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Original Article

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Gp120 binding with DC-SIGN induces reactivation of HIV-1 provirus via the NF-κB signaling pathway

Changzhong Jin[1,†], Jie Li[2,†], Linfang Cheng[1], Fumin Liu[1], and Nanping Wu[1,*]

[1]State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310003, China, and [2]Department of Infectious Disease, Second Affiliated Hospital of Wenzhou Medical University, Wenzhou 325027, China

[†]These authors contributed equally to this work.
*Correspondence address. Tel: +86-571-87236580; Fax: +86-571-87236582; E-mail: flwnp2013@163.com

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Abstract

The reactivation mechanism of latent human immunodeficiency virus type 1 (HIV-1) infection is unclear, especially in dendritic cells (DC). DC-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) binds with HIV-1 and other pathogens to activate the extracellular regulated protein kinase (ERK) and nuclear factor-kappa B (NF-κB) pathways and regulate cytokine expression. We hypothesized that DC-SIGN-induced signaling pathways may activate HIV-1 provirus. To investigate this hypothesis, we generated a model by transfecting 293T cells with a DC-SIGN expression plasmid and an HIV-1 5′ long terminal repeat (LTR) reporter plasmid, and then stimulated the 293T cells with HIV-1 gp120 protein, wild-type HIV-1 or VSV-G-pNL4.3 pseudotype virus (without gp120 protein). It was found that the HIV-1 5′LTR was reactivated by HIV-1 gp120 in DC-SIGN-expressing 293T cells. Then the HIV-1 chronically infected CEM-Bru cells were transfected with DC-SIGN expression plasmid and stimulated by HIV-1 gp120 protein. It was found that early and late HIV-1 provirus replication was reactivated by the HIV-1 gp120/DC-SIGN stimulation. We then investigated the involvement of the ERK, p38 mitogen-activated protein kinases and NF-κB signaling pathways in HIV-1 gp120/DC-SIGN-induced activation of HIV-1 provirus by inhibiting the pathways specifically. Our results indicated that HIV-1 gp120/DC-SIGN stimulation reactivates latent HIV-1 provirus via the NF-κB signal pathway.

Key words: HIV-1, DC, DC-SIGN, viral latency, signaling pathways, NF-κB

Introduction

The course of human immunodeficiency virus type 1 (HIV-1) pathogenesis is marked by the creation of a transcriptionally inert proviral reservoir. In this process, the HIV-1 cDNA is integrated into the human genome and viral gene transcription is silenced, thereby achieving a permanent latent infection [1]. The latent HIV-1 escapes host immune responses and is insensitive to antiretroviral therapy, thus representing a major challenge to the eradication of the virus in infected patients [1,2]. One strategy to eradicate the reservoirs of HIV-1 is to break down the latency and to reactivate the provirus [3]. Therefore, further elucidation of the molecular mechanisms involved in this process is required to facilitate the development of novel therapies to eradicate the reservoirs of HIV-1.

The main reservoirs of latent HIV-1 are resting memory CD4+ T cells [4], although certain subsets of dendritic cells (DCs), such as those found in the lymph nodes, have been proposed as reservoirs of viral latency [5]. However, the mechanism of latent HIV-1 reactivation in DC is poorly understood. DCs are thought to be the first cells that encounter HIV-1 at the mucosa, and it has been postulated that they are critically involved in the initial stages of HIV-1 infection and dissemination. Infected DCs migrate to the lymph nodes, where HIV-1 is efficiently transmitted to CD4+ T cells [6–9]. DC infection is also essential to the long-term survival of HIV-1 [10]. Therefore, eradication of DC reservoirs is an important strategy for the treatment of HIV-1 infection.

Reactivation of latent HIV-1 requires the stimulation of several signaling pathways, such as the extracellular regulated protein kinase (ERK) and nuclear factor-kappa B (NF-κB) signaling pathways, which are involved in many cellular events [11–13]. DC-specific intercellular adhesion molecule (ICAM)-3-grabbing non-integrin (DC-SIGN), which is an important C-type lectin expressed on DC, binds to HIV-1 gp120 with high affinity, captures HIV in the periphery, and promotes infection of target cells [14,15]. As a pattern recognition receptor (PRR), DC-SIGN recognizes and binds with many pathogens including HIV-1 to induce several signaling pathways, such as the ERK and NF-κB pathways, regulating cytokine secretion and immune responses [16,17]. Based on the signaling pathways which are common to both HIV-1 reactivation and DC-SIGN signaling, we hypothesized that DC-SIGN-induced signaling pathways may be involved in HIV-1 provirus activation.

In this study, we investigated the effects of the interaction between HIV-1 gp120 and DC-SIGN on HIV-1 production and the pathways involved. It was found that the binding of gp120 with DC-SIGN activated HIV-1 5′ long terminal repeat (LTR) expression and HIV-1 production predominantly via the NF-κB pathway.

