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Heme oxygenase-1 induction alters chemokine regulation and ameliorates human immunodeficiency virus-type-1 infection in lipopolysaccharide-stimulated macrophages

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Abstract

We have elucidated a putative mechanism for the host resistance against HIV-1 infection of primary human monocyte-derived macrophages (MDM) stimulated with lipopolysaccharide (LPS). We show that LPS-activated MDM both inhibited HIV-1 entry into the cells and were refractory to post-entry productive viral replication. LPS-treated cells were virtually negative for mature virions as revealed by transmission electron microscopy. LPS activation of MDM markedly enhanced the expression of heme oxygenase-1 (HO-1), a potent inducible cytoprotective enzyme. Increased HO-1 expression was accompanied by elevated production of macrophage inflammatory chemokines (MIP1α and MIP1β) by LPS-activated MDM, significantly decreased surface chemokine receptor-5 (CCR-5) expression, and substantially reduced virus replication. Treatment of cells with HO-1 inhibitor SnPP IX (tin protoporphyrin IX) attenuated the LPS-mediated responses, HIV-1 replication and secretion of MIP1α, MIP1β, and LD78β chemokines with little change in surface CCR-5 expression. These results identify a novel role for HO-1 in the modulation of host immune response against HIV infection of MDM.

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Keywords

HIV-1; Heme oxygenase-1; Macrophages; CCR-5; Chemokines

1. Introduction

Macrophages are a major reservoir for HIV-1 *in vivo*, and they play a central role in the pathogenesis of HIV-1 disease [1]. They are among the first cells infected by the virus, facilitate the transmission of virus to target cells causing persistent infection, and contribute to spread of the virus [2]. Productive HIV-1 replication in macrophages is dependent on host transcriptional machinery and is closely regulated by a complex pathway of pro-inflammatory cytokines that are produced during HIV infection or via systemic induction of an inflammatory response to bacterial infections [3,4].

Previous and emerging studies have shown a critical role of HO-1 in host defense against a variety of physiological insults such as oxidative stress, hypoxia, and pro-inflammatory cytokines [5–7], as well as roles in microbial host defense [8–14] and regulating normal immune function [15]. However, despite mounting evidence supporting the immunomodulatory capacity of HO-1 in host response to infectious diseases, the underlying mechanisms have not yet been clearly elucidated. In the present study, we show that HO-1-dependent sustained inhibition of HIV-1 infection of LPS-stimulated MDM was associated with chemokine production and down-regulation of CCR-5 surface expression, indicating putative regulatory roles for these host factors in a defense mechanism against HIV-1 infection.

2. Materials and methods

Human MDMs were generated from normal donors [16] and infected with HIV-1_{BaL} as described previously [12,13]. HIV replication was quantified by measuring cell-free HIV-p24 in culture supernatants using an NEN/Dupont ELISA analysis kit (Perkin Elmer Life Sciences, Inc., Boston, MA) [13]. For immunofluorescence assays, MDM were cultured on Permanox chamber slides (Fisher Scientific) in the absence or presence of 100 ng/ml LPS, and infected with HIV-1_{BaL} at an MOI of 0.05. Five days post-infection, cells were fixed with 2% PFA, permeabilized using 0.05% saponin in PBS containing 2% FBS and 0.1% human immunoglobulins, and stained with RD1-conjugated HIV-1-p24 mAb (Beckman Coulter, Miami, FL) for intracellular HIV-1 antigen expression; the cells were examined by fluorescence and phase-contrast microscopy. The presence of virus particles in HIV-1-infected MDM cultured for 7 days in the absence or presence of 100 ng/ml LPS was examined by transmission electron microscopy (Advanced Biotechnologies, Inc., Columbia, MD).

Cell surface CCR-5 expression was determined by flow cytometry on non-permeabilized MDM. The presence of intracellular virus and HO-1 expression in HIV-infected MDM was determined by flow cytometry. Paraformaldehyde-fixed and permeabilized cells were simultaneously stained with FITC-conjugated anti-HO-1 monoclonal antibody (Enzo Life Sciences, Farmingdale, NY) plus RD1-conjugated anti-HIV-1-p24 monoclonal antibody for

30 min at 4 °C. After washing three times with cold PBS, cells were analyzed by two-color flow cytometry (BD Biosciences). The levels of MIP1α and MIP1β in culture supernatants were determined by ELISA (Invitrogen). LD78β was quantified by sandwich ELISA using anti-LD78β and biotin-conjugated LD78β antibodies (Abcam) using human LD78β as standard.

