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RNAi-directed Inhibition of DC-SIGN by Dendritic Cells: Prospects for HIV-1 Therapy

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Abstract

Drug-resistant human immunodeficiency virus (HIV) infections are increasing globally, especially in North America. Therefore, it is logical to develop new therapies directed against HIV binding molecules on susceptible host cells in addition to current treatment modalities against virus functions. Inhibition of the viral genome can be achieved by degrading or silencing posttranslational genes using small interfering (si) ribonucleic acids (RNAs) consisting of double-stranded forms of RNA. These siRNAs usually contain 21-23 base pairs (bp) and are highly specific for the nucleotide sequence of the target messenger RNA (mRNA). These siRNAs form a complex with helicase and nuclease enzymes known as "RNA-induced silencing complex" (RISC) that leads to target RNA degradation. Thus, siRNA has become a method of selective destruction of HIV now used by various investigators around the globe. However, given the sequence diversity of the HIV genomes of infected subjects, it is difficult to target a specific HIV sequence. Therefore, targeting nonvariable HIV binding receptors on susceptible cells or other molecules of host cells that are directly or indirectly involved in HIV infections may be an interesting alternative to targeting the virus itself. Thus, the simultaneous use of siRNAs specific for HIV and host cells may be a unique, new approach to the therapy of HIV infections. In this article, we present evidence that siRNA directed at the CD4 independent attachment receptor (DC-SIGN) significantly inhibits HIV infection of dendritic cells (DCs). This effect may be mediated by modulation of p38 mitogen activated protein kinase (MAPK).

Keywords: siRNA, DC-SIGN, HIV-1, dendritic cells, costimulatory molecules, HIV-LTR/U5, MAPK

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Introduction

RNA interference (i), using small interfering (si) RNAs,[1] provides a methodology for analyzing the role of cellular and viral regulatory factors in the human immunodeficiency virus (HIV) life cycle. RNAi is an evolutionarily conserved mechanism that is operative in insects, nematodes, plants, and mammalian cells.[2-5] In this process, sequence-specific, posttranscriptional silencing is initiated by the introduction into cells of double-stranded, annealed, sense and antisense RNAs that are homologous to the sequence of the silenced gene.[6] The ultimate mediators of RNAi-induced degradation are 21-(mer) siRNAs generated by RNase III cleavage of double-stranded RNAs extending up to several hundred nucleotides that had been introduced into cells.[7,8] To use RNAi in mammalian systems, 21-mer sense and antisense RNA oligonucleotides homologous to a portion of the gene of interest are synthesized, annealed, and introduced into cells by transfection.[2,4,5] These siRNAs bind specifically to the cellular messenger (m) RNA of interest and activate an RNA degradation process that leads to an 80% to 90% decrease in the levels of the corresponding protein. Thus, RNAi can be used to silence the gene of interest but not other genes.[9] The exquisite selectivity of RNAi makes its clinical applications almost endless because virtually any gene whose expression may contribute to disease is a potential target. For RNA-based therapies, delivery, stability, and potency have been the most significant obstacles. Attempts to develop antisense drugs have been largely disappointing. Antisense is a single strand of RNA or deoxyribonucleic acid (DNA), complementary to a target mRNA sequence; pairing it up with the antisense strand prevents translation of the mRNA. Pairing of the antisense oligonucleotide with the mRNA prevents subsequent translation of the latter. Early studies suggested that antisense drugs effectively reduced viral loads in clinical trials. However, closer examination revealed that these results were largely due to an increase in production of interferons by the host in response to high doses of exogenous RNA, rather than to specific silencing of any target genes. This realization led to disillusionment with antisense therapy. The emergence of the RNAi technology and the realization of its potential as a

more effective way to silence gene expression have led to rapid progress in the evolution of this technology in recent years. The extraordinary selectivity of RNAi, combined with its potency (that is, only a few double-stranded RNAs are needed per cell), quickly made it the tool of choice for functional genomics and for targeted drug discovery and validation. By "knocking down" a gene with RNAi and determining the subsequent effect on cells, a researcher can, in the course of only a few days, develop significant insight into the function of the gene and determine if reducing its expression will be therapeutically useful. Whether or not RNAi has a better chance to succeed as a drug than the antisense therapy will ultimately be determined by clinical trials. RNAi technology is several orders of magnitude more potent at gene silencing than antisense technology because RNAi harnesses an endogenous, natural pathway.