Materials and Methods

Materials and antibodies

The luciferase reporter vectors, pGL-3 and pRL-TK, and the Dual Luciferase Reporter assay system were purchased from Promega (Madison, USA). Mouse anti-human phosphorylated and total NF-κB p65, p38 mitogen-activated protein kinases (MAPK) and ERK1/2 antibodies, anti-HIV-1 Tat antibody, goat anti-HIV-1 gp120 antibody, fluorescein isothiocyanate (FITC)-conjugated donkey anti-goat IgG H&L secondary antibody, horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG H&L secondary antibody and recombinant HIV-1 gp120 protein were obtained from Abcam (Cambridge, USA). Mouse anti-human DC-SIGN, CXCR4 and CCR5 neutralizing antibodies, mouse anti-human DC-SIGN, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) monoclonal antibodies were obtained from R&D Systems (Minneapolis, USA). Phycoerythrin (PE)-conjugated rat anti-human DC-SIGN for flow cytometry analysis was obtained from eBioscience (San Diego, USA). The NF-κB inhibitor helenalin, the p38 MAPK inhibitor SB202190, and the ERK1/2 inhibitor PD98059 were purchased from Merck (Gibbstown, USA) and used at the final concentration of 1 μM (helenalin), 5 μM (SB202190), and 50 μM (PD98059), respectively. HIV-1 p24 enzyme-linked immunosorbent assay (ELISA) kits were obtained from Shuangying Biological Technology Co. (Shanghai, China).

Construction of the DC-SIGN expression plasmid and HIV-1 5′LTR reporter vector

The DC-SIGN expression plasmid and HIV-1 5′LTR reporter vector were constructed as described previously [18]. Briefly, total RNA was extracted from whole blood obtained from healthy donors or from HIV-1 HXB2-infected H9 cells, and then was reverse transcribed into cDNA in a total volume of 20 μl with PrimeScript RT reagent Kit (TaKaRa, Dalian, China). The DC-SIGN and HIV-1 5′LTR fragments were amplified by polymerase chain reaction (PCR) using the forward and reverse primers containing *Bam*HI/*Xba*I and *Mlu*I/*Xho*I restriction sites, respectively (**Table 1**). The DC-SIGN and HIV-1 5′LTR fragments were double-digested with *Bam*HI and *Xba*I or *Mlu*I and *Xho*I (New England Biolabs, Singapore, Singapore), gel purified and ligated into *BamH*I and *Xba*I-digested pCDNA3.1(+) plasmid or *Mlu*I and *Xho*I-digested pGL-3 luciferase reporter vector to generate the DC-SIGN expression plasmid and HIV-1 5′LTR reporter vector.

Cell culture and cell transfection

293T cells were obtained from American Type Culture Collection (Rockville, USA). CEM-Bru cells that are a CEM-SS cell line chronically and unproductively infected by HIV-1 (Bru strain) [19]. H9 cells infected by HIV-1 (HXB2 strain), and VSV-G-pNL4.3 pseudotype virus were kindly provided by Dr. Paul Zhou (Institut Pasteur of Shanghai, Shanghai, China). 293T and CEM-Bru cells were cultured in complete Dulbecco's Modified Eagle's Medium (DMEM) at 37°C under a humidified atmosphere containing 5% CO_2. 293T cells were co-transfected with DC-SIGN expression plasmid (0.5 μg/10^5 cells), HIV-1 5′LTR reporter vector (0.5 μg/10^5 cells), and pRL-TK vector internal control (0.05 μg/10^5 cells) using Lipofectamine 2000 (Life Technologies, Carlsbad, USA). CEM-Bru cells were transfected with DC-SIGN expression plasmid (0.5 μg/10^5 cells). Transfected or untransfected 293T and CEM-Bru cells were treated with recombinant HIV-1 gp120 protein (1 μg/ml), HIV-1 HXB2 or VSV-G-pNL4.3 (HIV-1 p24 100 ng/10^6 cells) for 24 h. Cells were washed to remove unbound HIV-1 gp120 or virus, and then fresh medium was added. For antibody-neutralizing or signaling-blocking experiments, cells were treated with neutralizing antibodies or signaling inhibitors for

Table 1. Primers used in amplification of DC-SIGN, HIV-1 5′LTR, and Tat

Primers	Sequence
DC-SIGN forward primer	5′-CTCGGATCCATGAGTGACTCCAAGGAAC-3′
DC-SIGN reverse primer	5′-GCTCTAGATGAAGTTCTGCTACGCAGGA-3′
HIV-1 5′LTR forward primer	5′-TATTACGCGTTGGAAGGGCTAATTTGGTC-3′
HIV-1 5′LTR reverse primer	5′-GTGCTCGAGTGCTAGAGATTTTCCACACT-3′
HIV-1 *Tat* forward primer	5′-ATGGCAGGAAGAAGCGGAG-3′
HIV-1 *Tat* reverse primer	5′-ATTCCTTCGGGCCTGTCG-3′
GAPDH forward primer	5′-CCATGTTCGTCATGGGTGTG-3′
GAPDH reverse primer	5′-GGTGCTAAGCAGTTGGTGGTG-3′

Restriction sites: GGATCC, *Bam*HI; TCTAGA, *Xba*I; ACGCGT, *Mlu*I; CTCGAG, *Xho*I.

