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Original Article

Inhibitory effects of human immunodeficiency virus gp120 and Tat on CpG-A-induced inflammatory cytokines in plasmacytoid dendritic cells

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Plasmacytoid dendritic cells (pDCs), not only inhibit viral replication, but also play an essential role in linking the innate and adaptive immune system. In this study, we explored the effects of human immunodeficiency virus (HIV) gp120 and tat on CpG-A-induced inflammatory cytokines in pDCs. The results provided fundamental insights into HIV pathogenesis that may hold promise for preventative and even curative strategies. pDCs were isolated using blood DC antigen 4 (BDCA-4) DC isolation kit, and the purity was analyzed using BDCA-2 antibody by flow cytometry. pDCs and peripheral blood mononuclear cells (PBMCs) were stimulated by either CpG-A (5 μg/ml), gp120 (0.5 μg/ml), tat (0.5 μg/ml), or CpG-A treatment combined with gp120 or tat. The production of type I interferons (IFNs) and other inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interlukine-6 (IL-6), and interferon-gamma-inducible protein-10 (IP-10) in the culture supernatant, was determined by enzyme-linked immunosorbent assay. The results showed that CpG-A induced high levels of type I IFNs and other inflammatory cytokines, including TNF-α, IL-6, and IP-10, in pDCs. Concomitant treatment with gp120 reduced the levels of IFN-α, IFN-β, TNF-α, IL-6, and IP-10 induced by CpG-A in pDCs by 79%, 53%, 60%, 50%, and 34%, respectively, while tat suppressed them by 88%, 66%, 71%, 64%, and 53%, respectively. Similar results were demonstrated in CpG-A-treated PBMCs. In conclusion, gp120 and tat are effective inhibitors of the CpG-A-mediated induction of type I IFNs and other inflammatory cytokines from pDCs and PBMCs.

Keywords Type I interferons; plasmacytoid dendritic cells; cytokines; gp120; tat

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Introduction

Human immunodeficiency virus (HIV) infection remains a global threat, which is characterized by intense viral replication and dissemination to lymphoid tissues, leading to acquired immune deficiency syndrome (AIDS) [1]. The combined pharmacological approach to the treatment of HIV infection, known as highly active antiretroviral therapy (HAART), has dramatically reduced AIDS-related morbidity and mortality. However, its use has been associated with serious adverse reactions [2]. Further advances in therapeutics and informative technologies, combined with a better understanding of the immunological and viral components of HIV, are needed.

Recent studies have shown that tat and gp120 are key immunomodulators in the pathogenesis of AIDS [1,3]. Their function in dysregulating the production of type I interferons (IFNs) and proinflammatory cytokines is mainly through their effects on dendritic cells (DCs), which results in the perturbation of the host immune response and the enhancement of retrovirus survival [4,5]. Type I IFNs secreted from plasmacytoid DC (pDCs) play an essential role in linking the innate and adaptive immune systems [6]. IFN-α is important in the activation, differentiation, and survival of B cells, T cells, and natural killer (NK) cells [7–9]. Furthermore, IFN-α can inhibit viral replication in CD4+ cells that are infected by HIV, and can enhance the cytotoxicity of CD8+ cells and NK cells [10].

Other proinflammatory cytokines, such as interlukine-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interferon-gamma-inducible protein-10 (IP-10), are important in the host defense and inflammatory response. TNF-α can inhibit virus replication through interfering with the synthesis of early viral proteins [11]. IL-6 can regulate various types of cells, such as B cells, T cells, and NK cells, all of which have important roles in the immune response [12].

Furthermore, IL-6 can promote B-cell differentiation and the secretion of immunoglobulin (Ig) in B cells [13]. IP-10 expression has been associated with HIV infection, and can contribute to the accumulation of activated T cells in the cerebrospinal fluid compartment of HIV-1-infected individuals and consequently inhibit HIV-1 replication [14]. Changes in the number and function of type I IFNs and proinflammatory cytokines may lead to a wide array of HIV-related immune dysfunction.

pDCs are a subset of pre-DCs that predominantly express toll-like receptor (TLR)7 and TLR9. They have the ability to produce extremely high amounts of IFN-α. In addition, they produce moderate amounts of inflammatory cytokines, including TNF-α, IL-6, and IP-10, in response to viruses, single-stranded RNA, or oligodeoxynucleotides (ODNs) that contain CpG motifs (CpG-ODN) [15,16]. The preferential production of type I IFNs or proinflammatory cytokines by pDCs depends on the different types of CpG motifs. CpG-A induces higher levels of type I IFN production compared with CpG-B. In contrast, CpG-B has been shown to be more active than CpG-A in stimulating IL-8 production and increasing co-stimulatory and Ag-presenting molecules [17]. DCs are postulated to be involved in the transmission of HIV-1 to T cells and in the stimulation of HIV-1-specific cell-mediated immunity [18].

