Large quantity production with extreme convenience of human SDF-1α and SDF-1β by a Sendai virus vector

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Abstract We describe a robust expression of human stromal cell-derived factor-1α (SDF-1α) and SDF-1β, the members of CXC-chemokine family, with a novel vector system based upon Sendai virus, a non-segmented negative strand RNA virus. Recombinant SDF-1α and SDF-1β were detected as a major protein species in culture supernatants, reached as high as 10 μg/ml. This remarkable enrichment of the products allowed us to use even the crude supernatants as the source for biological and antiviral assays without further concentration nor purification and will thus greatly facilitate to screen their genetically engineered derivatives.

Key words: HIV-1; Chemokine; Stromal cell-derived factor 1; Sendai virus; Virus vector; Mammalian expression system

1. Introduction

Stromal cell-derived factor (SDF-1), also named pre-B cell growth-stimulating factor (PBSF), is a member of the CXC-chemokine family. SDF-1 was initially defined as a bone marrow stromal cell-derived soluble factor [1], but is subsequently found to be considerably multi-functional as it is involved in B lymphopoiesis, bone marrow hematopoiesis and cardiac ventricular septal formation [2,3]. Human and murine SDF-1 is known to arise in two forms, SDF-1α and SDF-1β, by differential splicing from a single gene. They differ in four carboxy-terminal amino acid residues which are present in SDF-1β and absent in SDF-1α [1,4]. Chemokines constitute a large family of small chemotactic cytokines of 60–80 amino acid residues. The members of this family have four conserved cysteine residues which form two intra-molecular disulfide bridges [5]. There are two subfamilies of chemokines, CC-chemokines and CXC-chemokines, which differ in the spacing of the first two cysteine residues. The CC-chemokine subfamily includes macrophage inflammatory peptide-1α and -1β (MIP-1α and MIP-1β), regulated on activation normal T cell expressed and secreted (RANTES) and monocyte chemoattractant protein-1 (MCP-1). The CXC-chemokine subfamily includes interleukin-8 (IL-8), platelet factor-4 (PF-4), in addition to SDF-1. Of these chemokines, SDF-1 appears to be most efficacious as a chemoattractant on resting T lymphocytes and monocytes [6].

Recently, both CC- and CXC-chemokines are attracting the keenest attention, because their receptors were proved to be used by human immunodeficiency virus type 1 (HIV-1) as a co-receptor for its entry into the CD4+ cells [7–13], and because they competitively block this virus-cell interaction [14–18]. Co-receptor usage and hence inhibition of infection by chemokines is strain specific. For instance, macrophage tropic/non-syncytium inducing strains need CCR-5 for their entry into target cells [7,9–12] and their infection can be blocked by the corresponding ligands, MIP-1α, MIP-1β, and RANTES [14,16,18]. On the other hand, CXCR-4 serves as a co-receptor for T cell line tropic/syncytium inducing strains [8,13], and its ligand SDF-1 can block the infection [15,17]. These discoveries have not only greatly facilitated our understanding of HIV replication and pathogenesis but also opened a novel possibility to treat HIV infection with chemokines or chemokine derivatives.

MIP-1α, MIP-1β and RANTES, produced in *E. coli*, have become commercially available and now widely used to get more information on the mechanism of co-receptor mediated HIV entry and its block by chemokines [7,18–21]. However, *E. coli*-based production generally requires extensive, multi-step purification of the product before use, and therefore is not always feasible for testing many different, genetically engineered derivatives. Extensive aggregation is often inevitable particularly for such basic polypeptides as chemokines. Thus, chemical synthesis of the original and modified versions has been adopted. This approach is not only laborious, including careful refolding, but also expensive. Purification of recombinant chemokines produced in mammalian and other higher vertebrate cells by recombinant viruses has not been reported.

We recently established a system to recover infectious Sendai virus (SeV), a non-segmented negative strand RNA virus in the family Paramyxoviridae, entirely from cDNA [22], and further succeeded in applying the technology to insert a foreign gene of interest to SeV genome and express the gene in extremely large quantities from an infectious recombinant SeV [23,24]. Here, we made attempts to create recombinant SeV expressing SDF-1α and SDF-1β to know whether or not this

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system can be applied for large quantity production of biologically active cytokines which can be used with minimal purification procedures. The SDF-1α and SDF-1β expressed from recombinant viruses consistently reached in amounts as high as 10 mg or more per liter of tissue culture medium. This high level of accumulation of the products has allowed not only the use of crude tissue culture medium as probes for chemotaxis and antiviral assays but also their purification by a single-step column chromatography. Thus, our system represents a novel and useful option for providing SDF-1α and SDF-1β and probably other chemokines and cytokines.

