Processing by CD26/dipeptidyl-peptidase IV reduces the chemotactic and anti-HIV-1 activity of stromal-cell-derived factor-1α

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Abstract The chemokine stromal-cell-derived factor-1α (SDF-1α) chemoattracts lymphocytes and $CD34^+$ haematopoietic progenitors and is the ligand for CXCR4 (CXC chemokine receptor 4), the main co-receptor for T-tropic HIV-1 strains. SDF-1α was NH_2-terminally cleaved to SDF-1α(3-68) by dipeptidyl-peptidase IV (CD26/DPP IV), which is present in blood in soluble and membrane-bound form. SDF-1α(3-68) lost both lymphocyte chemotactic and CXCR4-signaling properties. However, SDF-1α(3-68) still desensitized the SDF-1α(1-68)-induced Ca^{2+} response. In contrast to CD26/DPP IV-processed RANTES(3-68), SDF-1α(3-68) had diminished potency to inhibit HIV-1 infection. Thus, CD26/DPP IV impairs the inflammatory and haematopoietic potency of chemokines but plays a dual role in AIDS.

Key words: Chemokine; CXCR4; HIV-1 inhibitor; Desensitization; SDF-1α; Exopeptidase

1. Introduction

Chemokines constitute a family of low molecular mass cytokines with leukocyte chemotactic and activating properties. Together with adhesion molecules, they play a crucial role in lymphocyte homing and in leukocyte migration to inflammatory sites. Depending on the position of the first cysteines, chemokines are divided in CC, CXC, C or CX_3C chemokine subfamilies [1–3]. Chemokines interact with their target cells through seven transmembrane spanning G-protein coupled receptors [4]. For five CXC chemokine receptors (CXCR) and ten CC chemokine receptors (CCR) the ligands have been identified. For various candidate ('orphan') chemokine receptors, the functional ligand has not yet been identified.

A number of NH_2-terminally truncated chemokines have been isolated from natural sources [5–10]. Depending on the chemokine, such posttranslational processing leads to entirely different biological effects ranging from conversion into a chemotactic protein (e.g. platelet basic protein into neutrophil-activating peptide-2) [5,6], over the increase of inflammatory activity in the case of interleukin-8 [11], to complete inactivation for the monocyte chemotactic proteins-1, -2 and -3 [10,12]. Recently, dipeptidyl-peptidase IV (DPP IV; EC 3.4.14.5) or the lymphocyte activation marker CD26 was found to be responsible for the NH_2-terminal truncation of RANTES into RANTES(3-68) [13,14]. RANTES(3-68) was inactive at physiological concentrations in chemotaxis and signaling assays on monocytes [9,13,14], but proved to be a CCR5-specific ligand with anti-inflammatory properties [9,13].

The CC chemokines MIP-1α (macrophage inflammatory protein-1α), MIP-1β and RANTES have been shown to inhibit HIV-1 infection in leukocytes [15] and the MIP/RANTES receptor, i.e. CCR5, was found to be the major co-receptor for M-tropic HIV-1 strains [16–20]. Surprisingly, CD26/DPP IV-truncated RANTES turned out to be a more potent inhibitor of M-tropic HIV-1 infection than intact RANTES [14].

LESTR/fusin or CXCR4, which is the receptor for SDF-1, has been shown to be the main co-receptor for T-tropic HIV-1 strains [21–23]. Mature SDF-1α isolated from natural sources [24] contains a Pro at the penultimate position corresponding to the consensus sequence for CD26/DPP IV-cleavage [25]. Since CD26/DPP IV is present in soluble form in plasma and as a membrane-bound protease on a variety of cells, including a subset of T lymphocytes, we investigated whether SDF-1α is a substrate for CD26/DPP IV, and, if so, whether SDF-1α truncation by CD26/DPP IV has any effects on the lymphocyte chemotactic and HIV-1-inhibitory properties of SDF-1α.

2. Materials and methods

2.1. Reagents, cells and virus

Recombinant carrier-free human SDF-1α was obtained from R and D Systems (Abingdon, UK). Soluble CD26/DPP IV (sCD26/DPP IV) was purified to homogeneity from total seminal plasma by anion exchange chromatography and affinity chromatography on immobilized adenosine deaminase [26]. The enzymatic activity was determined using the fluorogenic substrate Gly-Pro-4-methoxy-2-naphthylamide.