During HIV infection, the production of type I IFNs and proinflammatory cytokines and the number of pDCs are profoundly impaired; the total pDC cell count in peripheral blood correlates inversely with viral load and positively with the CD4 T-cell count [19–22]. The origin of these defects is unclear. Gp120 and tat proteins have been shown to have profound effects on the function and viability of immune cells [3,4]. However, little information is known about gp120- and tat-mediated effects on pDCs. Given the central role that pDCs play in both innate and adaptive antiviral immune responses, an understanding of the mechanisms by which pDCs interact with and respond to HIV may provide fundamental insights into the pathogenesis of HIV, and hold promise for the development of new preventative and even curative strategies.

In this report, we examined the effect of gp120/tat addition on the function of pDCs. We found that gp120/tat could inhibit TLR9-mediated activation in pDCs, and suppress the CpG-A-induced secretion of type I IFNs and other inflammatory cytokines.

Materials and Methods

Synthesis of ODNs

The following ODNs were provided by Sangon (Shanghai, China): CpG-A (5 μg/ml), 5′-ggGGGACGATCGTCgg gggg-3′; and CpG-A control (5 μg/ml), 5′-ggGGGAG CATGCTGgggggc-3′. The lowercase letters represent phosphorothioate linkage. No endotoxin could be detected in CpG-A preparations using the *Limulus* amebocyte lysate assay (lower detection limit, 0.03 endotoxin U/ml; Bio-Whittaker, Walkersville, USA).

Preparation of peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque density centrifugation (Lymphoprep: Cedarlane Laboratories, Burlington, USA) from fresh heparinized peripheral blood obtained with informed consent from healthy volunteers (negative for HIV, hepatitis B virus, and hepatitis C virus, with normal blood cell counts and serum enzymes/proteins, and who were without fever, medication, or symptomatic allergies). The human studies were approved by the Institutional Review Board of Zhejiang University School of Medicine.

Enrichment of pDCs

PBMCs were depleted of T cells, B cells, NK cells, red blood cells, and monocytes, and enriched for DCs using a blood dendritic cell antigen 4 (BDCA-4) dendritic cell isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany). Cells were prepared according to the manufacturer's instructions. In brief, PBMCs were washed and resuspended in MACS buffer [phosphate-buffered saline (PBS) with 0.5% bovine serum albumin, and 2 mM ethylenediaminetetraacetic acid], treated with FcR-blocking reagent, and labelled with BDCA-4 microbeads at 4°C for 30 min. Cells were washed twice and resuspended in MACS buffer. Then, the appropriate MACS column and MACS separator were chosen according to the number of total cells to be isolated. The column was placed in the magnetic field of a suitable MACS separator, and then PBMCs were added to the column, which had been pretreated with MACS buffer. Columns were then washed with three rounds of MACS buffer. The columns were removed from the separator and placed in a suitable collection tube. A total of 1 ml buffer was pipetted onto the column, and immediately the fraction with magnetically labeled cells was flushed out of the column by firmly applying the plunger supplied. This procedure was repeated twice. Following isolation, the purified pDCs were analyzed by flow cytometry.

pDC purity analysis

Cells were fixed in PBS with 2% paraformaldehyde and stained with FcR-blocking reagent and PE-conjugated anti-BDCA-2 antibody (Miltenyi Biotec). Cells were mixed well and incubated for 30 min in the dark at 4°C. Cells were washed and the cell pellet was resuspended in buffer for flow cytometry analysis. pDCs were identified by staining for BDCA-2.