2. Materials and methods

2.1. Viruses and cells

HIV-1 strains NL43 [25], SF33 [26], and TK11 [27] and SIV mac strain 239 [28] were grown in MT4 T cell line. HIV-1 strain SF162 [29], primary isolates #12, #15 and #37 [30,31] were propagated in phytohemagglutinin-stimulated peripheral blood mononuclear cells (PBMC). CV1 cells were grown in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS). MT4 cells were grown in RPMI-1640 supplemented with 10% FBS. Primary chicken embryo fibroblasts (CEF) were prepared as described previously [32], and maintained in MEM supplemented with 10% FBS. After virus infection, CEF were maintained in MEM without serum. PBMC from healthy seronegative donors were prepared and grown as described previously [30].

2.2. Generation of recombinant Sendai viruses

Human SDF-1α or SDF-1β cDNAs were inserted just upstream of the open reading frame of the 3′ proximal N gene of SeV according to the method described previously [22–24] to generate recombinant SeV, SeV/SDF-1α or SeV/SDF-1β.

2.3. Northern blot

Total RNA was extracted using RNAzol-B (Tel-Test Inc., Texas) from approximately 10^6 CEF infected with the SeVSDF-1α(+) at various time points post infection (p.i.). The RNAs were ethanol precipitated, dissolved in formamide/formaldehyde solution, then electrophoresed in 1% agarose-formamide/MOPS gels, and capillary transferred onto Hibond-N filters (Amersham, UK). They were hybridized with the SDF-1 specific *Not*I fragment from pSeVSDF-1α(+) probes that had been labeled with α-P^{32}-dCTP using Multiprime DNA Labeling System (Amersham).

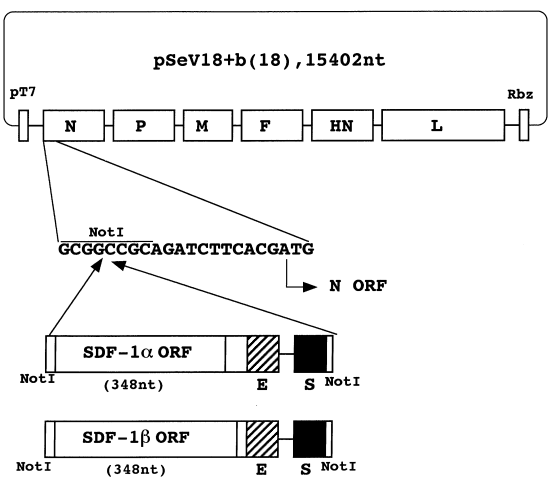

Fig. 1. Construction of the plasmids pSeV/SDF-1α(+) and pSeV/SDF-1β(+) which generate recombinant SeV/SDF-1α and SeV/SDF-1β antigenomic RNAs, respectively. The ORFs of human SDF-1α or SDF-1β followed by SeV transcriptional regulation signals E (hatched box) and S (filled box) were amplified with *Not*I-tagged primers and inserted to the *Not*I site in the parental pSeV18$^+$b(+) which generate a full length copy of the antigenomic positive sense of SeV RNA as described in Section 2.