Since RNAi was first recognized by Fire et al in 1998,[1] it has quickly become a powerful tool for HIV research. Several investigators have used siRNA specific for HIV genes to inhibit viral replication. These include siRNA specific for nef, gag, vif, tat, and rev genes.[10-14] However, given the significant sequence diversity of the HIV genome in infected subjects, it is difficult to target specific HIV sequences. Consequently, for potential therapeutic targets, investigators have turned to RNAi specific for critical genes of the host involved in the pathogenesis of HIV infections, such as the HIV receptor, CD4,[12,15,16] and the HIV co-receptors, CXCR4[15-18] and CCR5.[15,16,18,19] These studies have yielded some positive results in inhibiting HIV replication.

The novel dendritic cell (DC)–specific HIV-1 receptor, DC-SIGN, a C-type lectin, plays a key role in primary defense against HIV infection as well as in the dissemination of HIV-1 by DCs. DCs are essential for the early events of the immune response to HIV infection. Model systems of HIV sexual transmission show that DCs expressing DC-SIGN capture and internalize HIV at mucosal surfaces and are the first line of defense against HIV infection. Moreover, DCs efficiently transfer HIV to CD4+ T cells in lymph nodes, where subsequent viral replication occurs. DC-SIGN knockdown by siRNA in DCs selectively impairs infectious synapse formation between DCs and resting CD4+ T cells, but does not prevent the formation of DC–T cell conjugates. It was demonstrated that DC-SIGN is required downstream from viral capture for the formation of the infectious synapse between DCs and T cells.[20,21] DCs were unable to transfer HIV-1 infectivity to T cells in trans, demonstrating an essential role for the DC-SIGN receptor in transferring infectious viral particles from DCs to T cells.[20,21] Thus, the role of DC-SIGN in the transfer of HIV from DCs to T cells, a crucial step for HIV transmission and pathogenesis, is well established.

DCs also regulate the surface expression of the co-stimulatory molecules B7-1 and B7-2 (CD80 and CD86), which engage the CD28 molecule on the T cell. Optimal T-cell activation and viral replication occurs when the naive T cell receives 2 signals: the primary signal through the T-cell receptor recognizing foreign antigens; and the second signal through the B7-CD28 interaction.[22] Such a stimulus activates a cascade of genes, such as NFκB and mitogen activated protein kinase (MAPK), that lead to clonal expansion of antigen-specific T cells by binding to the viral promoter/enhancer (LTR), and driving high levels of transcription and consequently explosive virus production.[23] The current studies were undertaken to determine if DC-SIGN silencing modifies the expression of co-stimulatory molecules on the surface of DCs. In the present study, monocyte-derived, mature dendritic cells were transfected with siRNA specific for DC-SIGN. The kinetics of DC-SIGN gene suppression was investigated. Furthermore, the effects of DC-SIGN gene silencing on the expression of co-stimulatory molecules and HIV infectivity of DCs also were studied.

