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Impact of in vitro Costimulation with TLR2, TLR4 and TLR9 Agonists and HIV-1 on Antigen-Presenting Cell Activation

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Key Words
Toll-like receptors · Dendritic cells · Monocytes · HIV-1 · Cytokines · Co-stimulatory molecules

Abstract
Objective: HIV-1 infects several immune cells including dendritic cells (DCs) and monocytes, which contributes in both to dissemination of HIV-1 infection and induction of antiviral immunity. These cells produce high amounts of type I IFN and proinflammatory cytokines upon Toll-like receptor (TLR) stimulation. During HIV-1 infection, an altered production of proinflammatory cytokines has been reported. However, the mechanisms underlying cytokine modulation have not been well described. Here, we evaluated the production of proinflammatory cytokines and activation of myeloid and plasmacytoid DCs and monocytes costimulated in vitro with TLR agonists and HIV-1. ***Methods:*** Changes in cytokine expression by real-time PCR and activation of DCs and monocytes by flow cytometry were evaluated after costimulation with HIV-1 and TLR agonists. ***Results:*** We observed an upregulation of TNF-α expression after TLR4 stimulation, but a downregulation of IL-6 when TLR2/TLR9 were stimulated. Interestingly, the expression of CD80 and CD86 costimulatory molecules in monocytes and DCs were significantly increased in cells challenged with HIV-1 and TLR2/TLR4/TLR9 agonists. ***Conclusion:*** This regulation of TNF-α and IL-6 production and changes in the expression of costimulatory molecules can be critical in the context of HIV-1 infection, by favoring the antigen-presenting cell activation through the stimulation of TLRs.

Introduction

Pattern recognition receptors are critical in activation of innate immune response after microbial infection. The best characterized pattern recognition receptors are Toll-like receptors (TLRs) which sense pathogen-associated molecular patterns at the surface of the cell and endosomal compartments [1]. Pathogen-associated molecular pattern recognition by TLRs triggers an entire signal pathway leading to the expression of several molecules involved in a primary inflammatory response, which promotes adaptive immunity.

TLRs are expressed in a wide range of cell populations, including those that serve as HIV-1 targets/reservoirs, such as CD4+ T cells, monocytes/macrophages and dendritic cells (DCs) [2–5]. DCs play a very important role in HIV-1 transmission because they can deliver infectious HIV-1 particles to lymph nodes that contain a large number of CD4+ T cells [5, 6]. Close contact between these two cell types can facilitate cell-to-cell transmission and virus spread [7, 8]. Furthermore, DCs produce proinflammatory cytokines that generate a microenvironment

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that promotes virus replication [9]. Previous reports have described the importance of several cytokines in the activation of immune cells and pathogenesis of HIV-1 infection [10], particularly IL-6 and TNF-α [11]. However, the role of TLRs in HIV-1 infection is still not well known although it has been reported that microbial products can activate HIV-1 replication through the stimulation of TLRs [12]. Such stimulation induces the activation of NF-κB and the production of proinflammatory cytokines involved in HIV-1 replication through transactivation of viral promoters [13]. A report has shown an increase of TLR expression in monocytes from HIV-1-infected patients, which favors both an increase in viral replication and secretion of TNF-α [14]. On the other hand, a significantly lower expression of TLR1 and TLR2 was reported in monocytes and myeloid DCs (mDCs) from HIV-1-infected patients [15]. Recently it was demonstrated that HIV-1 gp120 inhibits the CpG-induced maturation of plasmacytoid DCs (pDCs) and the expression of TNF-α, IL-6, TLR9 and IFN-regulatory factor 7. These effects were mediated by interaction of gp120 with CD4 and mannose-binding C-type lectin [16, 17].

Furthermore, the activation of TLRs is important in the maturation of various cellular subpopulations such as DCs and monocytes since such stimulation leads to an increase in the expression of molecules like CD80 and CD86 that are required for an appropriate adaptive immune response [4, 18]. It has been reported that stimulation of DCs with HIV-1 derivatives such as ssRNA induces the upregulation of CD80 and CD86 [19]. Various studies have demonstrated that activation of DCs and monocytes with HIV-1-derived pathogen-associated molecular patterns such as ssRNA, through TLR7 and TLR8, induces the production of proinflammatory cytokines and the upregulation of costimulatory molecules (CD80, CD86 and CD40) [20–22]. In addition, lipopolysaccharide (LPS) stimulation of mDCs from uninfected neonates exposed in utero to HIV-1 induces the expression of the CD80 and CD86, but mDCs from unexposed infants do not [23].

Together these results show that HIV-1 upregulates CD80 and CD86 expression on DCs and monocytes mainly through TLR7/8. However, it is not well known whether HIV-1 stimulation promotes DCs and monocyte maturation and secretion of proinflammatory cytokines through TLR2, TLR4 and TLR9. In the present study we investigated mRNA expression of IL-6 and TNF-α in peripheral blood mononuclear cells (PBMCs) and monocytes/DC activation, based on CD80 and CD86 expression, from HIV-1-uninfected donors, costimulated with Pam_3CSK_4, LPS and CpG-containing oligodeoxynucleotides type B (CpG-B ODN), agonists of TLR2, TLR4 and TLR9, respectively, and in vitro challenged with HIV-1.