3. Results and discussion

Virus replication and progression of HIV disease depend upon host-cell transcription and gene regulation in virus-specific target cells. Thus, both viral and cellular gene transcription contribute to active viral replication. Emergence of drug resistance and frequent mutational changes in the viral genome often threaten effective treatment of HIV disease. Therefore, induction of cellular defense responses may afford an alternative or concurrent therapeutic strategy to overcome such challenges.

We tested this hypothesis by inducing HO-1, an inducible rate-limiting enzyme in heme catabolism implicated in host cellular defense, in MDM by LPS to determine whether HO-1-expressing MDM would be refractory to productive HIV-1 infection. MDM treated with LPS at various concentrations exhibited increased levels of HO-1 expression in a dose-dependent manner (Fig. 1A) with striking concomitant inhibition of HIV replication (Fig. 1B). Specifically, PCR analysis of cDNA synthesized from RNA samples isolated from monocyte-derived macrophages treated up to 24 h with 100 ng/ml LPS demonstrated that LPS treatment caused an up-regulation of HO-1 gene expression. The increased HO-1 gene expression could be detected as early as 4 h post-LPS treatment (Fig. 1C, top panel). We also found that treatment of monocyte-derived macrophages with LPS 24 h or 4 h prior to, at the time of, or even 4 h after infection inhibited HIV-1 replication (Fig. 1C, bottom panel). These results suggest that although pretreatment with LPS could block viral entry substantially, it also partially inhibited post-entry viral replication. That is, inhibition of viral replication was observed even when the monocyte-derived macrophages were treated 4 h after infection with HIV-1, suggesting that LPS treatment may not only interfere with viral entry but also obstructs post-entry events.

To establish a more direct correlation between the HIV infectivity and HO-1 expression, control and LPS-activated MDM were examined by flow cytometry 5 days after infection using FITC-labeled mAb against HO-1 and RD1-conjugated anti-p24 mAb. The results from this experiment shown in Fig. 1D demonstrate that most HIV-1-infected cells expressing viral p24 antigen were nearly negative for HO-1. In contrast, LPS-activated cells expressed a significantly reduced level of the viral antigen, indicative of low or undetectable infection, with significantly enhanced expression of HO-1. Consistent with the low virus replication, LPS-activated MDM exhibited substantially reduced HIV-associated cytopathic effects, as scored by multinucleated giant cell formation (Fig. 1E). These cells also expressed nearly undetectable viral antigen as revealed by immunofluorescence assay (Fig. 1F). In addition, ultra-structural examination showed LPS-activated MDM to be virtually negative for virus particles in contrast to untreated HIV-infected MDM, where a large number of mature virus particles were seen within intracytoplasmic vacuoles (endosomes) (Fig. 1G).

CCR5 is expressed by MDM and represents the most important co-receptor for macrophage-tropic HIV-1 strains to enter the cells. To explore whether HO-1-dependent inhibition of HIV-1 infection was related to altered levels of surface CCR-5, MDM were cultured at 37 °C for 24 h in the absence or presence of 100 ng/ml LPS and examined for intracellular HO-1 and surface CCR-5 expression by flow cytometry. As shown in Fig. 2 (panels A and B), reduced surface CCR-5 expression correlated with the increased levels of HO-1 expression in LPS-activated MDM, indicating reduced availability of CCR-5 for virus entry into MDM. Inhibition of virus entry has previously been attributed to lack of CCR-5 receptor expression on LPS-treated monocytes [17,18]. Although the lower abundance of CCR-5 may account for the reduced viral entry, suppression of post-entry virus transcription presents a plausible contributing factor to explain the sustained low level of HIV replication in HO-1-expressing LPS-activated MDM.

Macrophage inflammatory proteins are produced by macrophages after stimulation with LPS; these chemokines play a crucial role in immune response to HIV infection [19,20]. LPS-mediated HO-1 induction correlated with high expression of intracellular MIP1α, MIP1β, and LD78β (Fig. 2C), as well as markedly increased secretion of these chemokines by LPS-activated MDM (Fig. 2D). Treatment of monocytes with SnPP IX (tin protoporphyrin IX), an inhibitor of HO-1 activity, 2 h prior to LPS activation attenuated LPS-induced suppression of HIV replication (Fig. 2E) and reduced production of three chemokines: MIP-1α, MIP-1β and its isoform LD78β (Fig. 2F). Interestingly, surface CCR-5 expression on LPS-activated MDM was not significantly altered by SnPP treatment (Fig. 2G). Collectively, these results indicate a role for a novel HO-1-dependent host defense response in the LPS-activated MDM.