Materials and Methods

Purification of DCs

DCs were prepared as described by Dauer et al in 2002.[24] Briefly, human peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation on Ficoll-Paque (Amersham Pharmacia Biotech, Piscataway, NJ). $CD14^+$ monocytes were separated from PBMCs using plastic adherence; the monocytes were cultured in RPMI 1640 with 1% human, AB serum, 500 U/mL recombinant human interleukin-4 (IL-4; R&D Systems, Minneapolis, MN), and 1000 U/mL recombinant human granulocyte-macrophage colony-stimulating factor (GMCSF; Amgen, Thousand Oaks, CA) for 24 hours. DCs were allowed to mature by culturing for an additional 2 days in the presence of interleukin-1β (IL-1β, R&D Systems), interleukin-6 (IL-6, R&D Systems), tumor necrosis factor (TNF, R&D Systems) and prostaglandin E_2 (PGE_2; Sigma-Aldrich, St Louis, MO). Maturation cytokines were added at final concentrations of IL-1β, 10 ng/mL; IL-6, 1000 U/mL; TNF, 10 ng/mL, and PGE_2, 1 μg/mL. Maturation of DCs was verified by $CD83^+$ and major histocompatibility complex (MHC-HLA-DR, DQ-DP) marker staining.

siRNA Sequences (DC-SIGN)

The following are the sequences of the various siRNAs used: DC-SIGN antisense, 5′- ATTTGTCGTCGTTC-CAGCCAT-3′; DC-SIGN sense, 5′-ATGGCTGGAAC-GACACAAA-3′; DC-SIGN antisense scrambled control, 5′-CACACCACATCTTTCCGTCAC-3′: DC-SIGN sense scrambled control, 5′-GTGACGGAAAGATGTGGTG-3. RNA oligonucleotides were custom synthesized by Dharmacon Research Inc (Lafayette, CO) with an overhang

of 2 thymidine residues (dTdT) at the 3′ end. The RNA oligonucleotides were dissolved in Tris-EDTA (10 mM Tris-HCl, pH 8.0; and 1 mM EDTA) as 200 μM solutions and were stored at –20°C. Double-stranded siRNA molecules were generated by mixing the corresponding pair of sense and antisense RNA oligonucleotides in annealing buffer (30 mM HEPES-KOH, pH 7.9; 100 mM potassium acetate; and 2 mM magnesium acetate) at 20 μM and then by incubating the reaction mixture at 95°C for 2 minutes, followed by gradually cooling to room temperature. The siRNAs were then aliquoted and stored at –20°C.

Transfection of siRNAs

Twenty-four hours before siRNA transfection, 1×10^5 DCs were seeded in 6-well plates in OPTI-minimal essential medium (OptiMEM; Invitrogen, Grand Island, NY) containing 10% Fetal Bovine Serum (FBS) with no antibiotics. Cells were seeded per 6-well plate to give 30% to 50% confluency at the time of the transfection. The siRNAs were transfected at a final concentration of 50 nM using Oligofectamine (Invitrogen) according to the manufacturer's recommendations. The control (untransfected) cells received either Oligofectamine alone or Oligofectamine plus scrambled sequence. The siRNAs added to control cells were incubated for 24, 48, and 72 hours, and the cells were harvested and RNA was extracted.

RNA Extraction

Cytoplasmic RNA was extracted by an acid guanidinium-thiocyanate-phenol-chloroform method as described.[25] Cultured DCs were centrifuged and resuspended in a 4 M solution of guanidinium thiocyanate. Cells were lysed by repeated pipetting and then phenol-chloroform extracted in the presence of sodium acetate. After centrifugation, RNA was precipitated from the aqueous layer by adding an equal volume of isopropanol and the mixture was kept at –20ºC for 1 hour and then centrifuged to sediment the RNA. The RNA pellet was washed with 75% ethanol to remove any traces of guanidinium. The final pellet was dried and resuspended in diethyl pyrocarbonate (DEPC) water and the amount of RNA determined using a spectrophotometer at 260 nm. DNA contamination in the RNA preparation was removed by treating the RNA preparation with DNAse (1 IU/μg of RNA) for 2 hours at 37ºC, followed by proteinase K digestion at 37ºC for 15 minutes and subsequent extraction with phenol-chloroform and NH_4OAc/ETOH precipitation. DNA contamination of the RNA preparation was checked by including a control in which reverse transcriptase enzyme was not added in the polymerase chain reaction (PCR) amplification procedure. RNA devoid of any DNA contamination was used in subsequent experiments with semiquantitative PCR. The isolated RNA was stored at –70°C until used.