30 min before treatment with HIV-1 gp120, HIV-1 HXB2, or VSV-G-pNL4.3.

Western blot analysis

Levels of DC-SIGN expression, HIV-1 Tat protein, and phosphorylated signaling kinases were determined by western blot analysis. GAPDH were detected as the internal reference, and the relative levels of phosphorylated kinases were determined by comparing phosphorylated kinases with their total kinases. Briefly, 10 μg of each sample was subject to sodium dodecyl sulfate polyacrylamide gel electrophoresis under reducing conditions and transferred onto an immobilon polyvinylidene difluoride membrane (Millipore, Bedford, USA). After being blocked with 5% non-fat dried milk in 50 mM Tris–HCl, pH 7.6, 150 mM NaCl, 0.1% Tween 20 for 1 h, anti-phosphorylated and total NF-κB p65, p38 MAPK, ERK1/2 antibodies, anti-HIV-1 Tat (Abcam), anti-DC-SIGN, or anti-GAPDH antibodies (R&D Systems) (1–2 μg/ml for per antibody) were added and incubated overnight at 4°C. After incubation with HRP-conjugated goat anti-mouse secondary antibodies (Abcam) for 2 h, the membrane was washed three times, treated with ECL reagent (GE Healthcare, Pittsburgh, USA), and then exposed with the VersaDoc 5000 MP Image Analysis System (Bio-Rad, Hercules, USA). GAPDH were detected as the internal reference.

Flow cytometry analysis

The binding of gp120 with DC-SIGN was tested using flow cytometry by detecting DC-SIGN and gp120 double-positive cells. The harvested cells were washed twice with phosphate buffered saline, incubated with 10 μl PE-conjugated anti-human DC-SIGN antibody (eBioscience), and anti-HIV-1 gp120 antibody (Abcam) for 30 min and washed again. FITC-conjugated donkey anti-goat IgG secondary antibody (Abcam) was added and incubated for 30 min. Flow cytometry analysis was performed with a four-color FACScan flow cytometer (Becton Dickinson, Franklin Lakes, USA).

Dual-luciferase reporter assay

The luciferase activity of the HIV-1 5′LTR luciferase reporter plasmid and the pRL-TK vector internal control was detected using the Dual Luciferase Reporter assay kit with a GloMax96 microplate luminometer (Promega). The relative activity of the HIV-1 5′LTR was expressed as the ratio of the activity of the HIV-1 5′LTR luciferase reporter vector and that of the pRL-TK vector internal control.

Real-time quantitative PCR

Total RNA was extracted from CEM-Bru cells using a total RNA extraction kit (Life Technologies) and reverse-transcribed into cDNA in a total volume of 20 μl. Real-time quantitative PCR was performed with a DNA Engine Chromo 4 Real-time quantitative PCR system (Bio-Rad) using a SYBR Green kit (TaKaRa). Primers for amplification of HIV-1 *Tat* mRNA and the *GAPDH* internal reference are shown in Table 1. The reaction system was a mixture of 10 μl SYBR Green Master Mix, 0.2 μl of each oligonucleotides primers, and 2 μl of cDNA, and the final volume was adjusted to 20 μl with water. Real-