In conclusion, our study supports the notion of HO-1-dependent host defense as a protective mechanism against HIV infection. Our findings substantiate a role for the inducible isoform of heme oxygenase, HO-1, in the regulation of HIV infection of macrophages.

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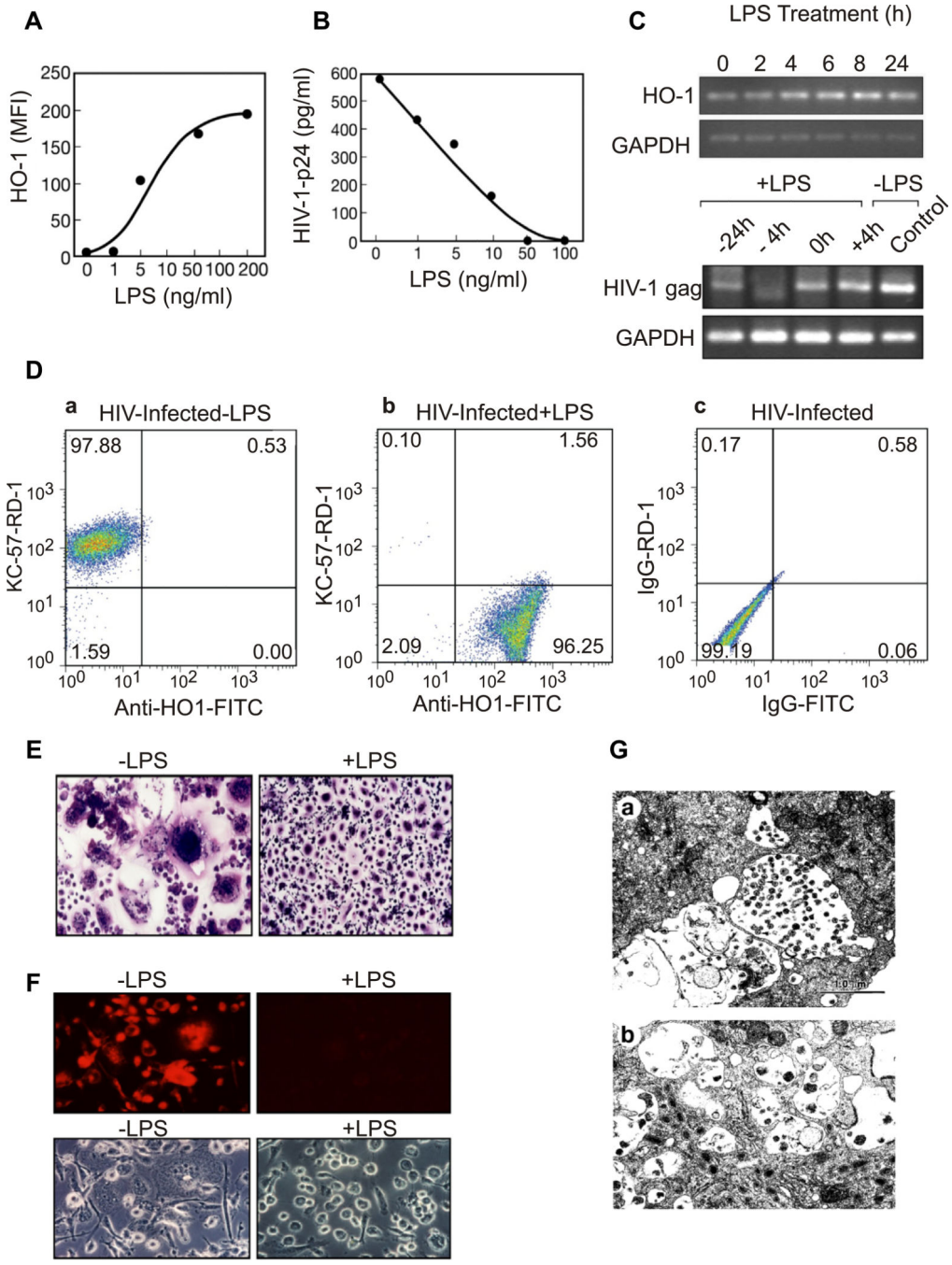

Fig. 1.