Real-Time Quantitative PCR

DC-SIGN, CD80, CD86, CD40, p38, and LTR- R/U5 gene expression were quantitated using real-time PCR using specific primers. Table 1 shows the primer sequences of the primers used in this study (All the primer sequences designed are proprietary). The relative abundance of each mRNA species was assessed using the SYBR green master mix from Stratagene (La Jolla, CA) to perform real-time quantitative PCR using the ABI Prism 5700 instrument that detects

Table 1. List of Primer Sequences

Primer	PCR Product Size	Primer Sequence
β-Actin	[548 bp]	5′ 5′-TGACGGGGTCACCCACACTGTGCCCATCTA-3′ 3′ 5-AGTCATAGTCCGCCTAGAAGCATTTGCGGT-3′
DC-SIGN	[205 bp]	5′ 5′-GAAGACTGCGCGGAATTTAG –3′ 3′ 5′-TCGAAGGATGGAGAGAAGGA -3′
HIV-LTR-R/U5	[180 bp]	5′ 5′-TCT CTC TGG TTA GAC CAG ATC TG-3′ 3′ 5′-ACT GCT AGA GAT TTT CCA CAC TG-3′
p38 MAPK	[560 bp]	5′ 5′-GGCAGGAGCTGAACAAGACAA-3′ 3′ 5′-TTCAGCATGATCTCAGGAGCC -3′
CD80	[216 bp]	5′5′−GGGAAAGTGTACGCCCTGTA-3′ 3′ 5′−GCTACTTCTGTGCCCACCAT-3′
CD86	[406 bp]	5′5′−ACAAAAAGCCCACAGGAATG-3′ 3′ 5′−ATCCAAGGAATGTGGTCTGG-3′
CD40	[156 bp]	5′5′−CCACTGGGTATGGTGGTTTC-3′ 3′ 5′−TCACCTTCTGCCTCCTGTCT-3′

and plots the increase in fluorescence versus PCR cycle number, thus yielding a continuous measure of PCR amplification. To provide precise quantification of initial target in each PCR reaction, the amplification plot is examined at a point during the early log phase of product accumulation. This is accomplished by assigning a fluorescence threshold above background and determining the time point at which each sample's amplification plot reaches the threshold (defined as the threshold cycle number or C_T). Differences in threshold cycle number are used to quantify the relative amount of PCR target contained within each tube. Relative expression of mRNA species was calculated using the comparative C_T method as described.[26]

Infection of DCs with HIV-1 Isolates

DCs were infected with HIV-1_{Ba-L} (NIH AIDS Research and Reference Reagent Program, Cat# 510) at a concentration of $10^{3.75}$ $TCID_{50}$/mL cells for 3 hours at 37ºC. Cells were washed and cultured for an additional 24 hours, and supernatants were collected for p24 analysis and cells were collected for RNA analysis. The HIV-LTR-R/U5 region was amplified by real-time quantitative PCR using primers specific for a 180–base pair (bp) fragment of the region as described.[27] This method is designed to detect early stages of reverse transcription of HIV.

RESULTS

Kinetics of DC-SIGN Silencing

DCs were transfected with DC-SIGN siRNA. Cells were harvested and RNA was extracted at 24, 48, and 72 hours following transfection. DC-SIGN gene expression was determined using real-time semiquantitative PCR. Results are expressed as the transcript accumulation index (TAI) with respect to the untransfected control. Data (Figure 1) demonstrate a 76% (TAI = 0.24 ± 0.078; P < .001), 66% (TAI = 0.34 ± 0.66; P < .001), and 53% (TAI = 0.47 ± 0.17; P < .001) inhibition of DC-SIGN expression in DCs transfected for 24, 48, and 72 hours respectively, compared with control, scrambled siRNA transfected cells. Scrambled siRNA had no effect on DC-SIGN gene expression and was similar to the untreated control culture (data not shown). These results demonstrate that DC-SIGN siRNA was effective in silencing gene expression in a time-dependent manner.

