APOBEC3G upregulation by alpha interferon restricts human immunodeficiency virus type 1 infection in human peripheral plasmacytoid dendritic cells

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APOBEC3G (A3G), a member of cytidine deaminase family, has potent anti-human immunodeficiency virus type 1 (HIV-1) activity. It has been demonstrated that alpha interferon (IFN-α) can significantly enhance the expression of A3G in human primary resting CD4+ T-cells, macrophages and primary hepatocytes, subsequently decreasing their viral susceptibility. Plasmacytoid dendritic cells (pDCs) are key effectors in innate host immunity, mediating adaptive immune responses and stimulating IFN-α production in reaction to various stimuli. In this report, we demonstrate that IFN-α, either exogenously added to- or endogenously secreted by pDCs, can enhance the expression of A3G and its family members such as A3A, A3C and A3F. We have also shown that IFN-α can inhibit HIV-1 expression in pDCs. This inhibitory effect could be countered by addition of an A3G-specific short interfering RNA, indicating that IFN-α-induced A3G plays a key role in mediating pDCs response to HIV-1. Given the central role played by pDCs in orchestrating the IFN-α/A3G intercellular network and intracellular signal pathway, our data indicate that pDCs themselves are also protected by an IFN-α/A3G-mediated innate immunity barrier from HIV-1 infection.

INTRODUCTION

The DNA-editing A3G enzymes have been identified as antiviral factors capable of inhibiting retroviruses such as human immunodeficiency virus type 1 (HIV-1), simian immunodeficiency virus, human T-lymphoma virus type 1, many type C retroviruses and hepatitis B virus. These findings shed light on antiviral approaches utilizing the innate immune system (Bishop et al., 2004; Doehle et al., 2005; Esnault et al., 2005; Noguchi et al., 2005; Sheehy et al., 2002; Yu et al., 2004). A3G belongs to the APOBEC family of cytidine deaminases, and its genetic locus is on human chromosome 22 (Jarmuz et al., 2002). A3G enzymes either edit newly synthesized viral DNA or have an inhibitory effect at other stage(s) of the viral life cycle (Mangeat et al., 2003, 2004; Mariani et al., 2003; Rosler et al., 2005; Sasada et al., 2005; Turelli et al., 2004; Zhang et al., 2003). Retroviruses encode various gene products that inhibit the effects of cytidine deaminases on viral replication. In the case of HIV-1, viral infectivity factor (Vif) effectively counters the antiviral effect of A3G/A3F by facilitating their degradation (Marin et al., 2003; Sheehy et al., 2003; Yu et al., 2003). Recent studies have demonstrated that the A3G gene is regulated by alpha interferon (IFN-α) in a cell-type-dependent manner. A3G upregulation by IFN-α has been observed in human resting CD4+ T-lymphocytes, macrophages and primary hepatocytes, but not in activated primary CD4+ T-cells, proliferating lymphoma-derived H9 cells or 293T cells (Bonvin et al., 2006; Chen et al., 2006; Peng et al., 2006; Sarks et al., 2006; Tanaka et al., 2006). Brief (6 h) treatment of newly infected resting CD4+ T cells with IFN-α has shown potent and irreversible inhibitory effects upon HIV-1 reverse transcription and viral infectivity (Chen et al., 2006).