HO-1-dependent inhibition of HIV-1 replication in LPS-activated MDM. (A) LPS-induced HO-1 induction. Human MDM (1×10^6 cells) were incubated at 37 °C for 24 h with increasing concentrations of LPS and examined for intracellular HO-1 expression. After 24 h of incubation, cells were washed three times with cold PBS and fixed in 2% paraformaldehyde for 2 h at 4 °C. Cells were washed three times with cold PBS, permeabilized using 0.05% saponin in cold PBS containing 2% FBS and 0.05% normal IgG, and stained for 30 min on ice with FITC-conjugated HO-1 mAb. After staining, cells were washed three times with cold PBS, fixed in 2% PFA for 2 h at 4 °C, washed again three times with cold PBS to remove PFA, and examined for intracellular HO-1 expression by flow cytometry. Data are expressed as mean channel fluorescence intensity (MFI) (solid

circles). (B) LPS activation inhibits HIV replication in MDM. Cells cultured at 37 °C with increasing concentrations of LPS for 24 h were then infected with HIV-1_{Ba-L} strain as described in Methods. After 5 days, culture supernatants were harvested and assayed for cell-free HIV-1-p24 levels by ELISA. (C) Time-dependent HO-1 expression in LPS-treated MDM. PCR amplification of HO-1 cDNA derived from total cellular RNA isolated from monocyte-derived macrophages treated with 100 ng/ml LPS for the indicated times. The cDNA samples were subjected to PCR using HO-1-specific primers and GAPDH primers, and the PCR products were separated on a 2% agarose gel and stained with ethidium bromide (top panels). Time-dependent inhibition of HIV-1 infection of LPS-treated MDM. Cells were treated with 100 ng/ml LPS prior to, at the time of (0 h), or post-HIV infection as indicated. After 48 h, total RNA was extracted and examined for HIV infection by RT-PCR using gag-specific primers. The PCR products were separated on a 2% agarose gel and stained with ethidium bromide (bottom panels). (D) HIV-1 antigen and HO-1 co-expression in control and LPS-activated MDM. Control and LPS-activated MDM were washed three times with cold PBS and fixed in 2% paraformaldehyde for 2 h at 4 °C. After washing three times with cold PBS, cells were permeabilized using 0.05% saponin in cold PBS containing 2% FBS and 0.05% normal IgG, and simultaneously stained with FITC-conjugated anti-HO-1 mAb and RD1-conjugated anti-p24-mAb, and examined by two-color flow cytometry. (E) LPS activation inhibits HIV-1-induced cytopathic effects in MDM. MDM cultured for 24 h at 37 °C in the absence or presence of 100 ng/ml LPS were infected with HIV-1_{Ba-L} strain. On day 5 post-infection, cells were Wright-stained and examined for HIV-associated cytopathic effects as the formation of multinucleated giant cells. (F) LPS-activated MDM express low intracellular viral antigen. HIV-1-infected MDM, cultured on Permanox plastic chamber slides in the absence or presence of 100 ng/ml LPS for 5 days, were washed with PBS and fixed in 2% PFA for 2 h at 4 °C. Cells were washed three times with cold PBS to remove PFA, stained with RD1-conjugated HIV-1-p24 mAb, and examined for the expression of HIV-1 antigen by fluorescence microscopy (top panels). Cell morphology in the same field was examined by a phase-contrast microscopy (bottom panels). (G) Electron micrographs of HIV-1-infected MDM cultured in the absence (a) or presence (b) of 100 ng/ml LPS.