Materials and Methods

Sampling and PBMC Purification

Blood samples were collected into EDTA tubes from 3 healthy donors. As required by the ethical guidelines, signed informed consent was obtained from all individuals before enrollment in the study. All biomedical investigations were approved by the bioethics committee of the Universidad de Antioquia and were conducted according to the principles of the Declaration of Helsinki. PBMCs were separated by density gradient using Ficoll-Hystopaque 1077 (Sigma-Aldrich, St. Louis, Mo., USA) according to the manufacturer's recommendation. The viability of PBMCs was determined by trypan blue exclusion.

Preparation of Virus Stocks

H9-HTLV-IIIcc chronically HIV-1-infected cell lines (NIH AIDS Research and Reference Reagent Program) were cultured over 7 days in RPMI 1640 medium (Invitrogen, San Diego, Calif., USA). The virus was prepared as described previously [24]. Briefly, the virus-containing supernatants were harvested, filtered through a 0.22-μm pore membrane and normalized for virion content by a sandwich ELISA assay specific for the major viral core p24 protein, using the Quick Titer Lentivirus Titer Kit (Cell Biolabs, San Diego, Calif., USA) and following the manufacturer's instructions. Values of p24 (pg/ml) were calculated on the basis of regression analysis of standards prepared from samples of known concentrations. The infectious/replication capacity of the viral stocks was determined by Western blot to detect Gag-derived proteins.

In vitro Stimulation of PBMCs with HIV-1 Together with TLR2-, TLR4- and TLR9-Specific Agonists

PBMCs were challenged with 3,000 pg/ml of HIV-1 p24 for 2 h at 37°. The cells were then washed twice with PBS 1X in order to remove excess virus and cultured a further 12 or 18 h at 37° and 5% CO_2 in the presence of 20 ng/ml of Pam_3CSK_4, 1 μg/ml of LPS or 2 μg/ml of CpG-B ODN (Invivogen, San Diego, Calif., USA), agonists of TLR2, TLR4 and TLR9, respectively.

Extraction of Total RNA from PBMCs

PBMCs challenged with HIV-1 and stimulated with TLR2, TLR4 or TLR9 ligands were used to extract total RNA with Trizol Reagent (Invitrogen, USA) according to the manufacturer's recommendation. Then, 1 μg of total RNA was treated with 2 UI RNase-free DNase I (Fermentas, Glen Burnie, Md., USA) for 1 h at 37° to remove traces of genomic DNA. The concentration and quality of the RNA was determined by spectrophotometery (λ = 260–280 nm) and agarose gel, and finally frozen at –70° until used.

mRNA Analysis of IL-6 and TNF-α by Real-Time PCR

For quantitative real-time PCR assays, specific primer for IL-6 and TNF-α genes were used, as previously described [24]. GAPDH was used as a reference housekeeping gene. The mRNA for both cytokines was determined in PBMC stimulated with HIV-1 in presence or absence of TLR agonists after 12 and 18 h of culture. How-