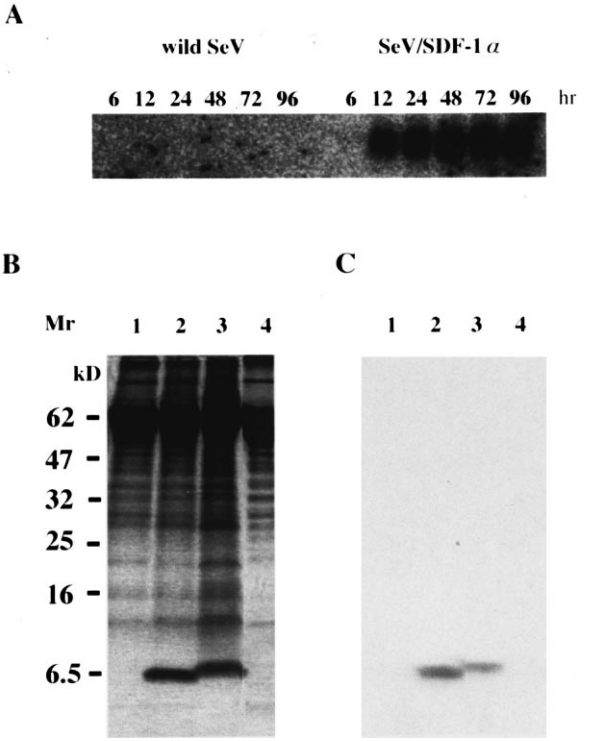

Fig. 2. Expression of human SDF-1α and SDF-1β genes from SeV/SDF-1α and SeV/SDF-1β. A: Northern blot hybridization. The RNA extracted at various hours after infection designated on the top of each lane from CEF infected with SeV/SDF-1α or wild-type SeV was analyzed by Northern blot hybridization with probe specific for SDF-1 gene. B: SDS-PAGE. Proteins in 100 μl of culture supernatant of CEF infected with wild-type SeV (lane 1), SeV/SDF-1α (lane 2), or SeV/SDF-1β (lane 3) were precipitated with ethanol together with 10 μg of BSA as carriers, and subjected to 15% SDS-PAGE. The gel was stained with Coomassie brilliant blue. Lane 4 shows 10 μg of BSA. C: Western blot analysis. Proteins resolved by SDS-PAGE were electrotransferred onto PVDF membrane and probed with anti-SDF-1 antiserum.

2.4. Western blot

Culture supernatants of infected cells were electrophoresed in 15% SDS-polyacrylamide gels [34]. The proteins in the gels were electrotransferred onto PVDF membranes (Millipore, Bedford) and probed with anti-SDF-1 antiserum, which was prepared by immunized rabbits with multiple antigen peptide containing residues 33–45 (RFFESHVARANVK) synthesized by Research Benetics Inc. (Huntsville, AL) [33].

2.5. Purification of SDF-1α and SDF-1β

Culture supernatants of CEF infected with SeV/SDF-1α or SeV/SDF-1β were harvested 72 h after infection and the SeV viruses were removed by centrifugation at 48 000×*g* for 1 h at 4°C. The supernatant was applied to an 1 ml Hi-Trap heparin column (Pharmacia, Uppsala, Sweden) equilibrated with 10 mM sodium phosphate buffer, pH 7.2, on an FPLC system (Pharmacia, Uppsala, Sweden). After washing with 5 ml of the same buffer, bound proteins were eluted with 10 ml of linear gradient of 0.4–1.0 M NaCl in the same buffer. Fractions were diluted more than 80-fold before assaying the chemotactic or anti-HIV activity. Active fractions were collected.

2.6. Chemotaxis assay

Lymphocyte chemotaxis assays were performed according to the method described by Bluel et al. [6]. Briefly, human peripheral blood lymphocytes were obtained from healthy donors by Ficoll-Histpaque method. Monocytes were removed by 1-h steps of plastic adherence.

