of the well, whereas iDCs remained in suspension. To more closely visualize the impact of shZ on the maturation process of monocytes, we studied four different specific makers by flow cytometry (Fig. 2B). Indeed, we measured surface expression of CD83, which is expressed by mDCs (34), DC-SIGN, which is a calcium-dependent pattern-recognition lectin reported to be predominantly expressed on iDCs (35), HLA-DR, which has been reported to be upregulated on DC maturation (26), and finally CCR7, which is associated with maturation of DCs and promotes their migration from tissues to lymphoid organs (36). When compared with iDCs, LPS/IFN-γ-induced maturation of DCs is characterized by a significant increase in CD83 (442 ± 62.4 versus 146 ± 3.3), HLA-DR (1807 ± 6351.4 versus 913 ± 153.9), and CCR7 expression (67 ± 14.8 versus 5 ± 1.5) and a downregulation of DC-SIGN (198 ± 46.9 versus 838 ± 126.8). A less pronounced enhancement of CD83 (72 ± 11.4 versus 14 ± 3.3) and CCR7 expression (17 ± 4.3 versus 5 ± 1.5) was detected in monocytes treated with shZ and GM-CSF/IL-4. Similarly, a less impressive diminution of DC-SIGN was observed in shZ- and GM-CSF/IL-4-treated monocytes compared with iDCs (555 ± 103.3 versus 838 ± 126.8). On the other hand, HLA-DR was not increased.
in monocytes subjected to a treatment with sHZ and GM-CSF/IL-4, which is in agreement with previous findings (37, 38). Interestingly, in contrast to stimulation with GM-CSF/IL-4 and LPS/IFN-γ, a treatment with sHZ and GM-CSF/IL-4 did not increase CD80 and CD86 expression (data not shown), thus suggesting that a semi-
mature phenotype is seen in DCs loaded with sHZ. To shed light on the mechanism underlying the sHZ-induced increase in CD83 and CCR7, we monitored mRNA and protein expression levels for both molecules. As expected, we found that LPS/IFN-γ treatment induced a significant increase in CD83 and CCR7 expression at both mRNA and protein levels (Fig. 3A, 3B).

Interestingly, sHZ was also capable of inducing an enhanced expression of the two cell surface constituents again at the mRNA and proteins levels. A more significant augmentation of CD83 and CCR7 mRNA levels was seen at an earlier time point (i.e., 5 instead of 6 d after treatment with sHZ) (Fig. 3C).

**DC-mediated transfer of HIV-1 is promoted by sHZ**

Given that our previous observations suggest that the differentiation process of monocytes in DCs is affected by sHZ, we studied the possible impact of those phenotypic changes on HIV-1 transfer to autologous CD4+ T cells. For that purpose, monocytes treated with GM-CSF/IL-4 only (i.e., iDCs) and those treated with sHZ and GM-CSF/IL-4 (i.e., semimature DCs) were initially pulsed with fully competent R5-tropic virus (i.e., NL4-3Balenv) and next co-cultured with autologous CD4+ T cells. A statistically significant increase in HIV-1 production was seen in sHZ-treated cells at early time points after initiation of the coculture (i.e., 2 and 4 d) (Fig. 4).

The subsequent sets of experiments were aimed at defining the mechanism by which sHZ can augment the process of DC-mediated transmission of HIV-1. To do so, iDCs and sHZ-loaded semimature DCs were acutely infected with HIV-1 and the extent of virus production was monitored over 12 d by measuring the p24 content in cell-free supernatants. As illustrated in Fig. 5A, virus replication is significantly lower in cells subjected to a treatment with sHZ. Virus production was almost completely abrogated in fully mDCs (i.e., treated with GM-CSF/IL-4 and LPS/IFN-γ) (data not shown). Importantly, cell viability is not affected by the chosen concentration of sHZ as monitored by the fluorescent cytotoxic mouse thymicstroma assay (data not shown). The sHZ-dependent reduced susceptibility to productive virus infection is accompanied by a lower surface expression of CCR5 coreceptor (Fig. 5B).
Although additional studies indicated that HIV-1 replication remains unaffected in sHZ-treated CD4+ T cells when compared with untreated cells (data not shown), we also tested if virus replication in HIV-1–infected CD4+ T cells was altered on a coculture step with sHZ-loaded semimature DCs. To this end, autologous mitogen-stimulated CD4+ T cells were first infected with NL4-3Bal and subsequently cocultured with iDCs or sHZ-loaded partially mDCs (Fig. 6).