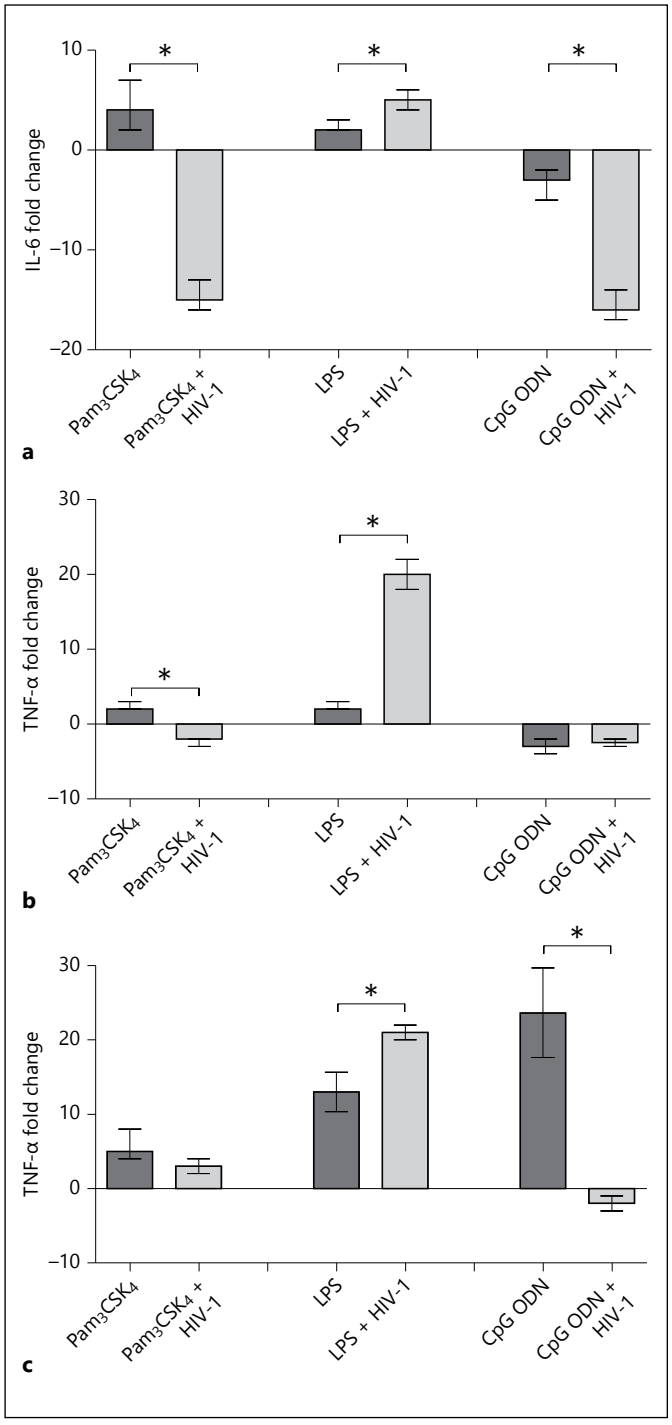

Fig. 1. Expression of IL-6 and TNF-α mRNA in PBMCs stimulated with TLR agonists and HIV-1. PBMCs were stimulated with TLR2, TLR4 or TLR9 agonists, in the presence or absence of HIV-1, then IL-6 and TNF-α mRNA determined by real-time RT-PCR, normalized to the uninfected control and to the housekeeping gene GAPDH (ΔΔCt method). **a** Modulation of IL-6 mRNA at 12 h after stimulation. **b**, **c** Modulation of TNF-α mRNA at 12 h (**b**) and 18 h (**c**) after stimulation. Data are shown as medians ± range of three individual experiments.

ever, since the IL-6 expression is an early event during inflammatory responses, the mRNA was not detected at 18 h. Briefly, real-time PCR was performed using the RevertAid Minus First Strand cDNA Synthesis Kit (Thermo Scientific, Wilmington, Del., USA) according to the manufacturer's instructions. Real-time PCR cycling was performed (5 min at 95°, followed by 38 cycles of 20 s at 95°, 30 s at temperature of annealing specified for each gene (TNF-α: 64°; IL-6: 61°; GAPDH: 60°) and 30 s at 72°) using Maxima SYBR green PCR master mix (Thermo Scientific). The relative expression of each target gene was normalized to an uninfected control and to the housekeeping gene GAPDH (ΔΔCt) and reported as fold change.

Quantification of the Costimulatory Molecules CD80 and CD86 in Monocytes and DCs by Flow Cytometry

To determine the effect of HIV-1 on the expression of costimulatory molecules CD80 and CD86, 1×10^6 PBMCs were cultured in 6-well polystyrene tissue culture plates, and challenged with HIV-1 and stimulated with TLR2, TLR4 or TLR9 ligands. The expression of CD80 and CD86 in monocytes, pDCs and mDCs was determined by flow cytometry using a FACS Canto Flow Cytometer (BD Biosciences, San Jose, Calif., USA) as described previously [24]. Logical gating was used to identify monocyte (CD14+), pDC ($Lin1^-/CD123^{high}$) and mDC ($Lin1^-/CD11c^{high}$) populations (online suppl. fig.; for all online suppl. material, see www.karger.com/doi//10.1159/000371765), as described previously [24]. The lineage cocktail 1 was used for negative selection of DCs. It contains a combination of antibodies against CD3, CD14, CD16, CD19, CD20 and CD56, in order to stain lymphocytes, NK cells, monocytes and neutrophils. The acquired events were analyzed using Cell Quest software. Each experiment was carried out with the respective controls (fluorochrome compensation, isotype controls and cells without staining).

Statistical Analysis

Data were plotted and analyzed using the Prism 5.0 software (GraphPad Software, La Jolla, Calif., USA). A Mann-Whitney test was used to compare the results between the different stimuli. $p < 0.05$ was considered statistically significant.

Results

Proinflammatory Cytokine Expression Is Modulated in PBMCs Exposed to HIV-1 in vitro and Treated with TLR Agonists

First, we evaluated whether the expression transcriptional of IL-6 and TNF-α cytokines is altered in PBMCs in vitro challenged with HIV-1 and treated with Pam_3CSK_4, LPS or CpG-B ODN. To achieve this objective, we first exposed fresh PBMCs to HIV-1 for 2 h. The prestimulated PBMCs were then cultured with the respective TLR agonist for 12 h to quantify IL-6 mRNA or for 12 and 18 h to quantify TNF-α mRNA, both by real-time RT-PCR. As one might expect, Pam_3CSK_4 treatment induces an increased expression of mRNA IL-6, compared to the mock. However, this response was significantly altered in those cells preexposed to HIV-1 and treated with Pam_3CSK_4 (fig. 1a).