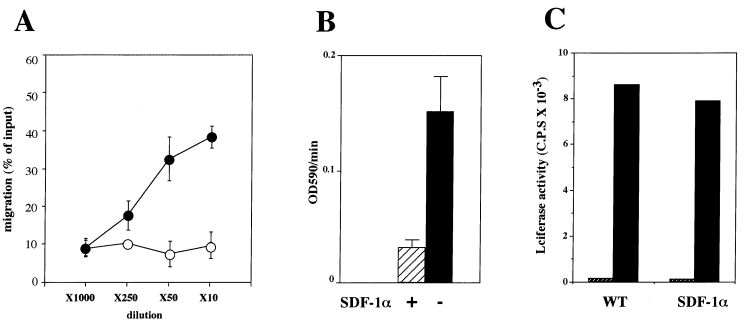

Fig. 3. Biological activities of SDF-1α. A: Chemotactic activity of SDF-1α. Culture supernatants of CEF infected with SeV/SDF-1α (○) or wild-type SeV (●) were serially diluted and examined for their chemotactic activity. Error bars indicate standard deviations of duplicated data. B: Inhibitory effect of SDF-1α on cell fusion mediated by gp160 of HIV-1 strain NL43. Filled and hatched bars indicate β-galactosidase activity within cells treated with the culture supernatants of CEF infected with wild-type SeV and SeV/SDF-1α, respectively. Error bars indicate standard deviations of duplicated data. C: Effect of SDF-1α on HIV-1 LTR-driven luciferase activity. MT4 cells were treated with culture supernatants of CEF infected with wild-type SeV (WT) of SeV/SDF-1α (SDF-1α) followed by transfection with both pHIV-1LTR/L-A-5′438 and pcDL-SRα/tat501 (filled bars), or pHIV-1LTR/L-A-5′438 alone (hatched bars). Luciferase activity within cells was assayed 40 h after transfection.

Cells (5×10^5) in 100 μl RPMI-1640 medium containing 0.25% human serum albumin (HSA) were added to the upper chamber of a 5-μm pore polycarbonate Transwell culture insert (Coaster, Cambridge, MA) and incubated with the indicated concentrations of proteins for 3 h. Transmigrated cells were counted with a FACScan (Becton Dickinson, San Jose, CA) for 20 s at 60 μl/min.

2.7. Anti-HIV-1 assay

PHA-stimulated PBMC or MT4 cells (5×10^5) were incubated with indicated concentrations of chemokines for 16 h, and then exposed to 1000 50% tissue culture infective dose of HIV-1 for 2 h at 37°C. The cells were washed twice with RPMI medium and maintained in the culture medium for each cell type. Culture supernatants of the infected cells were assayed for the levels of p24 core antigen (Abbott, Wiesbaden-Delkenheim, Germany). Data points are the means of duplicate cultures.

2.8. Cell fusion assay

A recombinant vaccinia virus-based gene activation assay using a β-galactosidase gene as a reporter was performed as described by Nussbaum et al. [35]. Briefly, L cells were transfected with plasmid pG1NT7β-gal with DOTAP and then infected with recombinant vaccinia virus expressing gp160 of HIV-1 strain NL43. MT4 cells were infected with vTF7-3 [36], and then treated with 2-fold diluted culture supernatant of CEF infected with SeV/SDF-1α or wild-type SeV. After 16-h incubation at 31°C, equal numbers (1×10^5) of L and MT4 cells were mixed and incubated at 37°C for 3 h. β-galactosidase activity within cell lysate was measured by using chlorophenol red-β-D-galactopyranoside as a substrate.

2.9. Luciferase assay

MT4 cells were incubated with or without 0.5 μg/ml of SDF-1α for 16 h, and then transfected with 5 μg of the plasmid carrying the luciferase reporter gene under the control of HIV-1 LTR, pHIV-1LTR/L-A-5′438, and 5 μg of the tat expression plasmid, pcDL-SRα/tat501, with DOTAP (Boehringer-Mannheim, Germany). Cells were maintained in the presence or absence of 0.5 μg/ml of SDF-1α for additional 40 h and then lysed for luciferase assay [22].

2.10. Ca^{2+} influx assay

Freshly prepared PBMC were stimulated with PHA for 3 days in RPMI without IL-2. Ca^{2+} influx into PHA-stimulated PBMC upon SDF-1α treatment was assayed as described previously [17].

3. Results

3.1. Generation of a recombinant SeV carrying human SDF-1α gene

The SeV genome of 15-kb long negative sense RNA is organized starting with a short 3′-leader region, followed by six