Plasmacytoid dendritic cells (pDCs) comprise a small [<1 % of total peripheral blood mononuclear cells (PBMCs)] but very special immune cell population that primarily resides in lymphoid tissues. They are able to produce 100–1000 times more type 1 IFNs, such as IFN-α, IFN-β and IFN-ω, than other blood cell types in response to certain stimuli (Cella et al., 1999; Dzinek et al., 2001; Grouard et al., 1997; Rissoan et al., 1997; Siegal et al., 1999). The pDCs strictly express toll-like receptor-7 (TLR-7), which recognizes single-stranded RNA, and TLR-9, which recognizes double-stranded viral DNA and CpG oligodeoxynucleotides (ODNs). Activation of TLR-7 and TLR-9 signalling stimulates pDCs to secrete large amounts of type 1 IFNs and leads to subsequent pDC...
differentiation and maturation. In addition, pDCs also produce moderate amounts of tumour necrosis factor alpha (TNF-α) and interleukin (IL)-6, but they do not produce IFN-γ (Abe et al., 2005; Gurney et al., 2004; Kadowaki et al., 2000). Endocytosis of HIV-1 particles into pDCs can trigger secretion of large amounts of IFN-α through TLR-7, possibly due to the interaction between TLR-7 and HIV-1 genomic RNA in the endosome (Beignon et al., 2005). HIV-1 gp120 can also stimulate the secretion of IFN-α from pDCs (Del Corno et al., 2005; Schmidt et al., 2005). Activation of pDCs can also result in the indirect and/or direct activation of many other cell types, such as monocytes/macrophages, myeloid DCs, B cells, NK cells and T cells (McKenna et al., 2005). In addition, depletion of pDCs from total blood mononuclear cells abolishes virus-specific polyclonal IgG production in PBMCs (Jego et al., 2003). Interestingly, the ability of virus-induced mature pDCs to induce production of IFN-γ by naïve CD4+ T cells is dependent on type 1 IFN, but not dependent on IL-12 or IL-15 (Kadowaki et al., 2000). Such observations have indicated a pivotal role for pDCs in linking innate and adaptive immunity (McKenna et al., 2005).

HIV-1 activates pDCs, causing both concomitant induction of bystander maturation of myeloid DCs (Fonteneau et al., 2004) and subsequent activation of T cells (Cella et al., 2000; Kadowaki et al., 2000), which in turn produce IL-10 and IFN-γ (Cella et al., 2000; Kadowaki et al., 2000). It has been reported that pDCs are productively infected by HIV-1, which is evidenced by HIV-1 infection of pDCs in cell culture and immunohistochemical staining of HIV-1 p24 antigen in pDCs from tonsils of HIV-infected individuals (Fong et al., 2002; Patterson et al., 2001; Schmidt et al., 2004). Also, the number of circulating pDCs is decreased in the advanced stages of HIV-1 infection (Chehimi et al., 2002; Feldman et al., 2001; Soumelis et al., 2001), and the loss of peripheral blood pDCs correlates with a high viral load (Donaghy et al., 2001; Soumelis et al., 2001). Furthermore, the decrease in pDCs is consistent with the fact that CD4 T-cells and IFN-α production are profoundly and transiently impaired in primary HIV-1 infection (Kamga et al., 2005; Killian et al., 2006). In fact, the pDC population and consequent IFN-α secretion remain low even after 1 year of suppressive highly active antiretroviral therapy (HAART) possibly due to residual replication of HIV-1 and sustained immune activation (Almeida et al., 2006). Because pDCs are susceptible to HIV-1 infection, it is important to explore antiviral mechanisms that protect pDCs from this infection. Our objective in this study was to determine whether the potent host antiviral factor A3G is expressed in human primary pDCs, and whether the expressed A3G is regulated by IFN-α. Furthermore, we investigated whether IFN/A3G-mediated innate immunity exhibits anti-HIV-1 activity in pDCs.

**METHODS**

**Isolation of pDCs.** Human PBMCs were isolated from HIV-1-seronegative individuals recruited by the blood bank of Thomas Jefferson University Hospital (Philadelphia, PA, USA) by Ficoll-Hypaque density-gradient centrifugation. The pDCs were enriched from PBMCs using microbeads coated with anti-CD304 (BDCA-4) mAb (Miltenyi Biotec), followed by magnetic cell sorting (Miltenyi Biotec). To examine their purity, the isolated pDCs were stained with anti-human Abs directed against FITC-conjugated CD3 (lineage marker), phycoerythrin (PE)-conjugated CD11c (myeloid dendritic cell surface marker), FITC-conjugated CD123 (DC surface marker) or PE-conjugated BDCA-2 (pDC detection marker), followed by FACS analysis, as described previously (Chen et al., 2006; Wang et al., 2005). The cells were then cultured in conditioned RPMI 1640 medium.