Effect of DC-SIGN Silencing on Co-stimulatory Molecule Expression

We examined whether silencing DC-SIGN also modulates the expression of co-stimulatory molecules by DCs. DCs were transfected with DC-SIGN siRNA; RNA was extracted

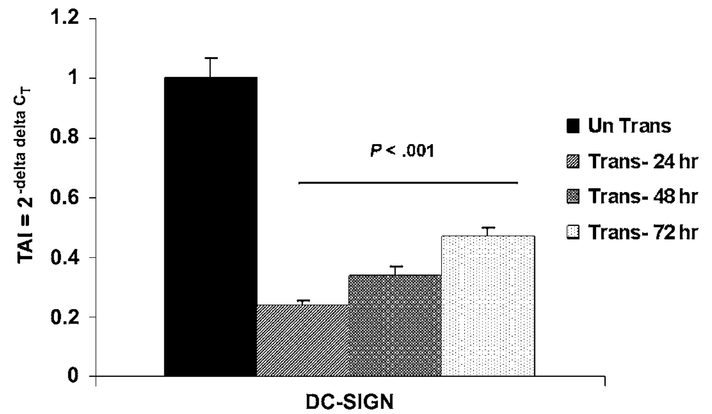

Figure 1. Kinetics of DC-SIGN siRNA transfection of DCs on DC-SIGN gene expression. DCs (1 × 10^5 cells/mL) were cultured in the absence or presence of DC-SIGN siRNA (50 nM) for 24, 48, and 72 hours. RNA was extracted and reverse transcribed, and DC-SIGN gene expression was analyzed by real-time quantitative PCR. Statistical significance was calculated by Student's 't' test (n = 2). (Un Trans indicates untransfected control; Trans indicates Transfected.)

following transfection; and CD40, CD80, and CD86 gene expression was determined. Data presented in Figure 2 show that 24 hours following transfection with DC-SIGN–specific siRNA, a 43% inhibition of CD40 gene expression occurred (TAI = 0.57 ± 0.19, P < .001); while a 12% inhibition of CD40 gene expression was observed at 48 and 72 hours of incubation (TAI = 0.88 ± 0.01, 48 hours; 0.88 ± 0.21, 72 hours; P < .05). Inhibition of CD80 expression was 48% (TAI = 0.52 ± 0.04, P < .001) at 24 hours and 4% (TAI = 0.96 ± 0.03, P = not significant [NS]) at 48 hours.

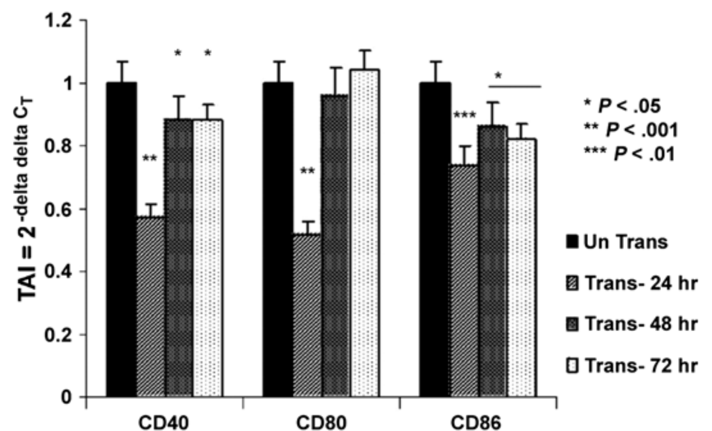

Figure 2. Kinetics of DC-SIGN siRNA transfection of DCs on the gene expression of the co-stimulatory molecules, CD40, CD80, and CD86. DCs (1 × 10^5 cells/mL) were cultured with or without DC-SIGN siRNA (50 nM) for 24, 48, and 72 hours. RNA was extracted and reverse transcribed, and CD40, CD80, and CD86 gene expression was analyzed by real-time quantitative PCR. Statistical significance was calculated by Student's 't' test (n = 2). (Un Trans indicates untransfected control; Trans indicates Transfected.)