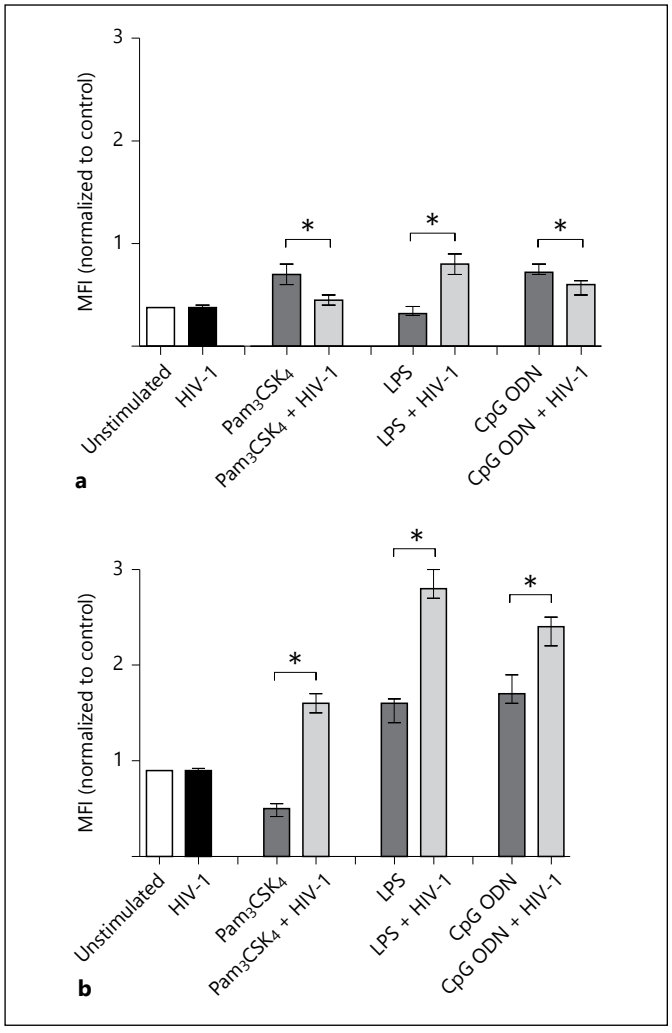

Fig. 2. Modulation of CD80 and CD86 expression through TLR2, TLR4 and TLR9 in monocytes. The PBMCs were stimulated only with the respective receptor agonist or preexposed to HIV-1 and then stimulated with the respective agonist, and the CD80 (**a**) and CD86 (**b**) expressions were determined by flow cytometry. Data are shown as medians ± range of three individual experiments. MFI = Mean fluorescence intensity.

For LPS stimulation, increased mRNA IL-6 was observed in both LPS alone and LPS after HIV-1 challenge, compared to the mock. In addition, this was significantly higher in the presence of HIV-1compared with LPS alone. In PBMCs treated with CpG-B ODN, IL-6 mRNA decreased (~3-fold), but a stronger downregulation was observed in the presence of CpG-B ODN and HIV-1 (~16-fold; fig. 1a).

For TNF-α mRNA on PBMCs challenged with HIV-1 in vitro and stimulated with LPS, a significant increase (~20-fold) was observed after 12 h of culture, compared to LPS alone. This is very interesting since in the presence of LPS alone, the change was only ~2-fold compared to the mock (fig. 1b). Stimulation with a TLR2 agonist and HIV-1 induced a significant increase in the production of TNF-α compared with stimulation only with a TLR2 agonist (fig. 1b). After 18 h of costimulation with HIV-1 and LPS, the TNF-α mRNA did not change compared with 12 h of stimulation, but LPS and HIV-1 stimuli continued to be significant compared with LPS stimulation alone. Interestingly, when the PBMCs were stimulated only with CpG-B ODN during 18 h, a strong production of TNF-α mRNA was observed (~23-fold), but in the presence of HIV-1 this effect was significantly decreased (fig. 1c). Taken together, our results suggest that HIV-1 can directly or indirectly upregulate the functionality of TLR4 leading to a change in TNF-α and IL-6 mRNA production.

In Presence of the TLR2, TLR4 or TLR9 Agonists, HIV-1 Induces the Maturation of Monocytes

CD80 and CD86 are costimulatory molecules used as molecular markers for activation/maturation of antigen-presenting cells. Although it has been described that TLR stimulation modulates the expression of the CD80 and CD86 costimulatory markers on monocytes [25], little is known about the role of HIV-1 infection on the modulation of the expression of both markers on monocytes CD14+ in the presence of TLR agonists. Therefore, we investigated whether expression of these two markers is affected by HIV-1 in the presence of Pam_3CSK_4, LPS or CpG-B ODN; cells stimulated only with TLR agonists served as controls. Considering a wide biological variability observed among individuals included in this study, for the analysis the data were normalized to unstimulated cells from each individual, allowing better visualization of the expression modulation. Values above/below 1 represent positive/negative regulation of CD80 or CD86 expression. As observed in figure 2a, in the presence of Pam_3CSK_4, LPS or CpG-B ODN, HIV-1 significantly decreased CD80 expression, based on the mean fluorescence intensity. In contrast, HIV-1 in the presence of Pam_3CSK_4, LPS or CpG-B ODN significantly increased the expression of CD86 (fig. 2b) compared with stimuli only with the agonists specific for each TLR. These results suggest that although the expression of CD86 is modulated in response to TLR agonists, HIV-1 can further enhance the maturation of monocytes.

Effect of the TLR2, TLR4 and TLR9 Agonists and HIV-1 on Activation of pDCs and mDCs

We next characterized mDCs and pDCs using lineage-specific and DC maturation markers. Interestingly, the