**Preparation of HIV-1/vesicular stomatitis virus (VSV)-pseudotyped viruses and viral infection.** HIV-1/VSV-pseudotyped viruses were generated by the transfection of 293T cells with 10 μg pNL4-3-ΔE-EGFP plasmid containing the HIV-1 proviral genome and a gfp expression cassette in the defective env region (Zhang et al., 2004) and 10 μg of plasmid encoding vesicular stomatitis virus envelope protein (VSV-G), with a calcium phosphate transfection kit (Promega). The supernatants were collected at 48 h post-transfection and filtered with a 0.45 μm pore size filter and were stored in aliquots at −80°C until further use. The pDCs (1 × 10^6) were infected with the HIV-1/VSV-pseudotyped viruses at the input viral concentration of 10 ng HIV-1 p24 antigen equivalent ml^-1. The media were replaced with fresh conditioned RPMI 1640 media at 12 h post-viral inoculation and the culture was allowed to grow for another 24 or 72 h. At the indicated time points, the cells were collected and washed three times with 1× PBS, followed by 0.2% trypsin/EDTA treatment for 10 min at 37°C to remove non-internalized viral particles. After two additional washings, including one with the serum-containing buffer, cell lysis buffer was added for intracellular HIV-1 p24 detection (Perkin Elmer). For viral DNA detection, the same procedure described above was followed, except the cell-free viruses were treated with DNase I (Promega) at 10 U ml^-1 for 15 min at 37°C before infection. The infected cells were then subjected to cellular DNA or RNA extraction and further PCR analysis, as described below.

For wild-type HIV-1 infection, pDCs (3 × 10^5) were infected with HIV-1 YU2 strain at the viral input concentration of 10 ng HIV-1 p24 antigen equivalent ml^-1. The media were replaced with fresh conditioned RPMI 1640 media at 12 h post-viral inoculation, and the viruses that had not infected the cells were washed off. The culture was maintained in conditioned RPMI 1640 medium. At the indicated time points, HIV-1 p24 antigen in the supernatant was determined using an ELISA kit (PBL Biomedical Laboratories). The manufacturer’s instructions were followed. The lowest limit of detection was 12.5 pg ml^-1.

**Endogenous IFN-α production from pDCs.** The CpG A-class ODN (ODN-A) 2336 (G^G^GGACGGCTGGTG^G^G^G^G^G) or ODN-A control 2243 (G^G^G^GACGCTGGTG^G^G^G^G^G^G) were provided by the Coley Pharmaceutical Group. The letters with asterisks represent phosphorothioate bonds, while the remaining nucleotides have phosphodiester bonds. The CpG ODN and the control, at various concentrations, were added to cultures of highly purified pDCs. At 24 h post-infection, the supernatants were collected and the level of IFN-α in the supernatants was determined using an ELISA kit (PBL Biomedical Laboratories). The manufacturer’s instructions were followed. The lowest limit of detection was 12.5 pg ml^-1.

**Short interfering RNAs (siRNAs) synthesis and transfection of pDCs.** The siRNAs used in the experiments were chemically synthesized by Dharmaco. The A3G-specific siRNA was siGENOME SMART pool (catalogue no. M-013072), while the luciferase-specific siRNA served as a negative control. pDCs were transfected with 100 pmol A3G-specific or control siRNA using an Amaxa nucleofector apparatus (Amaxa Biosystems). The procedures suggested by the manufacturer were followed. The cells...
were then maintained in conditioned RPMI 1640 medium before further treatments.