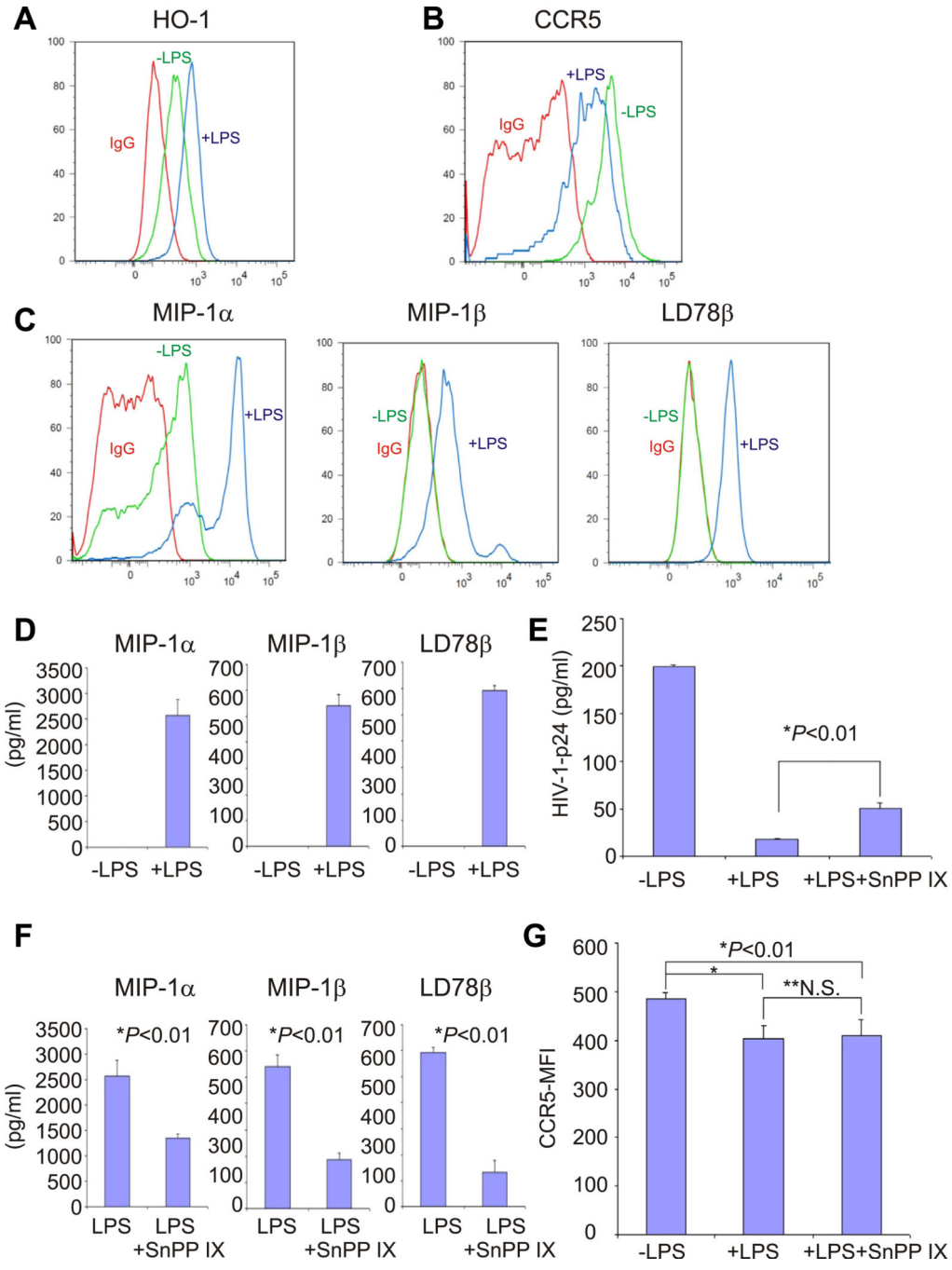

Fig. 2.

HO-1-dependent reduced viral infection is related to chemokine-dependent suppression of HIV-1 infection of LPS-activated MDM. (A) Increased intracellular HO-1 expression in LPS-activated MDM (shown in blue) relative to untreated control cells (shown in green). (B) Reduced surface CCR5 on LPS-activated MDM (shown in blue) relative to untreated control cells (shown in green). (C) Flow cytometric analysis of expression of the chemokines MIP1α, MIP1β, or LD78β in LPS-activated MDM (shown in blue) as compared to the untreated control cells (shown in green). Cells cultured for 24 h in the absence and presence of LPS (100 ng/ml) were fixed and examined for the intracellular expression of MIP1α, MIP1β, and LD78β by flow cytometry. (D) Increased chemokine secretion by LPS-activated MDM. Cells were cultured in the absence or presence of 100 ng/ml LPS

and chemokine production in culture supernatants was measured by ELISA. (E) Inhibition of HO-1 activity attenuates LPS-mediated protection against HIV infection of MDM. Cells were incubated with the HO-1 inhibitor SnPP IX 2 h prior to LPS activation for 24 h, infected with HIV-1_{Ba-L}, and examined for HIV-1-p24 in the culture supernatants 5 days after infection. (F) Inhibition of HO-1 activity attenuates chemokine production by LPS-stimulated MDM. Cells were incubated in the absence or presence of HO-1 inhibitor SnPP IX 2 h prior to LPS activation for 24 h, and the levels of secreted chemokines MIP1α, MIP1β, and LD78β in the culture supernatants from control and LPS-activated MDM were measured by ELISA. (G) Inhibition of HO-1 activity does not alter surface CCR-5 expression on LPS-activated MDM. MDM, pretreated for 2 h with HO-1 inhibitor SnPP IX prior to addition of 100 ng/ml LPS. Control cells were cultured in the absence of LPS. After 24 h, cells were washed with PBS, resuspended in PBS containing 2% PBS and 0.05% normal IgG, and stained with FITC-conjugated anti-CCR-5 mAb. After washing with cold PBS, cells were fixed in 2% paraformaldehyde (PFA), washed with PBS to remove the PFA, and examined for surface CCR-5 expression by flow cytometry. Data are expressed as MFI ± SD; $p < 0.01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)