**Discussion**

During the course of active malaria infection with *P. falciparum*, hosts’ erythrocytes help the parasite to develop and proliferate by degrading hemoglobin as a source of amino acids. As the parasite is growing, free HZ as well as HZ-fed erythrocytes are ingested by phagocytes and later accumulated in the reticuloendothelial system of the host (5, 38–40). It has been previously postulated that *P. falciparum*-infected erythrocytes could adhere to iDCs, inhibit their maturation, and subsequently reduce their capacity to stimulate both memory and naive T cell responses (41). Although phagocytosis of parasitized erythrocytes was shown to inhibit important cellular functions of human macrophages and DCs (42, 43), purified HZ was found to enhance DC maturation (44). With crude HZ preparation (i.e., unpurified), the precise role of malaria pigments and those of other contaminating proteins, such as membranes and GPI molecules, are difficult to discriminate (44). Other studies describe conflicting results on the impact of HZ on the host immune system, particularly on biological functions of DCs, but only in murine models (45–49). To our knowledge, studies focused on the impact of malaria pigments on HIV-1 infection and dissemination in and by human DCs are still lacking. In this study, we show that the interplay between HIV-1 and DCs is affected by the malarial pigment.

![FIGURE 3](http://www.jimmunol.org/)

**FIGURE 3.** sHZ enhances CD83 and CCR7 expression at both protein and mRNA levels. Adherent monocytes were subjected to the various treatments described in Fig. 1. Cells were harvested and protein (A) and mRNA levels (B) of CD83 and CCR7 were monitored by flow cytometry and real-time RT-PCR, respectively. Results from four different donors are shown and the median is represented by the horizontal bars. C, CD83 and CCR7 mRNA levels were assessed in semimature DCs treated for 5 and 6 d after sHZ treatment (i.e., 24 and 48 h after LPS/IFN-γ treatment) for the same donor.

![FIGURE 4](http://www.jimmunol.org/)

**FIGURE 4.** DC-mediated HIV-1 transfer is enhanced with sHZ. Monocytes treated with GM-CSF/IL-4 only (called Control) and monocytes treated with both GM-CSF/IL-4 and sHZ (called sHZ) were pulsed with NL4-3Balenv and next cocultured with activated CD4+ T cells at a 1:3 ratio. Virus replication was estimated by quantifying the p24 content in cell-free supernatants at the indicated time points. Virus production at 2 d after initiation of the coculture is depicted in the small insert. The data shown represent the mean ± SEM of quadruplicate samples from three distinct donors. Statistical analysis was performed on the results from all experiments. ***p < 0.001.
peripheral blood (44), we chose to isolate monocytes from healthy blood donors and differentiate them in the presence or not of sHZ by GM-CSF and IL-4 treatment to obtain iDCs. Usually, exposure of iDCs to danger signals triggers their maturation allowing them to migrate toward lymphoid tissues enriched in CD4+ T cells. Maturation of DCs is known to downregulate the expression of CCR5, a marker for peripheral tissue homing (51, 52), and to increase expression of the chemokine receptor CCR7, which is essential for their trafficking and subsequent entry into secondary lymphoid organs (51–53). During DC maturation, other markers are also increased including CD83, CD80, CD86, and HLA-DR, whereas others like DC-SIGN are diminished. It is now clear that iDCs can be partly activated leading to a semimature phenotype (54). Partially mDCs are characterized by a moderate expression of costimulatory molecules and low cytokine production. They also express CCR7 that permit their continuous migration to lymph nodes, a phenomenon contributing to the peripheral immune tolerance (55, 56). In the current study, we demonstrate that monocytes treated with sHZ and GM-CSF/IL-4 exhibit an intermediate state of maturation compared with iDCs (i.e., monocytes treated

**FIGURE 5.** sHZ reduces susceptibility of DCs to productive HIV-1 infection and diminishes CCR5 expression. A, Monocytes treated with GM-CSF/IL-4 only (called Control) and monocytes treated with both GM-CSF/IL-4 and sHZ (called sHZ) were inoculated with NL4-3Balenv. Virus production was evaluated by monitoring p24 levels in cell-free supernatants at various days after HIV-1 infection. The data shown represent the mean ± SEM of quadruplicate samples from three distinct donors. B, Surface expression of CCR5 was monitored by flow cytometry in monocytes treated with GM-CSF/IL-4 only (called Control), monocytes treated with GM-CSF/IL-4 and LPS/IFNγ (called LPS/IFNγ) and monocytes treated with both GM-CSF/IL-4 and sHZ (called sHZ). The small horizontal bars represent the means of the measurements obtained from five distinct human healthy donors. *p < 0.05; **p < 0.01.

