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Ingestion of the malaria pigment hemozoin renders human macrophages less permissive to HIV-1 infection

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ABSTRACT

Few studies have investigated the pathophysiologic mechanisms responsible for what seems to be a possible interaction between *Plasmodium falciparum*, the causative agent of malaria, and HIV-1 in dually infected patients. It has been shown that *Plasmodium* parasites detoxify heme molecules into a pigment called hemozoin (HZ), which can significantly modulate the immune system. The primary objective of this study was to determine whether exposure of human primary monocyte-derived macrophages (MDMs) to the malaria pigment influences the process of HIV-1 infection. We report here that HIV-1 replication is significantly diminished in HZ-loaded MDMs. The HZ-mediated reduction in virus replication is due to a block at a step in the virus life cycle occurring between the completion of full-length reverse transcripts and integration of viral DNA within the host chromosome. Understanding the pathological mechanisms involved in *P. falciparum* and HIV-1 co-infection is of high importance because of possible therapeutic ramifications.

Introduction

Malaria and acquired immune deficiency syndrome (AIDS) are responsible for major health problems in many regions of the world. By the end of 2007, an estimated 33.2 million people were living with human immunodeficiency virus type-1 (HIV-1), of whom 2.5 million were newly infected (WHO, 2007). *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 (Breman, 2001; WHO, 2002). Together, both infectious diseases cause more than 4 million deaths a year. To a substantial extent *P. falciparum* and HIV-1 infections are concentrated in the same geographical regions. The resulting co-infection and interactions between the two pathogens have major public health implications. Studies based on *P. falciparum* and HIV-1 co-infection, whether clinical or laboratory, have been inconclusive to fully understand the exact mechanisms by which those two microorganisms interact.

HIV-1 is a single-stranded RNA retrovirus which can infect a number of different target cells, including CD4-expressing macrophages and T-helper lymphocytes. The infection begins with the interaction of the virus-encoded external glycoprotein 120 (i.e., gp120) with the host's cell surface CD4 molecule and chemokine coreceptor CCR5 or CXCR4. As the virus enters the cell, the conversion of the viral genomic RNA into double-stranded DNA is achieved by the virus-produced reverse transcriptase. Once reverse transcription has occurred, the pre-integration complex, promoted by HIV-1 DNA Flap formation, undergoes uncoating at the nuclear pore (Arhel et al., 2007). Uncoating is an essential step in the viral replication cycle and the exact mechanism(s) involved remains controversial to this day. Thereafter, the viral DNA migrates to and enters the host cell nucleus and becomes integrated into the cell DNA with the help of the virus integrase. The provirus can then remain latent or be active, generating new virions emerging from the host cell membrane.

At the end of the 19th century, malaria was diagnosed by the presence of a dark pigment in various organs of patients (Hempelmann, 2007). Later, Brown (1911) began a long and continuing debate by suggesting that those pigments were involved in various physiopathological aspects of malaria. The parasite multiplies by ingesting hemoglobin found in the host's cytosol. This process leads to hemoglobin degradation as a source of amino acids and the formation of free heme, which is a byproduct known to be highly toxic to the parasite. The essential first step in detoxification of heme is its incorporation into an intracellular crystal called malarial pigment or hemozoin (HZ) (Pisciotta and Sullivan, 2008). Results from *in vitro* and *in vivo* studies indicate that, after the parasite's schizont rupture in red blood cells, crude HZ and a variety of attached components of parasite and host origin (e.g., lipids and phospholipids, lipid derivatives and proteins) are avidly taken up by phagocytes (i.e.,

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circulating neutrophils and monocytes and resident macrophages) (Arese and Schwarzer, 1997; Coban et al., 2002). Interestingly, it has been demonstrated that HZ can persist in macrophages of infected individuals for several months (Millington et al., 2007; Pisciotta and Sullivan, 2008; Schwarzer et al., 1998).

Blood-circulating monocytes when located in tissues can differentiate into macrophages and dendritic cells in response to danger stimuli (Mantovani, Sica, and Locati, 2005). Due to their migratory behavior and their key functions in immune system responses, it is not surprising that cells of the monocyte–macrophage lineage are the preferential targets of both *P. falciparum* and HIV-1 (Arfi et al., 2008; McGilvray et al., 2000). Like with *P. falciparum*, macrophages have been described as being important in HIV-1 infection by contributing to the pathogenesis of the disease throughout the course of infection (Kedzierska and Crowe, 2002). Indeed, tissue macrophages can be infected with HIV-1 under both *in vitro* and *in vivo* conditions. Following infection with HIV-1, macrophages are resistant to the virus-mediated cytopathic effects and serve throughout the course of infection as long-term stable viral reservoirs capable of disseminating the virus to tissues.

Some of the diseases seen in the setting of *P. falciparum* and HIV-1 co-infection have been well described in a recent review paper (Skinner-Adams et al., 2008). Although it is now appreciated that malaria and HIV-1 are causing bidirectional and synergistic interactions, there is still a paucity of data with regard to the mechanism(s) by which each pathogen impacts the other. Therefore, given the overlapping geographic distribution of both diseases and the urgent need for basic research in this area, the aims of this study were articulated as follows: (1) investigating whether exposure to the purified malaria pigment HZ influences HIV-1 replication in human primary macrophages and, if so, (2) understanding the mechanism(s) by which HZ can modulate the process of virus infection.