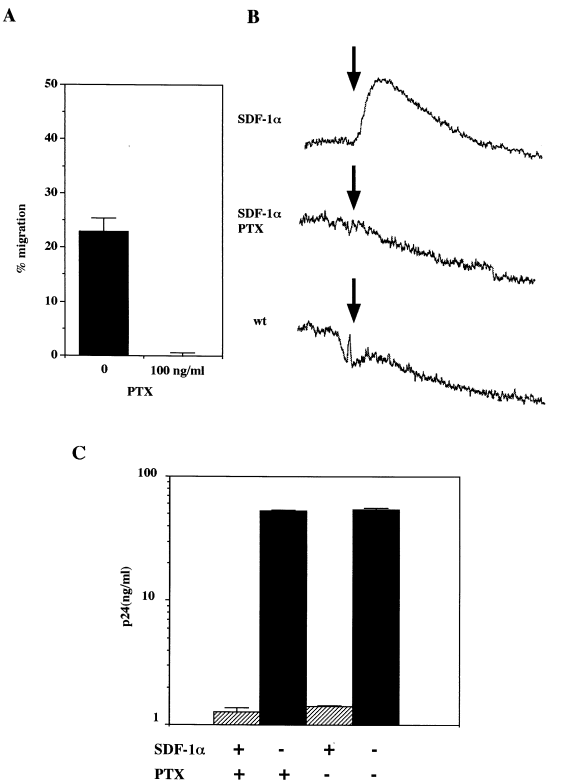

Fig. 4. A: Effect of PTX on chemotactic activity of SDF-1α. Freshly prepared PBL were incubated at 37°C for 16 h in the presence or absence of 100 ng/ml of PTX. Migration of PBL to 20-fold diluted culture supernatants of CEF infected with SeV/SDF-1α was performed as described in Section 2. Error bars indicate standard deviations of duplicated data. B: Effect of PTX on SDF-1α mediated Ca^{2+} influx into PBMC. Dialyzed culture supernatants of CEF infected with SeV/SDF-1α (SDF-1α) or wild-type SeV (wt) were added at the time point indicated by arrows. C: Effect of PTX on HIV-1 growth and SDF-1α mediated anti-HIV-1 activity. MT4 cells were incubated at 37°C for 16 h in the presence (+) or absence (−) of 100 ng/ml of PTX, and treated with culture supernatants of CEF infected with SeV/SDF-1α or wild-type SeV, and then infected with NL43 strain of HIV-1. The levels of p24 core antigen in the culture supernatants were assayed 3 days after infection. Data points are mean actual fluctuations of duplicated culture.

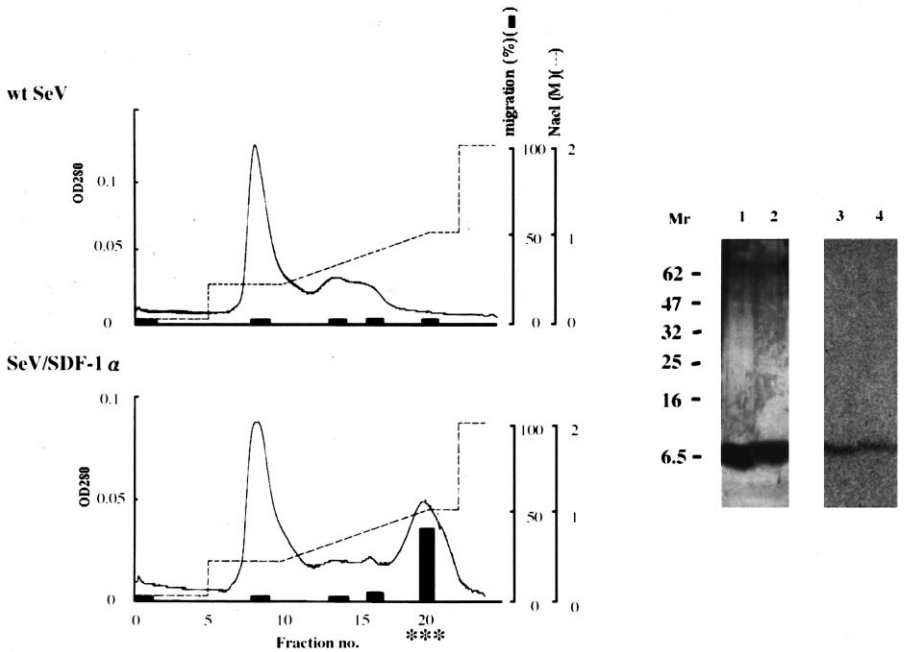

Fig. 5. Left panel: Purification of SDF-1α. SDF-1α was purified from the culture supernatant of CEF infected with SeV/SDF-1α by binding to Hi-Trap heparin column. Bars in the column chromatogram indicate chemotactic activity. Right panel: SDS-PAGE of purified SDF-1α and SDF-1β. Three active fractions shown by asterisks in the left panel were pooled and subjected to 15% SDS-PAGE. Proteins were silver-stained (lanes 1 and 2) or probed with anti-SDF-1 serum after electrotransferring to PVDF membrane (lanes 3 and 4). Positions of molecular weight markers are shown. Lanes 1 and 3, SDF-1α; lanes 2 and 4, SDF-1β.