SUP-T1 cells (American Type Culture Collection, Rockville, MD, USA) were cultured in RPMI 1640 (Boehringer Ingelheim, Heidelberg, Germany) supplemented with 10% fetal calf serum (FCS). Human osteosarcoma (HOS) cells transfected with CD4 and CXCR4 [17] were grown in DMEM with glutamax (Life Technologies, Paisley, Scotland) and 10% FCS. Puromycin (1 μg/ml; Sigma, St. Louis, MO, USA) was added to the medium as a selection agent. Peripheral blood mononuclear cells (PBMC) from healthy donors were purified by Lymphoprep density gradient centrifugation (Nycomed, Oslo, Norway) and stimulated for 3 days at 37°C with 1 μg/ml phytohaemagglutinin (PHA, Sigma) as previously described [27]. The activated cells (PHA-stimulated blasts) were washed three times with phosphate-buffered saline (PBS) prior to use in the antiviral assays. The T-tropic HIV-1 strain NL4.3 was obtained from the National Institute

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Abbreviations: $[Ca^{2+}]_i$, intracellular calcium concentration; CCR or CXCR, CC or CXC chemokine receptor; CI, chemotactic index; sCD26/DPP IV, soluble CD26/dipeptidyl-peptidase IV; SDF-1α, stromal-cell-derived factor-1α

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of Allergy and Infectious Disease, AIDS reagent program (Bethesda, MD, USA).

2.2. Incubation of chemokines with CD26/DPP IV and detection of proteolytic processing

A 1000 molar excess of SDF-1α was incubated for 18 h at 37°C with soluble CD26/DPP IV in 100 mM Tris/HCl pH 7.7. Chemokines were separated from CD26/DPP IV by SDS-PAGE on a Tris/Tricine gel system as previously described [28]. Proteins were electroblotted on PVDF (polyvinylidene fluoride) membranes (Problott, Perkin Elmer, Foster City, CA, USA) and stained with Coomassie brilliant blue R250. After destaining, membranes were rinsed at least five times with ultrapure water (Milli Q; Millipore, Bedford, MA, USA). To obtain sufficient amounts of pure truncated SDF-1α for biological assays, 30 μg of chemokine was treated with CD26/DPP IV and the cleavage product was purified by C-8 RP-HPLC as previously described for RANTES [14]. CD26/DPP IV-treated chemokines, purified by RP-HPLC or excised from PVDF blots, were NH_2-terminally sequenced by Edman degradation on a pulsed liquid phase 477A/120A protein sequencer (Perkin Elmer) using *N*-methylpiperidine as a coupling base.

2.3. Chemotaxis assays

Intact or truncated SDF-1α were tested for their lymphocyte chemotactic potency on lymphocytic SUP-T1 cells (2 days after subcultivation) in a Boyden microchamber at 5×10^6 cells/ml using 5 μm pore size fibronectin-coated polycarbonate membranes. After 4 h incubation at 37°C in the microchamber, the cells were fixed and stained with Diff-Quick staining solutions (Harleco, Gibbstown, NJ, USA) and the cells that migrated through the membranes were counted microscopically in 10 oil immersion fields at 500-fold magnification. The chemotactic index (CI) of a sample (duplicates in each chamber) was calculated as the number of cells that migrated to the sample over the number of cells that migrated to control medium [28].

2.4. Detection of intracellular Ca^{2+} concentrations

Intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) were determined as previously described [29]. Briefly, CD4/CXCR4-transfected HOS cells (10^7 cells/ml) were loaded in growth medium for 30 min at 37°C with 2.5 μM of the fluorescent dye fura-2 (Molecular Probes Europe BV, Leiden, The Netherlands). The cells were washed with growth medium and calcium buffer (HBSS with 1 mM Ca^{2+} and 0.1% FCS, buffered with 10 mM HEPES/NaOH at pH 7.4) and diluted to 10^6 cells/ml. Fura-2 fluorescence was measured at 510 nm in an LS50B luminescence spectrophotometer (Perkin Elmer) upon excitation at 340 and 380 nm. The $[Ca^{2+}]_i$ was calculated from the Grynkiewicz equation [30]. The K_d used was 224 nM. To determine R_{max} and R_{min}, the cells were lysed with 50 μM digitonin and subsequently Ca^{2+} was complexed with EGTA at pH 8.5. For desensitization experiments, cells were first stimulated with intact or CD26/DPP IV-truncated SDF-1α and 100 s later with intact SDF-1α at a concentration (0.3 nM) that induced a significant increase in $[Ca^{2+}]_i$ after prestimulation with buffer.