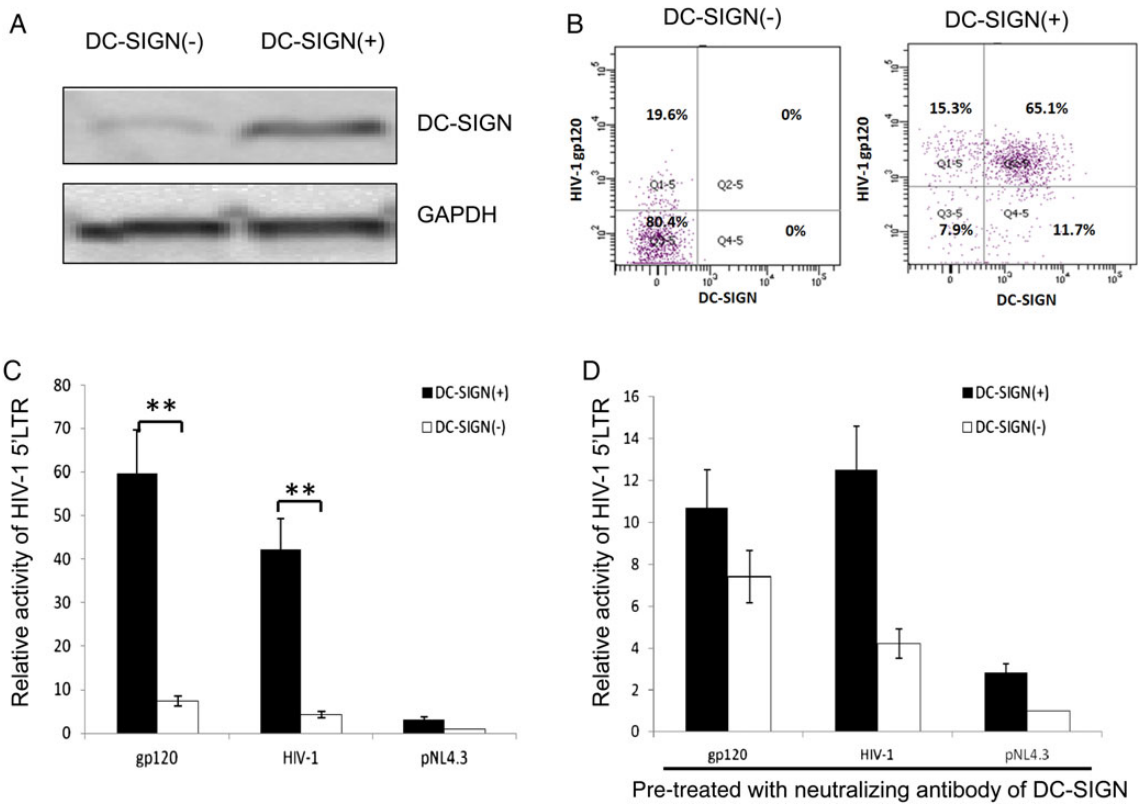

Figure 1. DC-SIGN induced HIV-1 5′LTR activation in 293T cells (A) DC-SIGN expression in 293T cells was detected by western blot analysis. GAPDH was used as an internal control. (B) DC-SIGN expression and gp120 binding were tested by flow cytometry, and double-positive cells were considered to be DC-SIGN(+) cells binding with gp120. (C) HIV-1 5′LTR activation in 293T cells stimulated by gp120 protein, wild-type HIV-1 (HXB2) and VSV-G-pNL4.3. The level of HIV-1 5′LTR activation in VSV-G-pNL4.3-stimulated DC-SIGN(−) cells was defined as 1. (D) Activation of HIV-1 5′LTR in 293T cells treated with neutralizing anti-DC-SIGN antibody. The level of HIV-1 5′LTR activation in VSV-G-pNL4.3 stimulated DC-SIGN(−) cells was defined as 1. DC-SIGN(+): 293T cells transfected with the DC-SIGN expression plasmid; DC-SIGN(−): 293T cells without DC-SIGN expression plasmid. $^{**}P < 0.01$.

time PCR was performed for 40 cycles of denaturation (95°C, 45 s), annealing (62°C, 30 s), and extension (72°C, 30 s), and double-stranded DNA was measured at 86°C after each cycle.

ELISA

HIV-1 p24 Ag levels were detected using an HIV-1 p24 ELISA kit (Shuangying Biological Technology Co). Briefly, 50 µl of Assay Diluent was added to the plate, and then 200 µl of supernatant from cell culture was added and incubated for 2 h at room temperature. The plate was washed three times with Tris-buffered saline containing 0.05% Tween 20. Then 200 µl of alkaline phosphatase-conjugated anti-HIV-1 p24 antibody was added and incubated for 2 h at room temperature. Finally, 200 µl of substrate solution was added, and after 20 min of incubation at room temperature, 50 µl of stop solution was added. The plate was read on an automatic microtiter plate reader at 450 nm (Bio-Tek Elx800; Winooski, USA), and the level of HIV-1 p24 was determined using a standard curve.

Statistical analysis

Data were presented as the mean ± standard deviation (SD) of triplicate tests. Student's *t*-test was used to compare the differences between two groups, while one-way ANOVA was for comparisons of more than three groups. All statistical analyses were performed using SPSS V.20 software (SPSS Inc., Chicago, USA). $P < 0.05$ was considered to be statistically significant.

Results

DC-SIGN induced HIV-1 5′LTR activation

First, we constructed a model to investigate the interaction between DC-SIGN and HIV-1 gp120 by transfecting 293T cells with a DC-SIGN expression plasmid and an HIV-1 5′LTR reporter vector. The transfected 293T cells were then stimulated with recombinant HIV-1 gp120 protein, wild-type HIV-1 (HXB2), or VSV-G-pNL4.3 pseudotype virus (without gp120 protein). 293T cells transfected with the DC-SIGN expression plasmid expressed high levels of DC-SIGN (**Fig. 1A**). The binding of DC-SIGN and gp120 was detected by flow cytometry and was indicated by the percentage of DC-SIGN and gp120 double-positive cells, since HIV-1 gp120 could bind to DC-SIGN with much higher affinity than other receptors [14]. There were 65.1% of DC-SIGN and gp120 double-positive cells in DC-SIGN (+) cells, and no double-positive cells in DC-SIGN (–)