Results

Chemical synthesis and phagocytosis of malaria pigments

Synthetic HZ (sHZ), when prepared *in vitro* under acidic conditions, is spectroscopically identical and morphologically similar to the native HZ isolated directly from *P. falciparum* cultures (Taramelli et al., 2001). Moreover, both synthetic and native HZ undergo the same process when internalized by phagocytes (Noland, Briones, and Sullivan, 2003; Olliaro et al., 2000; Pagola et al., 2000). Consequently, most of our experiments were performed with synthetic HZ. The biological activity of our sHZ stock was authenticated by measurement of nitric oxide levels following a brief exposure of the murine macrophage cell line J774 to IFN-γ as described previously (Bergeron and Olivier, 2006; Jaramillo et al., 2003). The sHZ preparation was found to be biologically active since a higher production of nitric oxide was seen in sHZ-treated cells upon the addition of IFN-γ (Fig. 1A). Moreover, sHZ was found to mediate a significant secretion of IL-1β in human primary MDMs (Fig. 1B), which confirms that the studied sHZ preparation is biologically active.

The next set of experiments was aimed at evaluating the ability of MDMs to engulf sHZ. To this end, cells were exposed to sHZ and phagocytosis of the malaria pigment was monitored during 24 h using a spinning disk confocal microscope. Acquisition was saved every 4 min and representative pictures were chosen. As shown in Fig. 2, malaria pigments were phagocytosed efficiently by MDMs. Furthermore, a single cell can phagocyte several crystals of malaria pigments, but the number of phagosomes containing sHZ was restricted (i.e., a maximum of one to three per individual cell). Phagocytosis was not only observed during the chosen time-range but also during the entire recording (data not shown). For that reason, treatment of MDMs with sHZ was made 24 h prior to HIV-1 infection, unless otherwise specified.

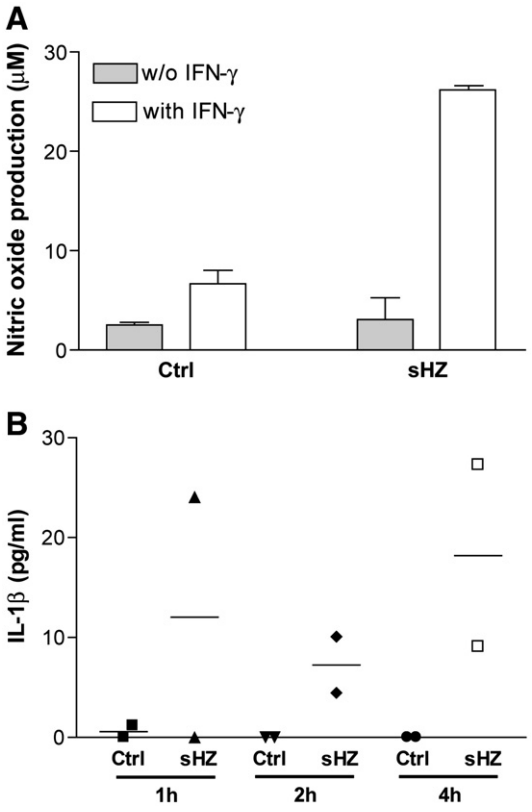

Fig. 1. sHZ is biologically active. Murine J774 cells (1.25×10^4) were initially treated with PMA and next either left untreated (Ctrl) or treated with sHZ (10 μg/ml). Finally, half of the samples were incubated with IFN-γ (100 U/ml). Nitric oxide production was assessed through the use of the Griess reagent (A). Results are a schematic representation of data from triplicate samples ± SEM of three independent experiments. Human primary MDMs (5×10^4) were either left untreated (Ctrl) or treated with sHZ (10 μg/ml) for the indicated time periods. Levels of IL-1β were assessed by using a commercial test (B). Measurements were done on two distinct donors.

sHZ inhibits HIV-1 replication in MDMs in a dose-dependent manner regardless of the viral preparations

Next, we utilized sHZ to measure its putative impact on HIV-1 infection in MDMs. First, MDMs were initially treated for 24 h with increasing concentrations of sHZ (ranging from 0.1 to 25 μg/ml) and infected with fully infectious R5-tropic virus (i.e., NL4-3Bal*env*) for 16 days. Results illustrated in Fig. 3A indicate that HIV-1 production in MDMs is significantly reduced in a dose-dependent fashion by concentrations of sHZ starting at 5 μg/ml. Importantly, cell viability was not affected by doses of sHZ that can diminish virus replication as monitored by the fluorescent cytotoxic MTS assay (Fig. 3B) and annexin V/7AA-D test (data not shown). To eliminate the possibility that sHZ could affect the phagocytic capability of MDMs, which could in turn reduce virus uptake, we estimated zymosan phagocytosis. As shown in Fig. 3C, sHZ is not altering the phagocytic capacity of MDMs.

Cells were then infected with four different R5-tropic virus preparations, i.e., fully infectious NL4-3Bal*env* and NL4-3Bal-HSA as well as single-cycle reporter virus pseudotyped either with JR-FL (NL4-3*Luc*$^+$*Env*$^-$*R*$^+$/JR-FL) or VSV-G envelope (NL4-3*Luc*$^+$*Env*$^-$*R*$^+$/VSV-G). It should be noted that, unlike most of the previous reporter constructs, the NL4-3Bal-HSA molecular construct leads to the production of fully competent viruses with no deletions in *env*, *vpr* or *nef*. Moreover, NL4-3Bal-HSA virus codes for a cell surface reporter molecule, namely, the murine heat-stable antigen (HSA) CD24, which permits to quantify the percentage of cells productively infected with HIV-1 (Imbeault et al., 2009). Virus infection was estimated at 6 days post-infection for all virus stocks tested by measuring either the p24 content, luciferase activity or percentage of HSA-expressing cells.