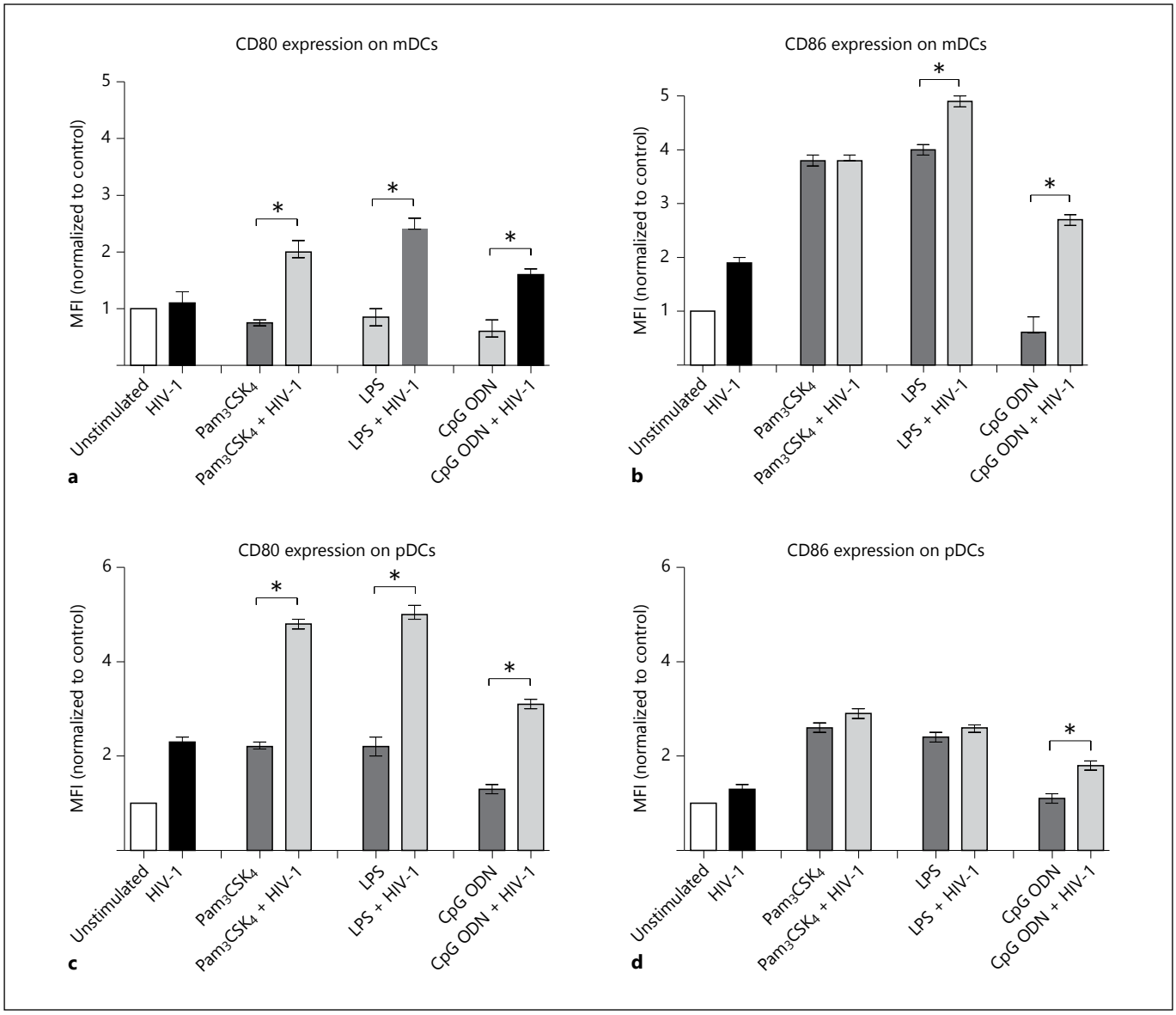

Fig. 3. Modulation of CD80 and CD86 expression through TLR2, TLR4 and TLR9 in DCs. The PBMCs were stimulated only with the respective receptor agonist or first exposed to HIV-1 and then stimulated with the respective agonist, and CD80 and CD86 expressions were determined by flow cytometry in mDCs (**a**, **b**) or in pDCs (**c**, **d**). Data are shown as medians ± range of three individual experiments. MFI = Mean fluorescence intensity.

challenge of PBMCs with HIV-1 in the presence of TLR2, TLR4 or TLR9 agonists results in a significant upregulation of CD80 expression in mDCs compared with the controls (fig. 3a). We also observed a significant increase in the expression of CD86 in mDCs when the cells were stimulated with HIV-1 in the presence of LPS or CpG-B ODN, but not in the presence of the Pam_3CSK_4 agonist (fig. 3b) compared to the stimulation only with the respective agonists.

Regarding CD80 and CD86 expression on pDCs, HIV-1 in the presence of Pam_3CSK_4, LPS or CpG-B ODN led to a significant increase in CD80 expression (fig. 3c) compared with the cells stimulated only with each agonist. Our results also show that in pDCs, CD86 expression only increased when the cells are challenged with HIV-1 in the presence of the TLR9 agonist (fig. 3d), but not with Pam_3CSK_4 or LPS. Together these results suggest that HIV-1, directly or indirectly, through TLR2,

TLR4 and TLR9 induces the maturation of mDCs and pDCs.

In conclusion, our results demonstrate that HIV-1 modulates the expression of CD80 in both DC populations, mainly through TLR4 and TLR2, and to a lesser proportion through TLR9. However, the modulation of CD86 expression occurs mainly through TLR9. In monocytes, the main way of inducing CD86 expression by HIV-1 may be through TLR4 and TLR2.

Discussion

Previous studies conducted in our laboratory have shown an increase of TLR4 expression in cells obtained directly from HIV-1-infected patients [3]. Furthermore, our in vitro experiments showed that stimulation of PBMCs with HIV-1 significantly increases IL-6 and TNF-α expression via TLR4 (fig. 1), suggesting that HIV-1 alters both the function and expression of this TLR. Based on these results and taking into account previous reports, we propose that increased expression and function of TLR4 may be a mechanism used by HIV-1 to promote the activation of infected cells and thereby favors its replication via NF-KB, a strong transactivator of HIV-1 promoter transcription [13]. On the other hand, this activation through TLR4 is important since microbial translocation, a common characteristic of chronic HIV-1, is related to systemic immune activation. In this context circulating LPS (TLR4 agonist) is increased in chronically HIV-infected individuals and is used as an indicator of microbial translocation [26], which contributes to the HIV-1 pathogenesis.