structural genes and ending with the short 5′-trailer region [37–39]. There is only a single promoter for RNA polymerase consisting of L and P proteins [40], at the 3′ end. By recognizing the stop or end (E) (termination/polyadenylation) and restart (S) signals, the polymerase gives rise to leader RNA and each mRNA species. The plasmid pSeV18^{+}(+) carries a cDNA copy of SeV full length antigenome (positive strand RNA), in which an additional 18 synthetic nucleotides containing unique *Not*I site was inserted. This insertion was done within the first gene locus (N gene) and just upstream of its ORF, and serves as the site for further insertion of a foreign gene of interest [23]. Placing the foreign gene in this 3′-terminal first locus expects the highest expression, because of polar attenuation of gene expression toward the 5′-terminus [41]. The entire viral sequence containing the 18-nucleotide insertion was placed between the T7 promoter and the hepatitis delta virus ribozyme. The latter was used to generate a precise 3′ end.

A 348-bp DNA fragment containing entire coding frame of human SDF-1α gene (267 bp) followed by a new set of synthetic E and S signals with intervening three nucleotides was amplified with *Not*I-tagged primers and inserted into the *Not*I site in pSev18^{+}b(+), generating pSeVSDF-1α(+) (Fig. 1). In the cells infected with recovered recombinant virus, the S signal originally used for the N gene start directs the initiation of inserted SDF-1α gene transcription, and the introduced synthetic E and S signals direct the termination of inserted SDF-1α gene and the transcription initiation of the downstream N gene, respectively. pSeVSDF-1α(+) was transfected to v-TF7-3 infected LLCMK2 cells and the T7-driven full length recombinant SeV RNA genomes were encapsulated with N, P and L proteins, which were derived from the cotransfected respective plasmids. Following a 40-h incubation to allow initiation of the infectious cycle and generation of progeny, the transfected cells were injected into embryonated chicken eggs to amplify the recovered virus. After a successive passage

Table 1
Effect of SDF-1α on the growth of several HIV-1 and SIV mac strains

Strain	Phenotype	p24 (ng/ml)	
		Control	SDF-1α (0.5 μg/ml)
NL43[a]	SI/T cell line tropic	54.44	1.40
SF33[a]	SI/T cell line tropic	78.50	6.40
TK11[a]	SI/T cell line tropic	355.00	26.00
#15[b]	SI	100.23	1.36
SF162[b]	NSI/macrophage tropic	10.43	12.35
#12[b]	NSI	27.91	17.70
#37[b]	NSI	22.01	20.00
SIV mac239[a]	T cell line tropic	4.81[c]	6.22[c]

SI and NSI indicate syncytium inducing and non-syncytium inducing phenotype, respectively. Data points are means of duplicate cultures.
[a]Inhibition by SDF-1α in MT4 cells was evaluated at day 3 after infection.
[b]Inhibition by SDF-1α in PBMC cultures was evaluated at day 7 after infection.
[c]SIV mac p27 core antigen levels are shown.

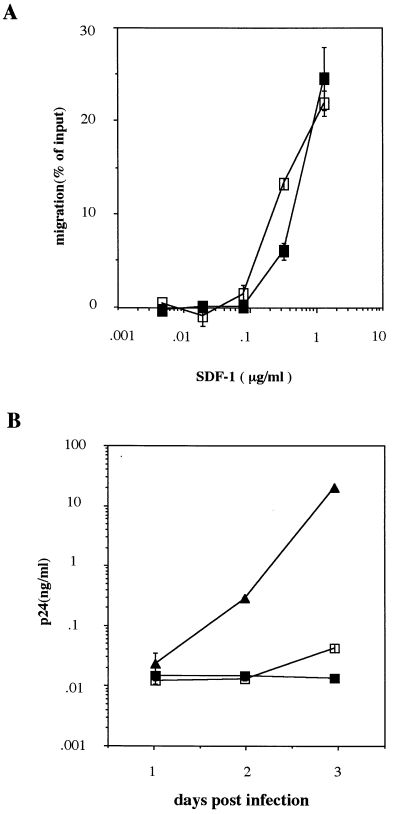

Fig. 6. A: Chemotactic activity of SDF-1α and SDF-1β. Purified SDF-1α (□) and SDF-1β (■) were serially diluted and assayed for the chemotactic activity. B: Anti-HIV-1 activity of SDF-1α and SDF-1β. MT4 cells were treated with 200 ng/ml of purified SDF-1α (□) or SDF-1β (■), and then infected with NL43 strain of HIV-1. The levels of p24 core antigen in the culture supernatants were assayed periodically. Data points are mean actual fluctuations of duplicate cultures. Filled triangles show cultures which were not treated with chemokines.