**Western blot analysis.** Proteins were extracted using CytoBuster protein extraction reagent (Novagen) and then quantified with a bicinchoninic acid protein assay reagent kit (Pierce). The procedure recommended by the manufacturer was followed. Up to 10 μg protein was used for electrophoresis (10 % polyacrylamide gel) and transferred onto PVDF membranes. After blocking with 5 % skimmed milk in PBS with 0.05 % Tween-20, membranes were probed with polyclonal anti-A3G antibody (NIH AIDS Research and Reference Reagent Program) at 1:1000 at 4 °C overnight. Membranes were washed with PBS three times, treated with secondary antibodies conjugated with horseradish peroxidase (Santa Cruz Biotechnology) and visualized by chemiluminescence (Pierce Chemical).

**Real-time PCR and RT-PCR analysis.** To clarify viral infection, HIV-1 late reverse transcription (RT) products were detected. DNA from HIV-1/JVSV-pseudotyped virus-infected pDCs was prepared and Taqman real-time PCR was performed. Primers MH531 and MH532 and probe LRT-P were used for detecting HIV-1 late RT products, as Taqman real-time PCR was performed. Primers MH531 and MH532 from HIV-1/VSV-pseudotyped virus-infected pDCs was prepared and SYBR green real-time PCR assays were then examined to determine the mRNA expression level of the APOBEC3 cytidine deaminase family, purified pDCs were pre-treated with or without IFN-α (300 U ml⁻¹) for 24 h. Total cellular RNAs were extracted by using the Trizol reagent (Invitrogen) followed by cDNA synthesis using the Superscript pre-amplification system (Invitrogen). SYBR green real-time PCR assays were then carried out in a 25 μl PCR mixture consisting of 12.5 μl SYBR Green Supermix (Bio-Rad) containing hot-start enzyme, iTaq DNA polymerase at 95 °C for 3 min, and subsequently 40 cycles in two phases consisting of 95 °C for 10 s and 60 °C for 45 s in an iCycler thermal cycler (Bio-Rad). Relative quantification ($2^{-ΔΔCt}$ method) was used in the analysis of relative changes of gene expression. Target genes were normalized using the endogenous GAPDH gene as a reference.

**Alu-PCR analysis.** The Alu–HIV-1 DNA junctions present in the chromosomal DNA of infected pDCs were amplified by primers Alu-S (5'-GGCTCCCAAAAGTGCTGATTACAG-3') and Alu-A (5'-GCTGAGAGAGAGGAGGCACAAATCAGACAGTGAAGAGGCTCTCTGGG-3'). The amplification products were subjected to analysis with PCR with HIV-1 long-terminal repeat (LTR)-specific primers LTR-F (5'-GGATGTGTGCTACGACGATCG-3') and LTR-R (5'-TGAGGGATCTCTACGTACAGTAGTCTGAGAG-3') (Butler et al., 2001). Amplification followed the following PCR regime was used: one cycle of 5 min at 95 °C, 3 min at 61 °C and 5 min at 72 °C, followed by 35 cycles of 30 s at 95 °C, 1 min at 61 °C and 3 min at 72 °C. PCR products were visualized with ethidium bromide after electrophoresis in a 1.5 % agarose gel. Genomic DNA from ACH2 cells (obtained from the NIH AIDS Research and Reference Reagent Program) carrying one copy of an integrated form of HIV-1 was serially diluted and utilized as an Alu-PCR copy-number standard.

**RESULTS**

**A3G expression in pDCs is upregulated by exogenous IFN-α**

Isolation of pDCs from PBMCs of normal blood donors was performed using microbeads coated with mAb against BDCA-4 (CD304). The purity of CD123⁺, BDCA-2⁺, CD11c⁻ and CD3⁻ pDCs reached 95.5 % (Fig. 1). To examine the effects of IFN-α on the expression of APOBEC family members, freshly isolated pDCs were incubated in the presence or absence of exogenous IFN-α (300 U ml⁻¹) for 24 h before cellular mRNA extraction. Because pDCs secrete IFN-α, TNF-α and other cytokines during standard cell culture, non-cultured pDCs were used for baseline analysis. SYBR green real-time RT-PCR was performed, and the level of the mRNA of interest was determined relative to untreated cells. As shown in Fig. 2(a), exogenous IFN-α enhanced the expression of all APOBEC3 family members examined, including A3A, A3C, A3F and A3G. Interestingly, A3G and A3F only increased in cells cultured for 24 h, as compared with uncultured pDCs, possibly due to stimulation of endogenous IFN-α. However, cells...
cultured for 24 h also displayed decreased A3A and A3C expression, which may be due to downregulation by another cytokine(s).