**FIGURE 6.** HIV-1 replication in CD4+ T cells is promoted by a contact with sHZ-loaded DCs. Autologous CD4+ T cells were initially infected with NL4-3Balenv and next cocultured with monocytes treated with GM-CSF/IL-4 only (called Control) and monocytes treated with both GM-CSF/IL-4 and sHZ (called sHZ) at a 3:1 ratio. Virus replication was estimated by quantifying the p24 content in cell-free supernatants at the indicated time points. Virus production at 2 d after initiation of the coculture is depicted in the small insert. The data shown represent the mean ± SEM of triplicate samples from three distinct donors. Statistical analysis was performed on the results from all experiments. ***p < 0.001.

**FIGURE 7.** Proposed hypothetical model of the consequences of HZ phagocytosis by monocytes on HIV-1 dissemination. In humans, malaria infection begins with the bite of an infected female *Anopheles* mosquito (1). After standard development of sporozoites in hepatocytes (2) and invasion of the merozoite form of *Plasmodium* species in RBCs (3), free HZ is phagocytosed by circulating monocytes (4). HZ-loaded monocytes can be recruited to peripheral tissues during inflammation or under steady-state conditions where they will differentiate into semimature DCs or macrophages on some specific stimuli (5). Thereafter, HZ-loaded semimature DCs can come in contact with HIV-1 particles present in the tissue, capture them and emigrate from the tissue to regional draining lymph nodes where they may encounter uninfected and/or virus-infected CD4+ T cells and promote their activation (6). At this stage, activation of CD4+ T cells increases the possibility of infected cells to become productively infected by cell-free virions or through a trans-infection process (7). Alternatively, the capacity of HZ-loaded semimature DCs to induce cell activation can also drive virus-gene expression in CD4+ T cells carrying integrated viral DNA (8). A similar scenario can occur with HZ-loaded macrophages either uninfected or infected with HIV-1 because they can also interact with both uninfected and HIV-1-infected CD4+ T cells (7, 8).
with GM-CSF/IL-4 only) and mDCs (i.e., monocytes treated with GM-CSF/IL-4 and LPS/IFN-γ). This is based on the sHZ-mediated upregulation of maturation markers CD83 and CCR7, an induction that was less prominent that the one seen in monocytes treated with the two powerful maturing agents LPS and IFN-γ. Monocytes, macrophages, and iDCs contain preformed intracellular CD83, and its rapid surface expression on activation is posttranslationally regulated in a process involving glycosylation (57). We demonstrate in this study that sHZ induces an increased expression of CD83 and CCR7 at the mRNA level. However, it cannot be excluded that sHZ could also induce redistribution of CD83 from an intracellular pool. Additional studies are warranted to solve this issue. Although its precise function(s) in DC biology remains to be defined, it has been reported that CD83 is involved in Ag presentation and CD4+ T cell differentiation in the thymus (58). Nonetheless, the possible contribution of CD83 in the activation of CD4+ T cells has yet to be confirmed (59). In light of our findings, it can be postulated that a contact between sHZ-loaded semimature DCs and CD4+ T cells will lead to T cell activation, a process promoting HIV-1 gene expression and virus replication.

Although treatment of monocytes with sHZ and GM-CSF/IL-4 was not sufficient per se to mediate a complete maturation profile, we wanted to corroborate previous reports suggesting that malaria pigments prevent DC maturation when HZ-loaded iDCs are challenged with the widely used maturation agent LPS. Indeed, it was previously established that monocytes-derived DCs exposed to sHZ before LPS treatment expressed lower surface levels of CD83 and CD80, two DC maturation markers, than LPS-challenged cells untreated with sHZ (43). Therefore, we used a modified experimental approach where sHZ-loaded DCs were treated with both LPS and IFN-γ for 2 d before performing flow cytometry analyses. Our results indicate that the presence of the malarial pigment did not prevent increased expression of the tested maturation markers in response to LPS/IFN-γ treatment (data not shown). However, as previously reported by others, levels of CD83, CD80, CD86, and CCR7 measured in monocytes treated with sHZ and GM-CSF/IL-4 were slightly decreased when compared with monocytes treated with GM-CSF/IL-4 and LPS/IFN-γ. Hence, phagocytosis of sHZ by monocytes regulates their subsequent differentiation pattern leading to the establishment of DCs bearing a semimature phenotype. Supplemental experiments are needed to clarify the biological functions of such semimature DCs and the type of immunological response(s) they induce.