in eggs, the recombinant virus reached a titer of over 10^9 PFU/ml comparable to that of the wild-type SeV. This second passage, initiated at a dilution of 10^{-6}, resulted in complete elimination of helper vTF7-3 present in an amount of 10^4 to 10^5 PFU/ml. Nucleotide sequencing of the recombinant virus revealed that there was no accidental nucleotide substitution within the inserted SDF-1α gene. The recovered virus was named SeV/SDF-1α.

3.2. *Anti-HIV-1 and chemotactic activities of SDF-1α expressed from SeV/SDF-1*

SeV has a broad host range and reaches quite a high copy number in susceptible cells. In previous experiments, we found that expression levels were the highest in CV1, a monkey kidney cell line [23,24]. Here, we found that primary chicken embryo fibroblasts (CEF) were still more productive, and used in all subsequent studies. In CEF infected with SeV/SDF-1α, the transcripts specific to SDF-1α with an expected size of approximately 400 bases were detected (Fig. 2A), and a polypeptide with a molecular mass of 8 kDa was observed as a major protein constituent in the culture supernatant (Fig. 2B). This protein species was absent in wild-type SeV infected fluid, and reacted specifically with rabbit serum immunized with 13-mer peptides derived from human SDF-1α sequence in Western blotting (Fig. 2C). These results demonstrated that substantial amounts of SDF-1α were produced from the recombinant SeV and secreted into the culture supernatant. After a 72-h incubation, the amount of SDF-1α in the culture fluid reached over 10 μg/ml.

Because the culture fluid efficiently attracted freshly prepared human peripheral blood lymphocytes (PBL) in chemotaxis assays (Fig. 3A), the recombinant SDF-1α was functionally authentic. The recombinant SDF-1α suppressed the replication of three different T cell line tropic HIV-1 strains, NL43, SF33 and TK11, in the MT4 T cell line, and one syncytium inducing primary isolate #15 in PBMC culture (Table 1). The SDF-1α did not suppress the replication of macrophage tropic strain SF162, nor two non-syncytium inducing primary isolates, #12 and #37, in PBMC cultures (Table 1). The recombinant SDF-1α did not show any inhibitory activity for simian immunodeficiency virus (SIV) strain mac239 in MT4 cells (Table 1). These results are consistent with the expected specificity of antiviral activities of SDF-1α and thus confirmed the biological authenticity of the SeV-derived recombinant SDF-1α. We further demonstrated that the recombinant SDF-1α indeed inhibited the step of membrane fusion, but not the viral transcription (Fig. 3B and C).

3.3. *Effect of a G protein blocker on SDF-1 mediated inhibition of HIV-1 growth*

Pretreatment of PBL with 100 ng/ml of pertussis toxin (PTX) for 18 h completely abolished the chemotactic activity of SDF-1α (Fig. 4A). Ca^{2+} mobilization into PBL mediated by SDF-1α was also inhibited by this reagent (Fig. 4B), confirming that SDF-1α transduces signals mediated by Gαi-coupled receptor. However, the same concentration of PTX did not affect the inhibitory activity of SDF-1α in HIV-1 growth (Fig. 4C). The blocker alone did not affect HIV-1 replication at all. These results indicated that the G protein mediated signal transduction is neither a prerequisite for HIV-1 entry nor essential for the inhibitory action of SDF-1α.

3.4. *Purification of the recombinant SDF-1α from culture supernatants*

An affinity heparin column chromatography was performed to purify SDF-1α from the culture supernatant of SeV/SDF-1α infected CEF. The chemotactic and anti-HIV-1 activity were co-eluted with a single 8 kDa polypeptide in the chromatography (Fig. 5). We consistently obtained at least 50 μg of purified SDF-1α from 50 ml crude culture supernatant. Amino acid sequencing demonstrated the NH_2-terminal KPVSLSYRXPXR, identical to the reported sequence of SDF-1α. In this NH_2-terminal peptide, X should be read as C, because it cannot be resolved by the sequencing method employed. In the purified SDF-1α preparation, we detected a small amount of protein with the N-terminal sequence, SYRXPXRFFE, which lacked the first five amino acid residues of the SDF-1α sequence. This minor species of the NH_2-terminal sequence was also reported to be present in the SDF-1α preparation purified from a bone marrow stromal cell line [6] and has been shown to be functionally inactive in both chemotactic and HIV-1 inhibition [15].