2.5. Antiviral activity assay

PHA-stimulated lymphoblasts were treated with varying concentrations of intact or CD26/DPP IV-treated SDF-1α at the time of infection with the T-tropic NL4.3 HIV-1 strain (10^5 pg of p24). After 1 h, non-adsorbed virus was removed by washing three times with PBS and HIV-1-infected blasts were cultured in the presence of 25 U/ml IL-2, as previously described [27]. At day 10, HIV-1 titers were determined in the culture supernatant with a commercial p24 Ag ELISA (duPont, Wilmington, DL, USA).

3. Results

3.1. CD26/DPP IV truncates intact SDF-1α into SDF-1α(3-68)

Recombinant SDF-1α was treated overnight with sCD26/DPP IV, separated from the protease by SDS-PAGE, blotted on a PVDF membrane and Coomassie blue stained. Edman degradation of the 7 kDa protein yielded the sequence VSLSYR which corresponds to the NH_2-terminal sequence of SDF-1α(3-68), that is intact SDF-1α without the first two amino acids Lys and Pro. Substantial amounts of SDF-1α(3-68) were generated by incubating 30 μg of intact SDF-1α with sCD26/DPP IV. The obtained SDF-1α(3-68) was purified by C-8 RP-HPLC and the NH_2-terminal sequence (VSLSYR) was confirmed by Edman degradation. No intact SDF-1α was recovered after CD26/DPP IV treatment and no truncation of SDF-1α occurred in the absence of CD26/DPP IV, indicating the specificity of the cleavage.

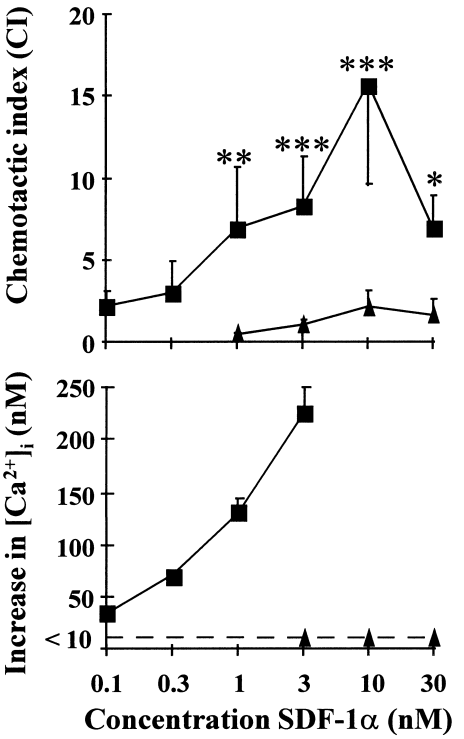

Fig. 1. Effect of CD26/DPP IV-cleavage on the chemotactic and Ca^{2+}-mobilizing activity of SDF-1α. Varying concentrations of intact SDF-1α(1-68) (squares) and truncated SDF-1α(3-68) (triangles) were compared for their lymphocyte chemotactic activity on SUP-T1 cells in the Boyden microchamber (upper panel). Results represent the mean CI ± S.E.M. of four or more independent experiments. Statistically significant difference between the two SDF-1α forms was calculated with the Student's *t*-test (*, $P<0.1$; **, $P<0.05$; ***, $P<0.01$). SDF-1α(1-68) (squares) and truncated SDF-1α(3-68) (triangles) were also compared for their potency to induce an increase in the $[Ca^{2+}]_i$ (mean ± S.E.M. of three or more independent experiments) in CD4/CXCR4-transfected HOS cells (lower panel). The dotted line at 10 nM represents the detection limit of the Ca^{2+} assay.