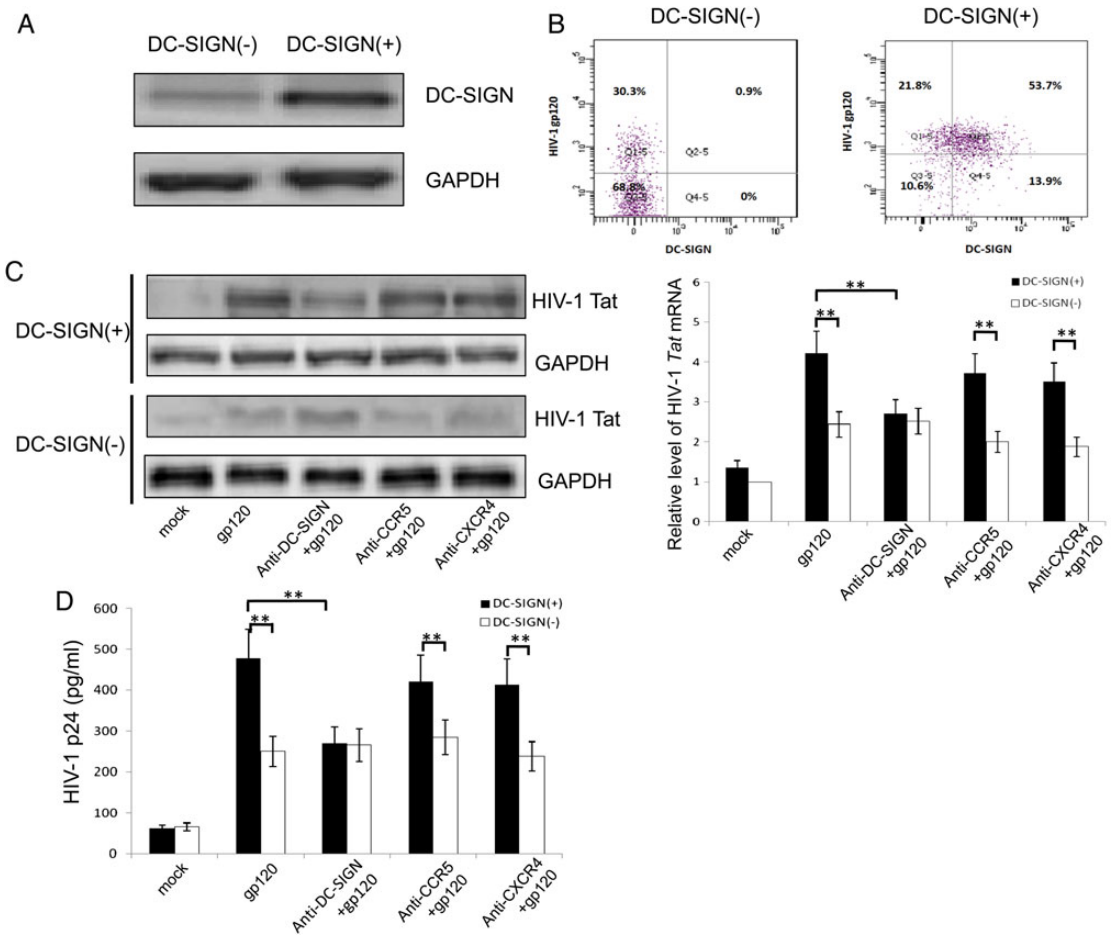

Figure 2. DC-SIGN induced replication of HIV-1 provirus CEM-Bru cells were transfected with DC-SIGN expression plasmid and then stimulated with HIV-1 gp120. (A) DC-SIGN expression in CEM-Bru cells was detected by western blot analysis. GAPDH was used as an internal control. (B) DC-SIGN expression and gp120 binding were tested by flow cytometry, and double-positive cells were considered to be DC-SIGN(+) cells binding with gp120. (C) HIV-1 Tat protein in gp120 stimulated CEM-Bru cells with or without blocking antibodies treatment was detected by western blot analysis. The relative expression of HIV-1 *Tat* mRNA was detected by qRT-PCR. The level of HIV-1 *Tat* mRNA in mock DC-SIGN(–) cells was defined as 1. GAPDH protein and mRNA were used as an internal control. (D) Relative level of HIV-1 p24 protein in cell culture supernatant was detected using an ELISA kit. DC-SIGN(+): CEM-Bru cells transfected with the DC-SIGN expression plasmid; DC-SIGN(–): CEM-Bru cells without the DC-SIGN expression plasmid. $^{**}P < 0.01$.

cells (**Fig. 1B**). HIV-1 gp120 and wild-type HIV-1 stimulated much higher HIV-1 5′LTR activation in DC-SIGN(+) 293T cells than in DC-SIGN(−) 293T cells. In contrast, VSV-G-pNL4.3 virus without gp120 did not stimulate high HIV-1 5′LTR activation in either DC-SIGN(+) or DC-SIGN(−) 293T cells (**Fig. 1C**). Interestingly, the HIV-1 5′LTR activation stimulated by VSV-G-pNL4.3 virus in DC-SIGN(+) 293T cells was higher than in DC-SIGN(−) cells, indicating the binding of VSV-G glycoprotein with DC-SIGN (**Fig. 1C**).