Whereas, no inhibition of CD80 gene expression occurred at 72 hours, which was comparable to control values (TAI = 1.04 ± 0.07 vs TAI = 1.00 ± 0.04, P = NS). A 26% (TAI = 0.74 ± 0.02, $P < .01$), 14% (TAI = 0.86 ± 0.04, $P < .05$), and 18% (TAI = 0.82 ± 0.04, $P < .05$) inhibition of CD86 gene expression occurred at 24, 48, and 72 hours, respectively (Figure 2). Scrambled siRNA had no effect on CD40, CD80, and CD86 gene expression (data not shown). These results show that silencing of DC-SIGN gene expression with siRNA also decreased the gene expression of the co-stimulatory molecules, CD40, CD80, and CD86.

Effect of DC-SIGN Silencing on Signaling Molecule Expression

We investigated whether silencing DC-SIGN modulates the expression of the intracellular signaling protein, p38 MAPK in DCs. Data presented in Figure 3 show the effect of DC-SIGN–specific siRNA on the gene expression of p38 MAPK by DCs at 24, 48, and 72 hours as determined by real-time semiquantitative PCR. Data demonstrate a 31% (TAI = 0.69 ± 0.06, $P < .05$) inhibition of p38 MAPK gene expression at 24 hours and a 28% (TAI = 0.72 ± 0.12, $P < .05$) inhibition at 48 hours. At 72 hours, the p38 MAPK gene expression was comparable to the untransfected, control culture (TAI = 1.05 ± 0.07 vs TAI =1.00 ± 0.13, P = NS). Scrambled siRNA had no effect on p38 MAPK gene expression (data not shown). These results demonstrate that silencing of DC-SIGN gene expression with specific siRNA also decreased the gene expression of the signaling molecule p38 MAPK.

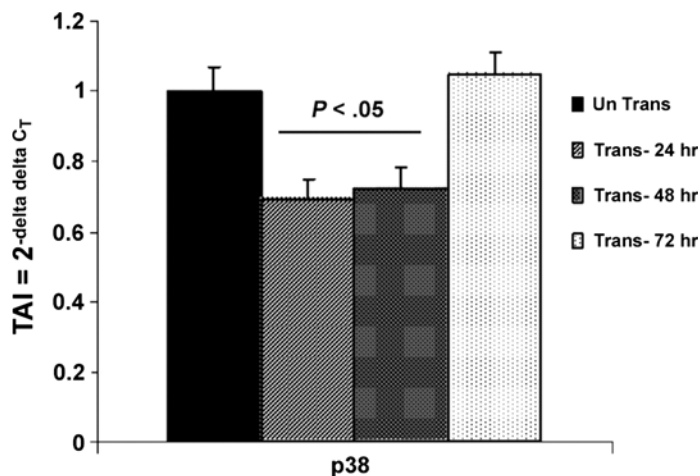

Figure 3. Kinetics of DC-SIGN siRNA transfection of DCs on the gene expression of p38 MAPK. DCs (1 × 10^5 cells/mL) were cultured with or without DC-SIGN siRNA for 24, 48, and 72 hours. RNA was extracted and reverse transcribed, and p38 MAPK gene expression was analyzed by real-time quantitative PCR. Statistical significance was calculated by Student's 't' test (n = 2). (Un Trans indicates untransfected control; Trans indicates Transfected.)