A3G protein expression in pDCs treated with IFN-α in the absence or presence of an antibody against human IFN-α/β receptor was examined by Western blot analysis. Exogenous IFN-α significantly increased A3G expression, and anti-human IFN-α/β receptor antibody substantially inhibited the increment of A3G expression induced by IFN-α (Fig. 2b), indicating that IFN-α induces A3G expression by binding to IFN-α/β receptor.

**A3G expression in pDCs is upregulated by endogenous IFN-α**

To clarify the role of endogenous/autocrine-stimulated IFN-α in regulating A3G expression, ODN-A, which activates TLR-9 for subsequent induction of IFN-α production from pDCs, was utilized. A3G mRNA expression was enhanced by ODN-A treatment (Fig. 3a). The concentration of endogenous IFN-α in the culture supernatant was correlated with ODN-A dosage (Fig. 3b). The intracellular expression of A3G also precisely correlated with the concentration of IFN-α in the supernatant. To examine whether enhancement of A3G expression upon ODN-A treatment is through the IFN-α pathway, human anti-IFN-α/β receptor was added to the culture in combination with ODN-A. This antibody partially decreased the enhancement of A3G expression (Fig. 4a, lane 4), indicating that, in addition to stimulation of the IFN/A3G signalling pathway, ODN-A might also have an alternative pathway(s) for enhancement of A3G expression. Because pDCs can also secrete endogenous TNF-α and other cytokines upon ODN-A treatment (Djeu et al., 1982), these cytokines could also induce the expression of A3G.

**IFN-α inhibits RT and integration of HIV-1 in pDCs**

To examine the possible correlation of intracellular A3G expression with susceptibility to HIV-1 infection, freshly isolated pDCs were first treated with exogenous IFN-α (300 U ml⁻¹), ODN-A (5 μg ml⁻¹) or ODN-A plus human anti-IFN-α/β receptor and then infected with HIV-1/VSV-pseudotyped viruses. VSV envelope can help viruses to avoid the possible effects of IFN-α on the entrance of wild-type viruses to pDCs. The expression of A3G was detected by Western blotting. Exogenous IFN-α or ODN-A treatment significantly increased A3G expression (Fig. 4a). Simultaneously, levels of the HIV-1 late reverse transcript products were 7.3-fold lower in IFN-α pre-treated pDCs and 8.1-fold lower in ODN-A pre-treated cells compared with untreated cells (Fig. 4b). Furthermore, viral integration was analysed by Alu-PCR and was undetectable in IFN-α pre-treated pDCs (Fig. 4c). Because IFN-α does not affect the entrance of HIV-1/VSV-pseudotyped viruses (see below), these data indicate that IFN-α most likely inhibits HIV-1 replication at the RT step, and the inhibitory effect is inversely correlated with the level of A3G in primary pDCs.