Cell viability assay

Determination of the cytotoxic effect of recombinant proteins, gp120 (Abcam, Cambridge, UK), tat (Abcam), or CpG-A, was imperative before further studies and was carried out. pDCs (1×10^4 cells/well in 96-well plate) were treated with either gp120 or tat (0, 0.1, 0.2, 0.5, and 1 μg/ml) or CpG-A (0, 0.1, 0.5, 1, 5, and 10 μg/ml) for 48 h, and cell viability was evaluated by the 3,4-(5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium salt (MTS) assay. The tetrazolium compound, MTS, and an electron coupling reagent, phenazine methosulfate (PMS), were used in the MTS assay. The cultures were seeded with 1×10^4 cells/well and incubated overnight to allow cell attachment. After incubation for the indicated time in the appropriate medium, 20 μl MTS/PMS mixture was added to each well. Then, the cells were incubated for 1 h, viable cells reduced MTS to formazan, and absorbance was measured at 490 nm using a spectrophotometer. The background absorbance from the control wells was subtracted from the actual absorbance value. Three duplicate studies were performed for each experimental condition.

Culture and treatment of pDCs and PBMCs

PBMCs and purified pDCs were cultured in RPMI-1640 medium that contained 10% fetal bovine serum, 2 mM L-glutamine, 1% penicillin, 1% streptomycin (Invitrogen, Carlsbad, USA), and were counted electronically with a Coulter Counter series Z1 (Coulter Electronics, Hialeah, USA). Cells were stimulated by CpG-A (5 μg/ml) in the presence or absence of recombinant gp120 protein (0.5 μg/ml) or recombinant tat protein (0.5 μg/ml) at 37°C under a humidified atmosphere with 5% CO_2 in air in 96-well plates (PBMCs: 2×10^5 cells in 200 μl medium/well pDCs: 5×10^4 cells in 200 μl medium/well) for 24 h. After incubation, supernatants were collected by spinning the plates for 5 min at 300 *g* at 4°C to remove cells and stored at −20°C until analysis. All tests were carried out in strict accordance with manufacturer's instructions, and each sample was analyzed in duplicate.

Cytokine analysis

Supernatants collected from cell cultures were stored at −20°C until further use. The concentration of IFN-α in the supernatants was then measured using a human IFN-α platinum enzyme-linked immunosorbent assay (ELISA) kit (eBioscience, Vienna, Austria). The limit of detection of human IFN-α was 7.8 pg/ml. The amounts of IFN-β in the supernatants were detected using a VeriKine human IFN-β ELISA with the minimum detection level of 50 pg/ml (PBL Interferon Source, Piscataway, USA). Supernatants were then analyzed for TNF-α, IP-10, IL-6 levels using ELISA kits from R&D Systems (Minneapolis, USA) with the minimum detectable levels of 15.6, 7.8, and 3.12 pg/ml, respectively. All tests were carried out in strict accordance with manufacturer's instructions, and each sample was analyzed in duplicate.

Statistical analysis

All experiments were repeated at least three times. The results were expressed as the means ± standard error of the mean (SEM). Statistical significance was calculated by one-way analysis of variance. P value <0.05 was considered to be statistically significant.

Results

Identification of pDCs in human PBMCs

BDCA-2 and BDCA-4 are expressed only on the surface of human pDCs in blood, lymphoid, and non-lymphoid tissues [23]. Its specific expression allows the direct identification of pDCs. Staining with BDCA-2 antibody is recommended for flow cytometric evaluation when pDCs are isolated using BDCA-4. Before separation, the purity of pDCs was only 0.56% [**Fig. 1(A)**], but after separation, the purity of pDCs was enriched to 87.42% [**Fig. 1(B)**].

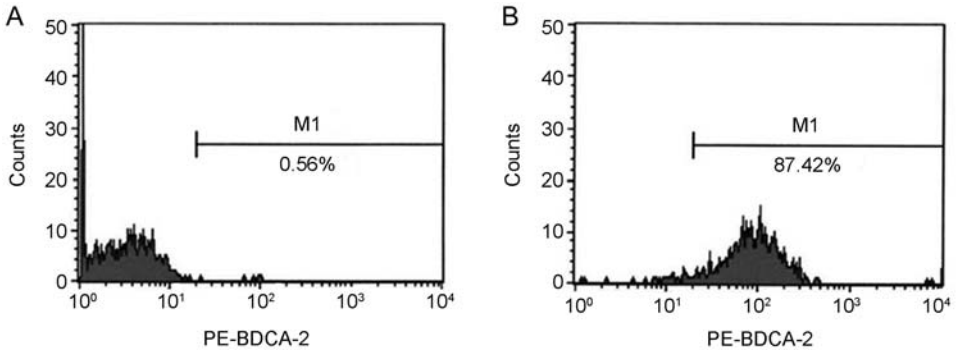

Figure 1 Identification of pDC in PBMCs PBMCs were prepared from the peripheral blood of normal healthy volunteers and analyzed by staining. The BDCA-2 antibody is recommended for flow cytometric evaluation when pDCs are isolated using the BDCA-4 antibody. Before separation, the purity of pDCs was 0.56% (A), after separation the purity of pDCs was enriched to 87.42% (B).