Effect of DC-SIGN siRNA on p24 Levels and HIV-LTR-R/U5 Gene Expression

We examined whether siRNA-directed inhibition of DC-SIGN decreased the levels of p24 antigen production and HIV-LTR gene expression by HIV infected cells. DCs were transfected with DC-SIGN siRNA for 24 hours, then infected with HIV-1_{BA-L}. Twenty-four hours after infection, culture supernatants were assayed for p24 using ELISA and HIV-LTR-R/U5 region gene expression by real-time semiquantitative PCR. A 76% inhibition of p24 levels (Figure 4A) following DC-SIGN gene silencing was observed in harvested culture supernatants (3.95 pg/mL, $P < .009$) compared with untransfected, control cultures (17.4 pg/mL) infected with HIV. Furthermore, our results demonstrate (Figure 4B) that silencing DC-SIGN gene expression also significantly (86%) inhibited HIV-LTR-R/U5 gene expression (TAI = 0.14 ± 0.07, $P < .001$) compared with untransfected control cultures infected with HIV

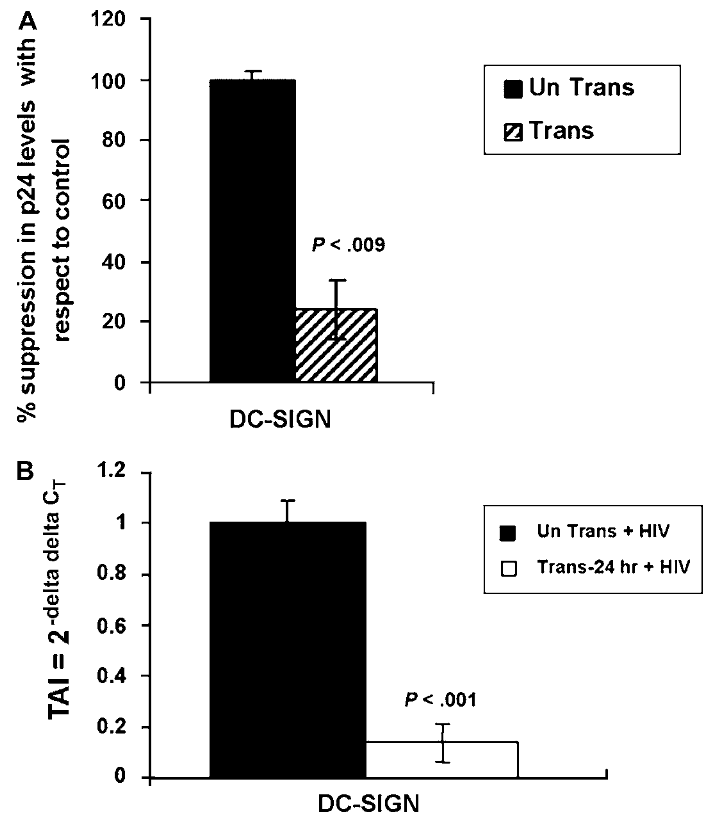

Figure 4. Effects of DC-SIGN siRNA transfection on p24 levels and HIV-LTR-R/U5 region gene expression by DC. DCs (1 × 10^5 cells/mL) were transfected with or without DC-SIGN siRNA for 24 hours. DCs were then infected with HIV-1 $_{Ba-L}$ for 3 hours at 37ºC, washed, and cultured for an additional 24 hours. **(A)** Culture supernatants were collected and assayed for p24. **(B)** RNA was extracted from cells and HIV-LTR-R/U5 gene expression was analyzed by real-time quantitative PCR. Statistical significance was calculated by Student's 't' test (n = 2). (Un Trans indicates untransfected control; Trans indicates Transfected.)

(TAI = 1.00 ± 0.12) (Figure 2). Scrambled siRNA had no effect on p24 levels or HIV-LTR-R/U5 region gene expression (data not shown). These data demonstrate that DC-SIGN–specific siRNA can inhibit significantly the production of HIV by infected DCs as demonstrated by decreases in p24 antigen production as well as HIV-LTR-R/U5 gene expression.