**A3G mediates IFN-α inhibition upon HIV-1 infection in human pDCs**

To examine whether knockdown of intracellular A3G expression would increase the susceptibility of pDCs to
HIV-1 infection, purified pDCs were transfected with A3G- or luciferase-specific siRNAs followed by IFN-α treatment for 24 h. The cells were then subjected to infection by the HIV-1/VSV-pseudotyped viruses. With or without exogenous IFN-α treatment, A3G mRNA was effectively downregulated by A3G-specific siRNA (Fig. 5a). After infection, the non-internalized viral particles were removed by treatment with 0.2% trypsin/EDTA followed by vigorous washing. Using ELISA, cell infection was evaluated by comparing intracellular HIV-1 p24 antigen levels following the indicated cell treatments, at 24 and 72 h post-infection. At 24 h post-infection, the levels of p24 were not significantly different, indicating a similar viral entry with various treatments. However, there was a significant increase in the amount of p24 observed in
A3G-specific siRNA-transfected pDCs at 72 h post-infection (Fig. 5b, lane 1 versus lane 3), indicating an increased susceptibility to HIV infection due to downregulation of A3G expression. Furthermore, compared to the control, the restriction of viral infection by exogenous IFN-α could also be reversed by treatment with A3G-specific siRNA (Fig. 5b, lane 2 versus lane 4).

In addition, we have also examined the effect of the IFN-α/ A3G regulatory system on the replication of wild-type HIV-1 in pDCs. Again, purified pDCs were transfected with A3G- or luciferase-specific siRNAs followed by IFN-α treatment for 24 h. The cells were then subjected to infection by wild-type HIV-1YU2 viruses. With or without exogenous IFN-α treatment, A3G mRNA was effectively downregulated by A3G-specific siRNA (Fig. 6a). Fig. 6(b)

![Graph](http://vir.sgmjournals.org)

**Fig. 5.** (a) Downregulation of A3G mRNA in pDCs by A3G-specific siRNA. pDCs were transfected with A3G- or luc-specific siRNA and cultured for 48 h, followed by IFN-α (300 U ml⁻¹) treatment for 24 h. The cells were collected and the total RNA was extracted and real-time RT-PCR was performed to detect A3G mRNA. (b) A3G mediates IFN-α inhibition of HIV-1 in pDC. Cells from the same source populations subjected in parallel to the treatment described above were further infected with HIV-1/VSV-pseudotyped viruses. At 24 or 72 h post-infection pDCs were collected for detection of intracellular HIV-1 p24 antigen. A 100 µl volume of cell lysate was used in the HIV-1 p24 ELISA assay. **P < 0.01. These data represent the results of two independent experiments, with cells from two different donors.

again indicates that downregulation of A3G expression can enhance the viral infectivity in the pDCs. Moreover, exogenous IFN-α could restrict HIV-1 replication in pDCs. This effect can largely be counteracted by treatment with A3G-specific siRNA, indicating that IFN-α inhibits HIV-1 replication through regulating A3G. Combining the data in Figs 5 and 6, we have provided solid evidence that A3G is a potent inhibitor in HIV-1 infection in pDCs and
that the antiviral effect of IFN-α is achieved by upregulation of intracellular A3G.

**DISCUSSION**

We have demonstrated that human peripheral pDCs express the APOBEC3 DNA-editing enzymes and that their expression is upregulated by human IFN-α treatment at both the transcriptional and translational level. This phenomenon has also been observed in other primary cell types, including resting CD4<sup>+</sup> T-cells, macrophages and hepatocytes (Bonvin et al., 2006; Chen et al., 2006; Peng et al., 2006; Sarkis et al., 2006; Tanaka et al., 2006). It is interesting to note that the enhancement of RNA transcription of A3G by exogenous IFN-α is moderate (1.5–2.5-fold) compared with that without IFN-α treatment. However, the difference in protein expression is much higher. A similar phenomenon has been observed in resting CD4<sup>+</sup> T-cells (Chen et al., 2006). In resting CD4<sup>+</sup> T-cells, the RNA transcriptional enhancement by IFN-α is about three to four times higher, while the protein expression enhancement is at least eight to ten times higher. We have some preliminary data showing that A3G can be phosphorylated by IFN-α stimulation (data not shown). It is possible that phosphorylation of A3G slows down the turnover of A3G protein and subsequently increases the concentration of A3G in the IFN-α-treated cells.