In the present study, we evaluated the mRNA expression of IL-6 and TNF-α on PBMCs challenged with HIV-1 in the presence of the TLR2, TLR4 or TLR9 agonists. Indeed, mRNA expression of proinflammatory cytokines is altered in PBMCs in response to HIV-1 stimulation in the presence of TLR agonists. HIV-1 significantly decreases mRNA expression of IL-6 through TLR2 and TLR9. An increase in the mRNA expression of TNF-α through TLR4 was observed, suggesting a strong induction of TNF-α on PBMCs during early HIV-1 infection, as demonstrated in monocyte-derived macrophages through the activation of NF-κB [27]. Here, we showed that stimulation of PBMCs with HIV-1 induces a rapid and significant upregulation of TNF-α in response to LPS (fig. 1b), suggesting a possible role of this cytokine in HIV-1 infection. Our data indicates that the TNF-α response to the TLR9 agonist in the presence of HIV-1 is not affected. Previously we showed an increase of TLR expression and production of proinflammatory cytokines in monocyte-derived macrophages and PBMCs infected with HIV-1 in vitro and in response to TLR stimulation compared to mock-infected controls [24].

Previous studies have shown that monocytes from HIV-1-infected patients present altered TLR2 expression and function, suggesting that HIV-1 or its components can regulate the expression and function of TLRs, enhancing chronic activation of the immune system and thereby HIV-1 replication [28]. Consistent with these findings, we also found that HIV-1 is capable of regulating TNF-α expression in PBMCs through TLR stimulation. This result could explain the impaired activation of the innate immune response observed in HIV-1-infected patients and may represent a mechanism that favors HIV-1 infection and replication.

On the other hand, the TLR stimulation manages the generation of adaptive immunity through the induction of major histocompatibility complex and costimulatory molecules CD80/CD86 on antigen-presenting cells [29, 30]. Here, we demonstrated that CD80 was significantly upregulated in monocytes challenged with HIV-1 in the presence LPS. In contrast, in the presence of Pam_3CSK_4 and CpG-B ODN, a significant decrease in CD80 expression was observed. Moreover, the expression of CD86 was significantly increased in monocytes challenged with HIV-1 and stimulated with the TLR2, TLR4 and TLR9 agonists (fig. 2b), compared with the expression in monocytes challenged only with HIV-1 or TLR agonists. This indicates that HIV-1 induces monocyte activation through TLR2, TLR4 and TLR9 measured by the CD86 expression.

Immature DCs are unable to induce the activation of T cells; nevertheless, after microbial infection occurs, the microbe is sensed by TLRs which leads to DC maturation/activation [6]. In our study, in the presence of TLR2, TLR4 or TLR9 agonists, HIV-1 significantly upregulates CD80 expression in mDCs and pDCs, compared with the controls, suggesting that HIV-1 might induce the activation of DCs through a mechanism dependent of these TLRs (fig. 3a, c). The effect on the modulation of CD86 expression by HIV-1 is more varied: in mDCs the modulation is statistically significant through TLR4 and TLR9, but in pDCs the modulation of the expression is only statistically significant through the TLR9 agonist. This modulation through TLR9 is interesting because previously CpG ODN was described as having an immunomodulatory role, suggesting that TLR9 agonists are effective vaccine adjuvants for infectious disease, cancer or asthma/

allergy [31]. Furthermore, it has demonstrated a promising role in viral infections such as influenza, hepatitis C and hepatitis B, where it has been demonstrated that use of CpG ODN as an adjuvant increases the vaccine effectiveness [32]. Specifically for HIV-1, a study showed that rabbits immunized with gp140 proteins derived from HIV-1 and CpG ODN as an adjuvant enhanced humoral responses to HIV-1, demonstrating the importance of TLR9 as modulating immune response [33]. In conformity with our results, previous studies have also demonstrated that stimulation of TLR2 with zymosan and TLR4 with LPS induce an increase of the expression of CD80, CD86 and other biomarkers [34]. Nevertheless, the functionality of this cellular activation is unknown. Based on our results, we suggest that monocyte and DC activation have an important role in viral pathogenesis, but it is not yet possible to establish the mechanism. However, it has also been described that monocyte, mDC and pDC activation are highly important since this activation leads to the development of adaptive immune response, allowing an antiviral response [18]. Loré et al. [22] also showed that cells obtained directed from HIV-1-infected patients have a similar maturation phenotype when they are exposed to the respective TLR ligands. Furthermore, the authors observed a higher activation of DCs than monocytes, as we reported here. Both pDCs and mDCs in HIV-infected patients express high levels of CD80 and CD86, and can be further activated by TLR engagement [35]. Taken together, our results support the hypothesis about the importance of HIV-1 in monocyte, mDC and pDC maturation through TLRs, as already described for other cellular populations, which show an increase in CD80/CD86 expression and cytokine production [36]. In addition, we previously found an association between TLR expression and upregulation of CD80 in pDCs [24]. Increases in the expression costimulatory molecules have also been reported in circulating T cells during HIV-1 infection and are considered to be the consequence of chronic T cell activation [37, 38]. Our findings are very interesting because the costimulatory molecules, CD80 and CD86, are necessary for appropriate antigen presentation, and the subsequent establishment of either Th1 or Th2 adaptive response [39, 40]. However, it was recently demonstrated that HIV-1 Nef protein induces loss of CD80 and CD86 expression in antigen-presenting cells [41], as an evasion mechanism.