3.2. Effect of CD26/DPP IV on the lymphocyte chemotactic and CXCR4-signaling capacity of SDF-1α

Intact and CD26/DPP IV-truncated SDF-1α were compared in vitro for their ability to attract lymphocytic SUP-T1 cells in the Boyden microchamber assay and for their potency to signal (increase in $[Ca^{2+}]_i$) through CXCR4 (Fig. 1). Intact SDF-1α was significantly chemotactic from 1 nM onwards with a maximal chemotactic effect at 10 nM. On CXCR4 transfectants a clear increase in the $[Ca^{2+}]_i$ was already obtained with intact SDF-1α at 0.1 nM. In contrast, SDF-1α(3-68) was inactive at concentrations up to 30 nM both in the chemotaxis and Ca^{2+} assay.

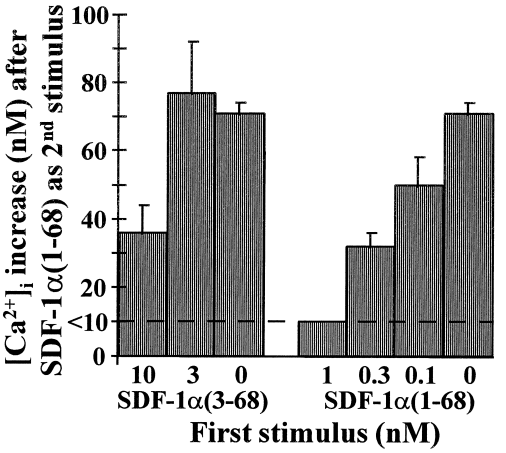

Fig. 2. Desensitization of the SDF-1α-induced Ca^{2+} response by SDF-1α(1-68) and SDF-1α(3-68). CD4/CXCR4-transfected HOS cells were prestimulated with buffer or varying concentrations of CD26/DPP IV-treated SDF-1α(3-68) or SDF-1α(1-68). After 100 s, cells were restimulated with 0.3 nM SDF-1α(1-68). Results represent the mean ± S.E.M. of three or more independent experiments. The dotted line at 10 nM represents the detection limit for the Ca^{2+} assay.

3.3. CD26/DPP IV-truncated SDF-1α(3-68) inhibits lymphocyte signaling by intact SDF-1α

Ca^{2+} responses towards 0.3 nM of SDF-1α(1-68) were completely desensitized by prestimulation of CXCR4 transfectants with 1 nM SDF-1α(1-68), whereas with an equimolar concentration about 50% desensitization of intact SDF-1α was obtained (Fig. 2). Although CD26/DPP IV-truncated SDF-1α(3-68) was inactive in the Ca^{2+} assay at 30 nM, 10 nM of this truncated chemokine led to a 50% reduction in the response towards a subsequent challenge with intact SDF-1α (Fig. 2). It must be concluded that although SDF-1α(3-68) failed to signal through CXCR4, the molecule is still able to bind and to desensitize for intact SDF-1α.

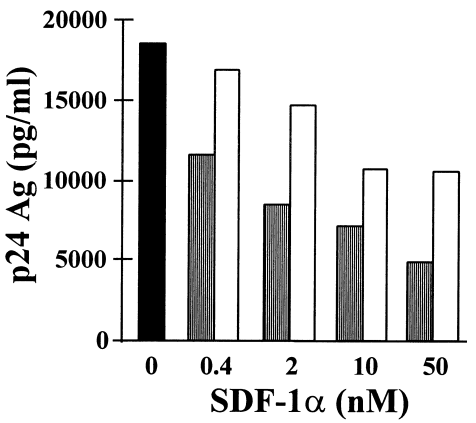

Fig. 3. CD26/DPP IV diminishes the anti-HIV-1 activity of SDF-1α. PHA-stimulated PBMCs were left untreated (black bar) or treated with varying concentrations of intact SDF-1α (dashed bars) or CD26/DPP IV-truncated SDF-1α(3-68) (open bars) at the time of infection with the NL4.3 strain of HIV-1. Virus replication was quantified by measuring the viral antigen p24 in a p24 ELISA. Results represent one representative experiment out of two.