Although IFN-α exerts its anti-HIV-1 activity through several pathways, recent studies have demonstrated that the antiviral activity of IFN-α can be achieved through the upregulation of intracellular A3G expression and the enhancement of the association of A3G with the low-molecular-mass (LMM) ribonucleoprotein complex in primary resting CD4<sup>+</sup> T-cells (Chen et al., 2006; Chiu et al., 2005). We also observed a decrease in HIV-1 reverse transcriptase products in pDCs when A3G expression was enhanced by IFN-α (Fig. 4b). More interestingly, down-regulation of A3G via A3G siRNA treatment released the inhibitory effect, as a higher level of HIV-1 p24 production was detected (Figs 5b and 6b). Previous studies have demonstrated that A3G exists in two distinct forms. One is an enzymically active form, when it is associated with the LMM complex, another is in enzymically inactive form, when it is associated with a high-molecular-mass (HMM) complex (Chiu et al., 2005). It would therefore be interesting to investigate the differences between the natural and IFN-α-enhanced forms of A3G in the cytoplasm of pDCs. Unfortunately, it is technologically difficult to perform a fast protein liquid chromatography study due to the extremely limited primary pDC population, which were directly isolated from PBMCs of human donors. Overall, our data provide evidence for the role of the IFN/A3G regulatory system in the inhibition of viral infection in pDCs, which are the major cells producing endogenous IFN-α and therefore play a key role in orchestrating the IFN-α/A3G intercellular network. It is notable that pDCs from only two individuals were examined; more experiments should further confirm and elaborate on these results.

Synthetic CpG oligodeoxynucleotide agonists can stimulate pDCs to produce a large amount of IFN-α through TLR-9, which is exclusively expressed in human pDCs and B cells (Bauer et al., 2001; Hemmi et al., 2000; Hornung et al., 2002; Kadowaki et al., 2001; Wagner, 2002). Furthermore, CpG ODN-A has been used in human clinical trials in the fields of infectious disease and cancer. Previous studies have demonstrated that the high level of IFN-α induced by ODN-A suppresses HIV-1 replication in human fetal thymus cells (Gurney et al., 2004), and B-class ODNs can also suppress HIV replication in cultured human cells (Scheepere et al., 2004). However, the mechanism of protection is largely unknown. Our data demonstrate that ODN-A can potently stimulate pDCs to secrete IFN-α, which upregulates the expression of A3G, an innate antiviral factor. This may provide a possible mechanism for the action of ODN-A in anti-HIV-1 activity. Interestingly, when we treated pDCs with anti-IFN-α/β receptor, only partial inhibition of A3G expression was observed (Fig. 4a), indicating that other TLR-9-mediated pathways participate in the upregulation of A3G. Further studies are required to explore this mechanism.

IFN-α, together with reverse transcriptase inhibitors, has been used to treat HIV-1-infected patients. However, its effect is not satisfactory (Berglund et al., 1991). With the understanding of the strong effect of IFN-α in A3G expression upregulation and the IFN/A3G pathway in antiviral RT and overall viral infectivity, re-evaluation of IFN-α for HIV-1 treatment in combination with powerful HAART could be needed. The potent and irreversible effect of IFN-α on the inhibition of HIV-1 infection has raised the possibility that enhancement of A3G expression may help completely eliminate residual HIV-1 replication in resting T cells and possibly in pDCs (Chen et al., 2006). Significantly, administration of IFN-α has toxic effects that many patients cannot tolerate. An alternative method with potentially fewer side effects may be to stimulate more IFN-α production specifically from pDCs. Here, we have shown that ODN-A can potently enhance IFN-α production and A3G expression in pDCs. It is likely that administration of a CpG oligodeoxynucleotide or other TLR-7 or TLR-9 agonists may increase IFN-α production from pDCs and thereby enhance the expression of A3G in other cells such as resting CD4<sup>+</sup> T-cells, macrophages, myeloid dendritic cells and pDCs themselves. These TLR agonists may therefore provide a novel therapeutic approach to control residual HIV-1 replication in the post-HAART era.

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