To further investigate the effect of DC-SIGN on HIV-1 5′LTR activation, the binding of gp120 or wild-type HIV-1 with DC-SIGN was blocked by the neutralizing anti-DC-SIGN antibody. Activation of HIV-1 5′LTR in DC-SIGN(+) 293T cells stimulated with gp120 or wild-type HIV-1 was significantly blocked by the neutralizing anti-DC-SIGN antibody (**Fig. 1D**). These results demonstrated that the binding of gp120 with DC-SIGN could induce high activation of HIV-1 5′LTR.

DC-SIGN induced HIV-1 provirus replication in CEM-Bru cells

To determine the effect of DC-SIGN on the reactivation of unproductive HIV-1 provirus, DC-SIGN expression plasmid was transfected into CEM-Bru cells followed by HIV-1 gp120 stimulation for 24 h. CEM-Bru cells transfected with DC-SIGN plasmid expressed high levels of DC-SIGN (**Fig. 2A**). There were 53.7% of DC-SIGN and gp120 double-positive cells in DC-SIGN(+) cells, which is much higher than that in DC-SIGN(−) cells (**Fig. 2B**). As reported by Gringhuis *et al.* [16], HIV-1 Tat protein and mRNA were detected as a measure of early replication, while HIV-1 p24 protein was detected as a measure of late replication. Compared with DC-SIGN(−) CEM-Bru cells, HIV-1 gp120 stimulation significantly increased both HIV-1 Tat and p24 levels in DC-SIGN(+) CEM-Bru cells, indicating a complete replication of HIV-1 provirus. When the binding of HIV-1 gp120 and DC-SIGN was blocked by the neutralizing anti-DC-SIGN antibody, HIV-1 Tat and p24 levels were decreased in DC-SIGN(+) CEM-Bru cells to levels similar to those detected in DC-SIGN(−) CEM-Bru cells (**Fig. 2C,D**).

HIV-1 gp120 can also bind to CCR5 or CXCR4 with high affinity [20]. To rule out this effect, this interaction was blocked with a neutralizing anti-CCR5 antibody or anti-CXCR4 antibody. It was found that the effect of HIV-1 gp120 on HIV-1 provirus reactivation was not significantly influenced by blocking of CCR5 or CXCR4 in both DC-SIGN(+) and DC-SIGN(−) CEM-Bru cells (**Fig. 2C,D**).

Signaling pathways involved in DC-SIGN-induced reactivation of HIV-1 provirus in CEM-Bru cells

To investigate the signaling pathways involved in DC-SIGN-induced reactivation of HIV-1 provirus, the NF-κB, p38 MAPK, and ERK pathways were inhibited in DC-SIGN(+) CEM-Bru cells with specific kinase inhibitors. The results showed that both early and late HIV-1 replication induced by DC-SIGN was decreased significantly (~50% reduction) by the NF-κB inhibitor compared with the cells treated with gp120 alone. However, p38 MAPK or ERK inhibitors had no effect on HIV-1 replication compared with that of cells treated with gp120 alone (**Fig. 3**).

We further investigated kinase activation in these different pathways. It was found that phosphorylated NF-κB p65 was increased