Effect of recombinant proteins (gp120 or tat) and CpG-A on pDC viability

The viability of pDCs following incubation with different recombinant proteins (gp120 or tat) for 48 h was determined by the MTS assay. The results demonstrated that pDCs retained almost the same viability when exposed to recombinant protein concentrations of 0–1 μg/ml [**Fig. 2(A)**] or CpG-A of 0–10 μg/ml [**Fig. 2(B)**] under our incubation conditions. Therefore, <1 μg/ml of recombinant proteins (gp120 or tat) or 10 μg/ml of CpG-A were suitable for use, without causing any interference with pDCs.

Gp120/tat inhibits the CpG-A-mediated production of type I IFNs from pDCs and PBMCs

CpG-A treatment can induce the high levels secretion of IFN-α from pDCs in a concertration-dependent manner ranging from 0.1 to 10 μg/ml (data not shown). In this study, we used 5 μg/ml CpG-A to treat the cells. The recombinant proteins gp120 or tat alone did not significantly change the level of IFN-α in the supernatant compared with the untreated group. However, when pDCs were treated with gp120 or tat combined with CpG-A, the level of IFN-α dramatically decreased compared with that in the cell treated with CpG-A alone. We evaluated several concentrations of gp120 and tat recombinant proteins (from 0.025 to 1 μg/ml), and found that their effects on IFN-α secretion were concentration-dependent (data not shown), and 0.5 μg/ml gp120 and tat consistently reduced IFN-α levels by at least 50%. According to the use of IFN-α as a reference, in the following experiments, we used 5 μg/ml CpG-A and 0.5 μg/ml gp120 or tat as the most suitable concentrations. The results showed that addition of gp120 (0.5 μg/ml) reduced the CpG-A-induced IFN-α levels by 79% ($P < 0.05$), and the addition of tat (0.5 μg/ml) reduced the CpG-A-induced IFN-α levels by 88% ($P < 0.05$) (**Table 1**). Next, we evaluated the secretion of IFN-β from pDCs under the same conditions. The results showed that gp120 and tat recombinant proteins could suppress CpG-A-induced IFN-β secretion in pDCs by 53% and 66%, respectively, ($P < 0.05$; **Table 1**). The study also showed that gp120 and tat proteins could suppress the

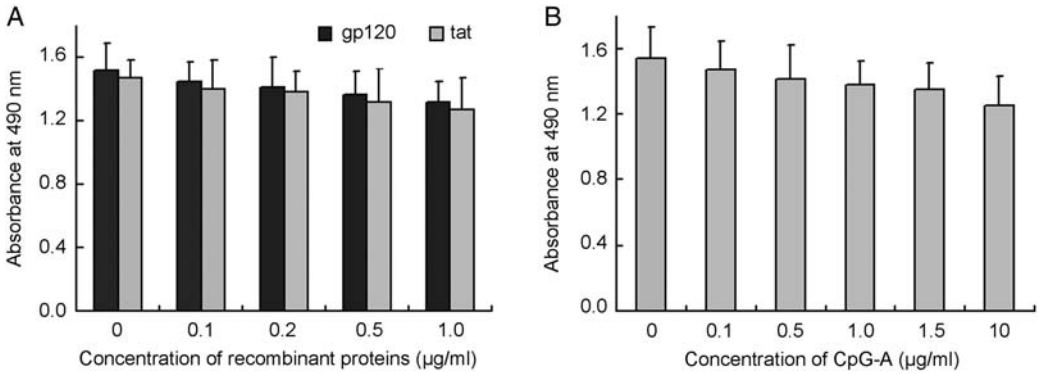

Figure 2 Effect of recombinant proteins (gp120 or tat) and CpG-A on the viability of pDCs (A) Effect of recombinant proteins (gp120 or tat) on the viability of pDCs. pDCs (1×10^4 cells/well) were incubated with recombinant proteins (gp120 or tat) of different concentrations for 48 h. Cell viability was determined by the MTS assay. Data represent means ± SEM ($n = 3$). (B) Effect of CpG-A on the viability of pDCs. PDCs (1×10^4 cells/well) were incubated with CpG-A of different concentrations for 48 h. Cell viability was determined by the MTS assay. Data represent means ± SEM ($n = 3$).