3.5. *Comparison of SDF-1α and SDF-1β*

The human and murine SDF-1 gene gives rise to two forms, SDF-1α and SDF-1β, by alternative splicing. They differ in

the carboxy-terminal four amino acid residues, which are present in SDF-1β and absent in SDF-1α [1,4]. We also successfully constructed a recombinant SeV expressing SDF-1β (SeV/SDF-1β) (Fig. 1). As in the case of SDF-1α, the amount of SDF-1β reached over 10 μg/ml in the culture supernatant of CEF infected with SeV/SDF-1β, and readily purified by heparin column chromatography (Fig. 5). Consistent with the fact that SDF-1β has additional four amino acid residues, it migrated slightly more slowly than SDF-1α in SDS-PAGE (Figs. 2B and 5).

The chemotactic and anti-HIV-1 activities of SDF-1β were examined and compared with those of SDF-1α. SDF-1β was found to attract freshly prepared PBL in chemotaxis assay and suppressed HIV-1 strain NL43 as efficiently as did SDF-1α (Fig. 6).

4. Discussion

In this paper, we have described SeV-based expression of both SDF-1α and SDF-1β. They were of equal efficacy in chemotactic activity on freshly prepared PBL and inhibiting HIV-1 replication. Their specificity was demonstrated by selective inhibition of T cell line tropic laboratory strains and SI type primary isolates. In most previous papers on SDF-1α, it was either chemically synthesized or physiologically produced from bone marrow stromal cells [6,15,17,19,42–45]. In the latter, native molecules can be obtained in conditioned media but in an estimated amount of only 1 μg/ml at most. In the former, the primary product should be oxidized to form disulfide bridges, and then carefully purified by high performance liquid chromatography. Moreover, the production is laborious and expensive. Only a single round of synthesis of 1 mg may require numerous days and cost. In our expression system, SDF-1α as well as SDF-1β accumulated extensively, reaching as high as 10 μg/ml or more, in the culture supernatants. Thus, the product existed as a major protein constituent in the supernatant, indicating its remarkable enrichment already in the crude material. Because of this high level of production and because an amount around 200 ng/ml is sufficient for chemotaxis and antiviral assay, culture supernatants without further condensation and purification could be conveniently used as the source of SDF-1α and SDF-1β for various experiments described above. It will be thus also easy to test the functions of derivatives produced from recombinant viruses following various engineering of their genes.

SDF-1α we generated efficiently suppressed replication of T cell line tropic or primary SI strains but not macrophage tropic or NSI strains of HIV-1. It was recently reported that simian immunodeficiency virus (SIV) strains do not utilize CXCR-4 as a co-receptor, although they can grow very well in T cell lines which lack another co-receptor, CCR-5 [46–48]. Co-receptors for SIV were recently identified and named BOB/gpr1, Bonzo and gpr15 [49,50]. Consistent with these findings, recombinant SDF-1α did not suppress replication of SIV strain mac239 at all even in the MT4 T cell line.

The transcripts of murine SDF-1α and SDF-1β were observed in many tissues including the brain, heart, lung, kidney, thymus, liver, and spleen, and the amount of transcripts of SDF-1α was always higher than that of SDF-1β [1]. In the present study, we found that SDF-1α and SDF-1β were equally effective in both chemotaxis and virus inhibition. On the other hand, it is reported that negative charges in the C-terminus of CC-chemokine MIP-1α were important for its tendency to form self-aggregation [51]. Since two out of four amino acid residues specific for SDF-1β are basic lysine residues, SDF-1α and SDF-1β may differ in their tendency to form self-aggregates. Thus, SDF-1α and SDF-1β may display different rates of in vivo spread, and therefore their contribution to in vivo physiology and the effects on HIV-1 containment may differ both quantitatively and qualitatively. It will be important to learn the physical properties and antiviral actions of recombinant SDF-1α and SDF-1β in vivo.

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