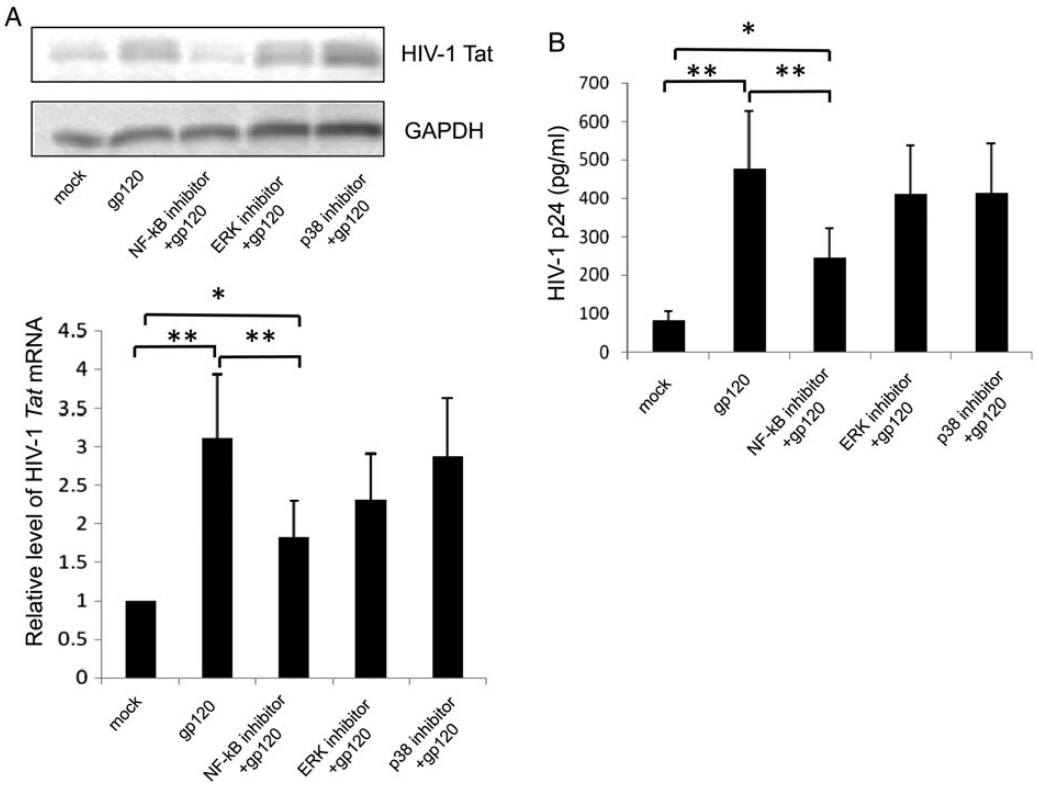

Figure 3. Effect of signaling pathway inhibitors on DC-SIGN-induced replication of HIV-1 provirus in DC-SIGN(+) CEM-Bru cells (A) HIV-1 Tat protein in gp120-stimulated CEM-Bru cells with or without signal inhibitors treatment was detected by western blot analysis. The relative levels of HIV-1 *Tat* mRNA in CEM-Bru cells were detected by qRT-PCR, and the level of HIV-1 *Tat* mRNA in mock cells was defined as 1. GAPDH protein and mRNA were used as an internal control. (B) Relative level of HIV-1 p24 protein in cell culture supernatant was detected using an ELISA kit. $*P < 0.05$, $**P < 0.01$.

markedly in gp120-treated DC-SIGN(+) CEM-Bru cells and was inhibited by NF-κB inhibitor helenalin, while phosphorylated ERK1/2 and p38 were not increased (**Fig. 4**). Taken together, our results indicated that the DC-SIGN-induced reactivation of HIV-1 provirus in CEM-Bru cells was mediated by NF-κB signaling pathway.

Discussion

A number of strategies have been developed to eliminate latent HIV-1 in infected patients, the most widely accepted of which involves reactivation of HIV-1 replication using T-cell activators combined with highly active antiretroviral therapy (HAART). Many factors are involved in reactivation, including physiological stimuli such as TNF-α, IL-1β, IL-2, IL-6, IFN-γ, and CD154, chemical compounds such as phorbol esters (PMA and prostratin), histone deacetylase inhibitors (trichostatin, valproic acid, sodium butyrate, and suberoylanilide hydroxyamic acid), histone methyltransferase inhibitors, p-TEFb activators, hydroxyurea, and some activating antibodies (anti-CD3) [3]. However, these methods are non-specific and can lead to global T-cell activation and serious side-effects [21]. In addition, current research is mainly focused on targeting resting CD4+ T reservoirs. However, there is no resting status for DC reservoirs; therefore, it is unclear whether the existing methods are also effective in DC. Myeloid cells, such as DC, are non-permissive for HIV-1, and the rate of infection is very low [22]. Studies of HIV-1 latency in DC are hindered by the difficulties associated with effectively developing replicative infection in DC, and the absence of models or cell lines for DC reservoirs [23]. To investigate the role of DC-SIGN-induced signaling pathways in HIV-1 provirus reactivation, we generated cell models of HIV-1 DC reservoirs using 293T cells transfected with a DC-SIGN expression plasmid and HIV-1 5′LTR reporters and CEM-Bru cells transfected with a DC-SIGN expression plasmid.

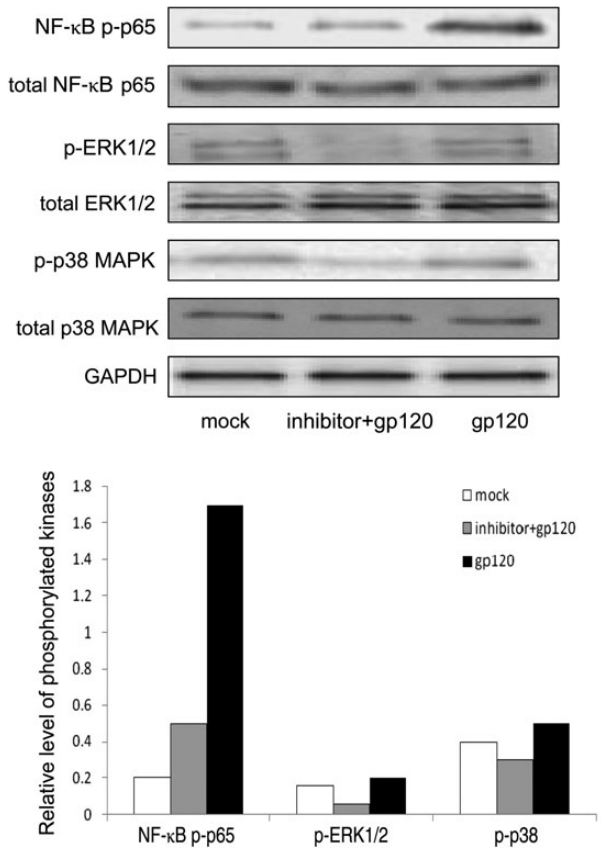

Figure 4. Activation of different signaling pathways in DC-SIGN(+) CEM-Bru cells induced by HIV-1 gp120 Phosphorylation of NF-κB p65, ERK1/2 and p38 in gp120-stimulated CEM-Bru cells with or without specific inhibitor treatment was detected by western blot analysis. GAPDH was used as an internal control. The relative levels of phosphorylated kinases were defined as the ratio of phosphorylated kinases to total kinases (the lower panels).