Table 1 The effects of gp120 and tat on type I IFNs induced by CpG-A

Groups	pDCs		PBMCs	
	IFN-α (pg/ml)	IFN-β (pg/ml)	IFN-α (pg/ml)	IFN-β (pg/ml)
CpG-A	8524.3 ± 724.6	547.4 ± 23.6	984.5 ± 273.4	128.6 ± 14.4
gp120	42.3 ± 10.7	73.4 ± 9.3	9.5 ± 3.8	64.2 ± 7.2
Tat	35.6 ± 8.7	67.5 ± 8.4	8.2 ± 4.3	56.3 ± 8.1
CpG-A + gp120	1748.3 ± 428.5*	257.3 ± 17.3*	387.2 ± 162.6*	84.3 ± 6.5*
CpG-A + Tat	993.7 ± 246.1**	184.3 ± 13.5**	218.1 ± 106.4**	75.6 ± 8.3**
Untreated	38.2 ± 5.8	75.2 ± 5.7	12.4 ± 3.5	68.5 ± 4.8

IFN-α and IFN-β secretion from pDCs and PBMCs under the stimulation with CpG-A (5 μg/ml) alone, or in the presence of gp120 (0.5 μg/ml) or tat (0.5 μg/ml).

*$P < 0.05$ vs. CpG-A alone, **$P < 0.05$ vs. CpG-A alone.

CpG-A-induced secretion of type I IFNs in PBMCs. However, the expression of type I IFNs and their inhibitory effect in PBMCs were weaker than those found in pDCs (**Table 1**).

We also studied the kinetics of IFN-α production in pDCs. The supernatants were collected after different times of incubation. Inhibition of CpG-A-induced IFN-α production by gp120 or tat could be seen within the first 12 h; and the effect was still present up to 48 h after the start of incubation (**Fig. 3**).

Gp120/tat inhibits CpG-A-mediated production of proinflammatory cytokines from pDCs and PBMCs

In addition to the secretion of IFN-α, activated pDCs can secrete moderate amounts of other inflammatory cytokines, such as TNF-α, IL-6, and IP-10. These inflammatory cytokines have important roles in both the innate and adaptive immunity. Using ELISA kits, we were able to detect TNF-α, IL-6, and the chemokine IP-10 after CpG-A-mediated stimulation. As with IFN-α, concomitant treatment with gp120 reduced the CpG-A-induced increase in the levels of TNF-α, IL-6, and IP-10 in pDCs by 60%, 50%, and 34%, respectively, ($P < 0.05$; **Table 2**). When pDCs were treated with tat combined with CpG-A, the levels of TNF-α, IL-6, and IP-10 in pDCs were reduced by 71%, 64%, and 53% compared with those under the treatment of CpG-A alone, respectively, ($P < 0.05$; **Table 2**). We also detected the secretion of all three cytokines in PBMCs when cultured under the same conditions, and found similar results with IFN-α expression (**Table 2**).

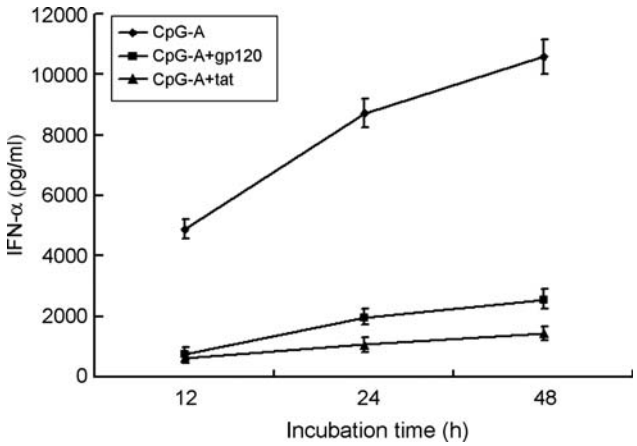

Figure 3 IFN-α production of pDCs stimulated with CpG-A (5 μg/ml) or in the presence or absence of gp120 (0.5 μg/ml) or tat (0.5 μg/ml) pDCs were isolated from PBMCs and cultured in 96-well plates (5×10^4 cells/well). Supernatants were collected and assayed by ELISA at 12, 24, and 48 h.