DISCUSSION

Because of the sequence diversity of the HIV genome, current approaches to control HIV infection are problematic and incomplete. Although siRNA is highly specific, a change in the target mRNA by even 1 bp, can dramatically reduce the efficiency of degradation of the target mRNA. Because of the high error rate of HIV reverse transcriptase (1 in 1000 nucleotides per replication cycle), there is a high likelihood of generating siRNA escape mutants. Thus, an alternate approach is to target the stable molecules on the host cell that bind HIV-1 such as HIV-receptors, co-receptors, or CD4 independent binding structures. Furthermore, the simultaneous use of siRNA targeted at viral genes may increase the anti-HIV activities of siRNA. DC-SIGN has been shown to be the first molecule that facilitates HIV infection independent of CD4 or viral co-receptors[28-30] and thus may be an ideal target for RNA interference. Arrighi et al[20,21] showed that DC-SIGN-negative DCs were unable to enhance transfer of HIV-1 infectivity to T cells in trans, demonstrating an essential role for DC-SIGN in the progression of HIV-1 infections. DCs increase the surface expression of the co-stimulatory molecules, CD80 and CD86, that engage the CD28 molecule on T cells.[29] Therefore, decreasing DC-SIGN gene expression also may alter the expression of these co-stimulatory molecules, further inhibiting DC–T cell interactions and the progression of HIV-1 infections. Our data demonstrate that silencing DC-SIGN decreases the gene expression of the co-stimulatory molecules CD40, CD80, and CD86 that, in turn, may inhibit the DC–T cell interactions needed for progression of HIV-1 infections.

CD80 (B7-1) and CD86 (B7-2) on DCs engage with the CD28 molecule on the T cell. This stimulus activates a cascade of genes such as MAPK, leading to clonal expansion of antigen-specific T cells and subsequently increased virus production.[23] Our studies show that siRNA induced–silencing of DC-SIGN and subsequent inhibition of co-stimulatory molecules and viral replication, as demonstrated by reduced p24 antigen production and LTR-RU- R/U5 gene expression, may be mediated by p38 MAPK inhibition.

Previous studies show that cytokines can induce differentiation or maturation of DCs.[31-34] Conflicting results were obtained on the replication of HIV-1 in DCs[35] because of differing stages of maturation. Immature DCs were found to be most susceptible to HIV infection and its progression. Frank et al[36] demonstrated that both mature and immature DCs pulsed with HIV-1 Ba-L were able to produce significant virus production as analyzed by p24 antigen expression. However, in co-cultures of DCs and T cells, virus replication was 3 times higher than in DCs alone. Ganesh et al[37] recently showed that DCs matured with poly(I-C) treatment could be transfected with HIV-1_{ADA} vector. Our previous studies demonstrate that although immature DCs are a more susceptible target for HIV infection, mature DCs also show a basal level of infection with HIV and the efficiency of infection is associated with the stage of DC maturation.[38] Furthermore, we demonstrated that mature DCs transiently exposed to syngeneic T cells become highly susceptible to HIV infection (data not shown). Interestingly, RNAi against DC-SIGN decreases the gene expression of HIV-LTR-R/U5 and levels of p24 following infection with HIV BAL. These data demonstrate that for efficient HIV infection of DCs, DC-SIGN must be present, and the data further support the role of DC-SIGN in transferring infectious viral particles from DCs to T cells. Thus, designing drugs specifically silencing DC-SIGN gene expression likely will inhibit initial HIV infection of DCs and DC–T cell clustering, which, in turn, leads to subsequent infection of T cells. A long-term goal of our study is to develop novel therapeutic approaches to prevent initial binding of HIV-1 to primary target cells as a first step in inhibition of HIV-1.

ACKNOWLEDGMENTS

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