Co-infection with non-HIV pathogens has been postulated to be an important exogenous factor that influences HIV replication [24]. The heterologous infectious agents include viruses (e.g. human herpesvirus-6, and herpes simplex virus −1), parasites (e.g. *Leishmania donovani*, *Toxoplasma gondii*, and *Plasmodium falciparum*), and bacteria (e.g. *Mycobacterium* ssp. and *Neisseria gonorrhea*) [24–27]. The term 'immune activation' has been used to refer to stimulation of the HIV LTR by signaling pathways triggered by the immune response to non-HIV antigens or infectious agents [28]. As a PRR expressed by DC, DC-SIGN can bind with many pathogens including HIV-1 gp120, to modulate the immune response and cytokine secretion [16]. The association between pathogens and DC-SIGN induces Raf-1-directed phosphorylation of the NF-κB subunit p65 and MEK/ERK [29].

The capacity of DC-SIGN-induced pathways to reactivate latent HIV-1 provirus has not yet been reported. In this study, we found that the binding of HIV-1 gp120 with DC-SIGN activated the expression of HIV-1 5′LTR and replication of HIV-1 provirus. Using different stimuli with or without gp120, we confirmed that gp120 is the active portion of HIV-1 that stimulates DC-SIGN signaling. To a certain extent, we found a weak increase of HIV-1 5′LTR activation in DC-SIGN(+) 293T cells stimulated by VSV-G-pNL4.3 virus when compared with in DC-SIGN(−) cells, indicating the binding of VSV-G glycoprotein with DC-SIGN. We also excluded the possibility that replication of HIV-1 provirus was mediated by the interaction of gp120 with CCR5 using a neutralizing anti-CCR5 antibody. Our results indicate that replication and reactivation of HIV-1 provirus is mediated by DC-SIGN.

We further investigated the signaling pathways involved in DC-SIGN-induced reactivation of HIV-1 provirus. We found that binding of HIV-1 gp120 with DC-SIGN induced the phosphorylation of NF-κB p65, which indicated the activation of NF-κB pathway. Furthermore, HIV-1 replication was blocked by inhibition of the NF-κB pathway. The NF-κB pathway also plays an important role in DC-SIGN expression. We and other researchers have shown that DC-SIGN expression is induced by IL-4 through multiple signaling pathways, including the ERK, JAK-STAT, and NF-κB signaling pathways [30,31]. Moreover, expression of DC-SIGN can also be regulated by some microbes, such as *Penicillium marneffei* [32], human herpesvirus 6 (HHV-6) [33], and human T-cell leukemia virus type I (HTLV-I) [34]. The signaling pathways triggered by these pathogens, such as the ERK and NF-κB pathways, may be responsible for the up-regulation of DC-SIGN expression. Based on our results, we speculate that in DC the expression of DC-SIGN and the reactivation of latent HIV-1 are stimulated by common signaling pathways, such as the NF-κB signaling pathway.

Although recurrent HIV-1 replication can be inhibited and controlled by effective HAART, the expression of DC-SIGN on DC with latent HIV-1 infection would be up-regulated, since DC-SIGN expression shares signaling pathways in common with those required for HIV-1 replication. This would allow further dissemination of HIV-1 in the host, thus limiting the control of the virus by

antiretroviral regimens and the host immune system, and attenuating the eradication of latent virus. On the other hand, the signals stimulating DC-SIGN expression in DCs may be hijacked by latent HIV-1, promoting virus replication and transmission. The roles played by DC-SIGN-induced HIV-1 provirus reactivation in the maintenance or eradication of HIV-1 DC reservoirs remain to be fully elucidated. It can be speculated that DC-SIGN expression and latent HIV-1 reactivation act in concert to promote the spread of HIV-1 infection. This may, in part, account for the existence of continued low-level HIV-1 replication and the maintenance of DC reservoirs in HIV-1 infected patients receiving HAART.

In summary, in this study, we identified a novel mechanism responsible for the reactivation of latent HIV-1 provirus in DC reservoirs involving the binding of HIV-1 gp120 with DC-SIGN. This interaction may induce activation of the HIV-1 5′LTR and HIV-1 replication via the NF-κB signaling pathway. The interaction between DC-SIGN expression and HIV-1 provirus replication may prevent the clearance of latent HIV-1 in DC reservoirs.

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