Discussion

The underlying mechanisms that govern the pathogenesis of AIDS from HIV remain unclear. Immune dysregulation, particularly an imbalance in cytokine secretion, and impaired antigen presentation by DC cells, appears to be a crucial hallmark in this process [24].

The biological function of pDCs is to produce large amounts of IFN-α following the ligation of TLR9 with bacterial DNA or a synthetic counterpart, such as the oligoDNA containing unmethylated CpG motifs (CpG DNA) [25]. In addition to type I IFNs, the pDCs produce IL-12, IL-6, IP-10, and TNF-α, as well as a number of inflammatory chemokines and chemokine receptors that stimulate the migration of pDCs to lymph nodes [26]. Type I IFNs can regulate various types of cells, such as B cells, T cells, and NK cells [7–9]. Among people with AIDS or those infected with HIV, type I IFNs and other inflammatory chemokines are profoundly reduced [20,21] and these cell populations also exhibit major functional defects [27]. Thus, the reduction of type I IFNs and other inflammatory cytokines may contribute to the HIV-induced immunodeficiency, resulting in the exacerbation of HIV

Table 2 The effects of gp120 and tat on other cytokines induced by CpG-A

Groups	pDCs			PBMCs		
	TNF-α (pg/ml)	IL-6 (pg/ml)	IP-10 (pg/ml)	TNF-α (pg/ml)	IL-6 (pg/ml)	IP-10 (pg/ml)
CpG-A	865.6 ± 56.1	652.4 ± 17.2	9678.3 ± 146.7	534.6 ± 23.7	387.5 ± 14.9	6845.2 ± 158.4
gp120	21.4 ± 3.6	18.3 ± 5.4	193.6 ± 23.8	16.3 ± 3.8	8.3 ± 4.2	135.8 ± 26.5
Tat	17.3 ± 2.7	16.7 ± 4.9	183.9 ± 34.6	15.9 ± 2.6	6.9 ± 3.4	116.8 ± 13.6
CpG-A + gp120	342.3 ± 16.4*	321.4 ± 34.6*	6385.6 ± 126.5*	356.2 ± 19.3*	243.1 ± 15.3*	4135.7 ± 145.7*
CpG-A + Tat	253.5 ± 12.8**	236.2 ± 16.3**	4538.4 ± 176.9**	298.3 ± 13.1**	208.3 ± 12.2**	3847.2 ± 158.3**
Untreated	34.2 ± 7.3	26.4 ± 7.6	223.5 ± 35.7	22.3 ± 4.1	11.5 ± 3.7	154.3 ± 34.6

TNF-α, IL-6, and IP-10 production from pDCs and PBMCs stimulated with CpG-A (5 μg/ml) or in the presence or absence of gp120 (0.5 μg/ml) or tat (0.5 μg/ml).

*$P < 0.05$ vs. CpG-A alone, **$P < 0.05$ vs. CpG-A alone.

infection. The potential role of pDCs in immune dysfunction in HIV has therefore generated substantial interest.

To better understand how HIV interacts with pDCs, we treated freshly isolated pDCs with recombinant gp120 alone. No change of IFN-α secretion was found compared with the untreated group (**Table 1**). Gp120 treatment did, however, produce specific alterations in the CpG-A-mediated function of pDCs. When pDCs were treated simultaneously with CpG-A and gp120 recombinant protein, CpG-A-mediated levels of type I IFNs were significantly suppressed (**Table 1**), which was consistent with the observations of Martinelli *et al*. [24]. Subsequently, we evaluated several concentrations of gp120, ranging from 0.025 to 1 μg/ml, over a time course from 12 to 48 h. It was determined that the effects of gp120 on IFN-α secretion were both concentration- and time-dependent.