In summary, in this study we have evaluated the response of human monocytes and DCs to HIV-1 stimulation in the presence of TLR agonists. According to previous reports, our results shown that TLR agonists induce proinflammatory cytokine expression such as IL-6 and TNF-α, but in this case HIV-1 stimulation is able to increase TLR responsiveness. Parallel results show increased expression of costimulatory molecules CD80 and CD86 on monocytes and two subpopulations of DCs (mDCs and pDCs) in the presence of TLR agonists and HIV-1.

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Disclosure Statement

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References

1 Ostuni R, Zanoni I, Granucci F: Deciphering the complexity of Toll-like receptor signaling. Cell Mol Life Sci 2010;67:4109–4134.
2 Caron G, Duluc D, Fremaux I, Jeannin P, David C, Gascan H, Delneste Y: Direct stimulation of human T cells via TLR5 and TLR7/8: flagellin and R-848 up-regulate proliferation and IFN-gamma production by memory CD4+ T cells. J Immunol 2005;175:1551–1557.
3 Hernandez JC, Arteaga J, Paul S, Kumar A, Latz E, Urcuqui-Inchima S: Up-regulation of TLR2 and TLR4 in dendritic cells in response to HIV type 1 and coinfection with opportunistic pathogens. AIDS Res Hum Retroviruses 2011;27:1099–1109.
4 Pasare C, Medzhitov R: Toll-dependent control mechanisms of CD4 T cell activation. Immunity 2004;21:733–741.
5 Steinman RM, Hemmi H: Dendritic cells: translating innate to adaptive immunity. Curr Top Microbiol Immunol 2006;311:17–58.
6 Banchereau J, Steinman RM: Dendritic cells and the control of immunity. Nature 1998; 392:245–252.
7 Cameron PU, Freudenthal PS, Barker JM, Gezelter S, Inaba K, Steinman RM: Dendritic cells exposed to human immunodeficiency virus type-1 transmit a vigorous cytopathic infection to CD4+ T cells. Science 1992;257: 383–387.
8 Pope M, Betjes MG, Romani N, Hirmand H, Cameron PU, Hoffman L, Gezelter S, Schuler G, Steinman RM: Conjugates of dendritic cells and memory T lymphocytes from skin facilitate productive infection with HIV-1. Cell 1994;78:389–398.