DCs and HIV-1 interact together through a complex molecular networking (16, 60). This is exemplified by the fate of HIV-1 on a contact with DCs. For example, viral particles captured by iDCs can remain bound onto the cell surface as intact virions through an association with the C-type lectin DC-SIGN, be stored within multivesicular bodies, or be preserved as an integrated provirus after entry by CD4- and CCR5-mediated fusion. It is well established that LPS/IFN-γ-induced mDCs express low amounts of surface CCR5, a process rendering these cells less susceptible to productive viral infection (12, 61–63). Moreover, the maturation agent LPS can also trigger synthesis of soluble factors, such as type I IFNs (e.g., IFN-α), that can limit HIV-1 replication (64–66). Although maturation renders DCs less permissive to virus infection, the ability of these cells to trans-infect CD4+ T cells is strongly enhanced. Accordingly, our results demonstrate that on sHZ-treatment, phenotypic modifications taking place in monocyte-derived DCs significantly increase viral transfer to CD4+ T cells. The mechanism(s) by which sHZ can facilitate the DC-mediated virus transmission is still unknown but we provide evidence that this is not due to a more important virus production in DCs because HIV-1 replication is reduced in sHZ-loaded DCs.

In response to P. falciparum protozoan parasites and malaria pigments, murine macrophages and human DCs have been found to synthesize IFN-γ, TNF-α, MCP-1, IL-6, and IL-12 (44, 67). Furthermore, DCs are known to produce higher levels of IL-12 than macrophages and monocytes. The fact that DCs are likely to deliver IL-12 to surrounding CD4+ T cells is of high interest particularly in light of the natural capacity of this cytokine to augment T cell proliferation (68). This might in turn result in a higher virus production when sHZ-loaded DCs are cocultured together with CD4+ T cells. Interestingly, our preliminary findings confirm our hypothesis because we found that exposure of monocyte-derived DCs to sHZ leads to production of IL-1β (unpublished observations), which is known to induce IL-12 secretion in DCs. The sHZ-mediated increase in CCR7 expression in DCs might also modulate the pathogenesis of HIV-1 infection by promoting migration of DCs in areas of peripheral lymphoid tissues rich in CD4+ T cells.

Based on the previously described data with DCs and findings with monocyte-derived macrophages (Supplemental Fig. 1), we propose a schematic representation about the impact of the uptake of the malarial pigment HZ by human DCs and macrophages on HIV-1 infection and dissemination processes (Fig. 7). In humans, malaria is transmitted when a female Anopheles mosquito takes a blood meal. After standard development of sporozoites in hepatocytes and invasion of the merozoite form of Plasmodium species in RBCs, HZ will be synthesized leading to a burst of infected RBCs and the release of free HZ in peripheral blood. Free HZ is then heavily engulfed by monocytes that circulate in the bloodstream until their migration into peripheral tissues where they will differentiate into semimature DCs or macrophages on inflammatory stimuli. Through an upregulation of CCR7 expression, HZ-loaded semimature DCs will migrate to secondary lymphoid tissues, which are known to act as preferential reservoirs for HIV-1. A very efficient propagation of HIV-1 particles from HZ-loaded DCs toward neighboring uninfected CD4+ T cells will ensue. Alternatively, virus production in HIV-1-infected CD4+ T cells will be augmented on a physical contact with HZ-loaded semimature DCs. With regard to the situation prevailing in macrophages, phagocytosis of HZ by monocytes prior to their differentiation in macrophages and migration to tissue will favor macrophage-mediated transmission of HIV-1 toward uninfected CD4+ T cells. Moreover, a contact between HZ-loaded uninfected macrophages and virus-infected CD4+ T cells will promote HIV-1 expression.

In conclusion, we propose a new mechanism by which malaria pigments can modulate HIV-1 disease progression. Indeed, we demonstrate that internalization of malaria pigments affects DC differentiation and maturation status rendering them more potent to trans-infect and prime CD4+ T cells for HIV-1 replication. Additional and more comprehensive basic studies are needed to fully understand the complex cellular consequences of such deadly interactions. This is essential to discover promising drug combinations and establish new prevention strategies to treat worldwide coinfected individuals.

Acknowledgments
We appreciate the excellent technical contribution of O. Simard, C. Côté, R. Tremblay, and M.-A. Roy. We express our gratitude to C. Gilbert for critical and constructive comments for this study. This study was performed by J.D. in partial fulfillment of a Ph.D. degree in the Microbiology-Immunology Program, Faculty of Medicine, Laval University.

Disclosures
The authors have no financial conflicts of interest.
by cross-linking surface Ig.
J. Exp. Med. 179: 1109–1118.

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References


17.

zerland.


Int. 57: 89–96.

Sci. USA 93: 11685–11170.


infected and can therefore not be used as a source of virus for cell culture. J. Clin.

deficiency virus type 1 replication in dendritic cell-T cell cocultures is increased upon incorporation of host LFA-1 due to higher levels of virus production in immature dendritic cells. J. Virol. 81: 7672–7682.