Tat is an important regulatory protein, which plays an important role in the pathogenesis of HIV. The role of tat in pDCs is a subject of great interest. Therefore, we treated freshly isolated pDCs with recombinant tat alone or in combination with CpG-A. The results showed that tat cannot induce IFN-α from pDCs, but can inhibit the CpG-A-mediated production of type I IFNs from pDCs (**Table 1**). These results differ from a previous report by Fanales-Belasio *et al.* [28] who observed that key co-stimulatory molecules including TNF-α and IL-6 were up-regulated after pDCs were exposed to tat. This discrepancy may reflect the fact that, before the action of tat, the pDCs were cultured in complete medium in the presence of GM-CSF and IL-4, which can cause the maturation of pDCs. El-Hage *et al*. [29] found that tat can reduce the expression of TLR9 in the astroglia. Tat also may contribute to the results described above by reducing the expression of TLR9 in pDCs and altering the expression of several important DC genes [30].

It has been reported that the ligation of the pDC-specific C-type lectin BDCA-2 with a monoclonal anti-BDCA-2 Ab rapidly increased intracellular Ca^{2+} levels and inhibited virally-stimulated IFN-α production [31]. Gp120 can bind C-type lectin BDCA-2 in pDCs and increased intracellular Ca^{2+} levels and consequently inhibit IFN-α production [31,32]. Both gp120 and tat can reduce the IFN-α secretion of pDCs. Given the central role that IFN-α plays in both the innate and adaptive antiviral immune responses, a reduction in the secretion of type I IFNs may lead in part to the immune deficiency observed in HIV, therefore promoting the progression of this virus.

The roles of other cytokines in immune dysfunction in HIV are very important. In the present study, TNF-α, IL-6, and IP-10 expression in pDCs treated with CpG-A were significantly suppressed by either gp120 or tat protein (**Table 2**). TNF-α promotes the expression of major histocompatibility complex class I antigens in T cells, and enhances the proliferation of IL-2-dependent thymocytes and T cells. TNF-α can prevent the synthesis of early proteins in the virus to inhibit virus replication [11]. IL-6 promotes the proliferation of a variety of cell types, such as B cells, T cells, and NK cells, all which have an important role in the immune response [12]. Furthermore, it can promote the differentiation of cells, for example, B-cell differentiation, Ig secretion, and cytotoxic lymphocyte differentiation [13]. IP-10 contributes to the accumulation of activated T cells in the cerebrospinal fluid compartment in HIV-1-infected individuals and inhibits HIV-1 replication there [14]. It can also induce the expression of IFN-γ and consequently enhance the cytotoxic activity of NK cells. Direct interactions between gp120/tat and pDCs may help to suppress responses to specific opportunistic infections. Martinelli *et al.* [24] found that gp120 suppressed pDC-induced cytolytic activity of NK cells. In light of the central role that type I IFNs and other cytokines secreted from pDCs play in regulating the immune system, the results reported herein provide insights into the role of HIV–pDC interactions in HIV-driven immune system dysfunction.

Our study also showed that the secretion of type I IFNs and other inflammatory cytokines (TNF-α, IL-6, and IP-10) decreased in PBMCs treated simultaneously with CpG-A and either gp120 or tat. Both the levels of CpG-A-induced type I IFNs and inflammatory cytokines (TNF-α, IL-6, and IP-10), and the inhibition caused by gp120/tat in PBMCs, were less than those in pDCs.

IRF-7 plays an essential role in the virus-activated transcription of IFN-α gene [33]. This discrepancy may account for the high constitutive levels of IRF-7 expression in pDCs compared with those in PBMCs [34]. Type I IFNs, TNF-α, IL-6, and IP-10 were mostly induced by the stimulation of TLR9 in pDCs. As Soriano-Sarabia *et al.* [35] reported, TLR9 polymorphisms might play a role in HIV clinical disease progression.

In conclusion, tat and gp120 induce the suppression of CpG-A-induced type I IFNs and proinflammatory cytokines production. The suppression in the secretion of type I IFNs and inflammatory cytokines production may lead to an impaired host defense, thereby increasing the risk of airway opportunistic infections, a major cause for HIV mortality.

As described above, pDCs play an important role in the pathogenesis of HIV. Stimulators of the TLR9-mediated pathway in pDCs may have a protective effect in HIV patients, which could lead to the development of new drugs to treat HIV infection. The stimulation of TLRs initiates the activation of an intracellular signaling network that results in the secretion of proinflammatory cytokines, mainly type I IFNs, TNF-α, and IL-6. Attempts to develop TLR agonists for the treatment of HIV are underway.

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