9 Kadowaki N, Ho S, Antonenko S, Malefyt RW, Kastelein RA, Bazan F, Liu YJ: Subsets of human dendritic cell precursors express different Toll-like receptors and respond to different microbial antigens. J Exp Med 2001; 194:863–869.
10 Kedzierska K, Crowe SM: Cytokines and HIV-1: interactions and clinical implications. Antivir Chem Chemother 2001;12:133–150.
11 Navikas V, Link J, Persson C, Olsson T, Hojeberg B, Ljungdahl A, Link H, Wahren B: Increased mRNA expression of IL-6, IL-10, TNF-alpha, and perforin in blood mononuclear cells in human HIV infection. J Acquir Immune Defic Syndr Hum Retrovirol 1995;9: 484–489.
12 Sundstrom JB, Little DM, Villinger F, Ellis JE, Ansari AA: Signaling through Toll-like receptors triggers HIV-1 replication in latently infected mast cells. J Immunol 2004;172:4391–4401.
13 Equils O, Schito ML, Karahashi H, Madak Z, Yarali A, Michelsen KS, Sher A, Arditi M: Toll-like receptor 2 (TLR2) and TLR9 signaling results in HIV-long terminal repeat transactivation and HIV replication in HIV-1 transgenic mouse spleen cells: implications of simultaneous activation of TLRs on HIV replication. J Immunol 2003;170:5159–5164.
14 Heggelund L, Müller F, Lien E, Yndestad A, Ueland T, Kristiansen KI, Espevik T, Aukrust P, Froland SS: Increased expression of Toll-like receptor 2 on monocytes in HIV infection: possible roles in inflammation and viral replication. Clin Infect Dis 2004;39:264–269.
15 Funderburg NT, Sieg SF: Diminished responsiveness to human β-defensin-3 and decreased TLR1 expression on monocytes and mDCs from HIV-1-infected patients. J Leukoc Biol 2012;92:1103–1109.
16 Chung NP, Matthews K, Klasse PJ, Sanders RW, Moore JP: HIV-1 gp120 impairs the induction of B cell responses by TLR9-activated plasmacytoid dendritic cells. J Immunol 2012; 189:5257–5265.
17 Gringhuis SI, den Dunnen J, Litjens M, van Het Hof B, van Kooyk Y, Geijtenbeek TB: C-type lectin DC-SIGN modulates Toll-like receptor signaling via Raf-1 kinase-dependent acetylation of transcription factor NF-kappaB. Immunity 2007;26:605–616.
18 Pasare C, Medzhitov R: Toll-like receptors and acquired immunity. Semin Immunol 2004;16:23–26.
19 Meier A, Bagchi A, Sidhu HK, Alter G, Suscovich TJ, Kavanagh DG, Streeck H, Brockman MA, LeGall S, Hellman J, Altfeld M: Upregulation of PD-L1 on monocytes and dendritic cells by HIV-1 derived TLR ligands. AIDS 2008;22:655–658.
20 Beignon AS, McKenna K, Skoberne M, Manches O, DaSilva I, Kavanagh DG, Larsson M, Gorelick RJ, Lifson JD, Bhardwaj N: Endocytosis of HIV-1 activates plasmacytoid dendritic cells via Toll-like receptor-viral RNA interactions. J Clin Invest 2005;115:3265–3275.
21 Gringhuis SI, van der Vlist M, van den Berg LM, den Dunnen J, Litjens M, Geijtenbeek TB: HIV-1 exploits innate signaling by TLR8 and DC-SIGN for productive infection of dendritic cells. Nat Immunol 2010;11:419–426.
22 Loré K, Betts MR, Brenchley JM, Kuruppu J, Khojasteh S, Perfetto S, Roederer M, Seder RA, Koup RA: Toll-like receptor ligands modulate dendritic cells to augment cytomegalovirus- and HIV-1-specific T cell responses. J Immunol 2003;171:4320–4328.
23 Velilla PA, Montoya CJ, Hoyos A, Moreno ME, Chougnet C, Rugeles MT: Effect of intrauterine HIV-1 exposure on the frequency and function of uninfected newborns' dendritic cells. Clin Immunol 2008;126:243–250.
24 Hernandez JC, Stevenson M, Latz E, Urcuqui-Inchima S: HIV type 1 infection up-regulates TLR2 and TLR4 expression and function in vivo and in vitro. AIDS Res Hum Retroviruses 2012;28:1313–1328.
25 Tsai CC, Lin CR, Tsai HY, Chen CJ, Li WT, Yu HM, Ke YY, Hsieh WY, Chang CY, Wu CY, Chen ST, Wong CH: The immunologically active oligosaccharides isolated from wheatgrass modulate monocytes via Toll-like receptor-2 signaling. J Biol Chem 2013;288: 17689–17697.
26 Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, Kazzaz Z, Bornstein E, Lambotte O, Altmann D, Blazar BR, Rodriguez B, Teixeira-Johnson L, Landay A, Martin JN, Hecht FM, Picker LJ, Lederman MM, Deeks SG, Douek DC: Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med 2006;12: 1365–1371.
27 Naif H, Ho-Shon M, Chang J, Cunningham AL: Molecular mechanisms of IL-4 effect on HIV expression in promonocytic cell lines and primary human monocytes. J Leukoc Biol 1994;56:335–339.
28 Mirlashari MR, Lyberg T: Expression and involvement of toll-like receptors (TLR)2, TLR4, and CD14 in monocyte TNF-alpha production induced by lipopolysaccharides from *Neisseria meningitidis*. Med Sci Monit 2003;9:BR316–BR324.
29 Medzhitov R, Janeway CA Jr: Innate immunity: impact on the adaptive immune response. Curr Opin Immunol 1997;9:4–9.
30 Pasare C, Medzhitov R: Toll pathway-dependent blockade of CD4+CD25+ T cell-mediated suppression by dendritic cells. Science 2003;299:1033–1036.
31 Jurk M, Vollmer J: Therapeutic applications of synthetic CpG oligodeoxynucleotides as TLR9 agonists for immune modulation. BioDrugs 2007;21:387–401.
32 Gupta K, Cooper C: A review of the role of CpG oligodeoxynucleotides as Toll-like receptor 9 agonists in prophylactic and therapeutic vaccine development in infectious diseases. Drugs R D 2008;9:137–145.
33 Burke B, Gómez-Román VR, Lian Y, Sun Y, Kan E, Ulmer J, Srivastava IK, Barnett SW: Neutralizing antibody responses to subtype B and C adjuvanted HIV envelope protein vaccination in rabbits. Virology 2009;387:147–156.
34 Yamamoto M, Sato S, Hemmi H, Sanjo H, Uematsu S, Kaisho T, Hoshino K, Takeuchi O, Kobayashi M, Fujita T, Takeda K, Akira S: Essential role for TIRAP in activation of the signalling cascade shared by TLR2 and TLR4. Nature 2002;420:324–329.
35 Martinson JA, Tenorio AR, Montoya CJ, Al-Harthi L, Gichinga CN, Krieg AM, Baum LL, Landay AL: Impact of class A, B and C CpG-oligodeoxynucleotides on in vitro activation of innate immune cells in human immunodeficiency virus-1 infected individuals. Immunology 2007;120:526–535.
36 Seki E, Brenner DA: Toll-like receptors and adaptor molecules in liver disease: update. Hepatology 2008;48:322–335.
37 Boasso A, Hardy AW, Landay AL, Martinson JL, Anderson SA, Dolan MJ, Clerici M, Shearer GM: PDL-1 upregulation on monocytes and T cells by HIV via type I interferon: restricted expression of type I interferon receptor by CCR5-expressing leukocytes. Clin Immunol 2008;129:132–144.
38 Kochli C, Wendland T, Frutig K, Grunow R, Merlin S, Pichler WJ: CD80 and CD86 costimulatory molecules on circulating T cells of HIV infected individuals. Immunol Lett 1999; 65:197–201.
39 Manicassamy S, Pulendran B: Modulation of adaptive immunity with Toll-like receptors. Semin Immunol 2009;21:185–193.
40 Takeda K, Akira S: Microbial recognition by Toll-like receptors. J Dermatol Sci 2004;34: 73–82.
41 Chaudhry A, Das SR, Hussain A, Mayor S, George A, Bal V, Jameel S, Rath S: The Nef protein of HIV-1 induces loss of cell surface costimulatory molecules CD80 and CD86 in APCs. J Immunol 2005;175:4566–4574.