3.4. Inhibitory effect of the CD26/DPP IV-truncated SDF-1α on HIV-1 infection

Intact and CD26/DPP IV-truncated SDF-1α were compared for their ability to inhibit infection of peripheral blood mononuclear cells with the T-tropic HIV-1 strain NL4.3, which uses CXCR4 as co-receptor (Fig. 3). NH_2-terminal processing of SDF-1α by CD26/DPP IV significantly diminished the anti-HIV-1 potency of the chemokine. For intact SDF-1α, an IC_{50} value of 1.5 nM was obtained, while no 50% inhibition was reached with SDF-1α(3-68) at a concentration up to 50 nM. Despite the 40-fold decrease in antiviral activity compared to intact SDF-1α, SDF-1α(3-68) at 10 nM still caused a 43% reduction in viral production.

4. Discussion

A number of NH_2-terminally truncated CXC and CC chemokine forms have been isolated from cellular sources [5–10]. Most of these NH_2-terminal modifications significantly increase or reduce the biological activity of the molecules. Although the first reports date from almost 2 decades ago, only little is known about the enzymes which are responsible for the NH_2-terminal processing of chemokines. Plasmin and trypsin convert the connective tissue activating peptide III into β-thromboglobulin which in turn is further processed by chymotrypsin and cathepsin G into the CXC chemokine neutrophil-activating peptide-2, a neutrophil chemoattractant which interacts with CXCR2 [31–34]. Recently, the dipeptidylpeptidase CD26/DPP IV has been reported to be able to process the CC chemokine RANTES into RANTES(3-68) [13,14]. RANTES(3-68) is a chemotaxis inhibitor and has impaired signaling properties through CCR1 and CCR3, but not through CCR5 [9,13,14]. Moreover, RANTES(3-68) is a more potent inhibitor of HIV-1 infection than intact RANTES [14].

Another chemokine with a penultimate Pro at the NH_2-terminus, the consensus motif for potential CD26/DPP IV substrates, is the lymphocyte chemotactic molecule SDF-1 [24]. SDF-1 is the ligand for CXCR4, the main co-receptor for T-tropic HIV-1 strains [21–23]. Here, we report that CD26/DPP IV cleaves the two NH_2-terminal residues from SDF-1α, selectively generating SDF-1α(3-68). Processing of SDF-1α by CD26/DPP IV results in loss of lymphocyte chemotactic activity and signaling properties through CXCR4. However, inactive SDF-1α(3-68) still desensitizes for SDF-1α-induced Ca^{2+} responses through CXCR4. In a report on the solution structure of SDF-1, synthetic SDF-1 variants, including SDF-1(3-67), have been used to investigate the influence of NH_2-terminal truncation on the biological activity of SDF-1 [35]. The data have led to a two-site model for SDF-1 binding to CXCR4. The two receptor binding sites are situated in SDF-1(1-17). The region SDF-1(12-17) is thought to initiate binding to CXCR4, and this docking step could facilitate access of the NH_2-terminal residues to a more buried receptor site. SDF-1(3-67) still interacts with CXCR4, but with lower affinity. The reduced binding affinity probably explains why SDF-1α(3-68) is able to desensitize for Ca^{2+} responses towards intact SDF-1α and also why we still observed, albeit reduced, antiviral activity against T-tropic strains in PBMC when SDF-1α was processed by CD26/DPP IV.

The findings that CD26/DPP IV is able to process the NH_2-terminus of both RANTES [13,14] and SDF-1α with differ-

ential effects on their anti-HIV-1 activity, brings novel insights into the role of CD26/DPP IV in HIV-1 infection. Although significantly lower numbers of $CD26^{+}$ memory cells have been detected in HIV-infected persons [36], which can be explained by the observation that CCR5 is expressed by $CD26^{high}$ T cells [37], CD26/DPP IV remains present in plasma [36]. Thus, during all stages of the HIV disease, significant amounts of active CD26/DPP IV are present. M-tropic CCR5 using HIV-1 strains predominate during the asymptomatic phase. Higher levels of CD26/DPP IV may therefore be beneficial during this initial stage by increasing the anti-HIV-1 potency of RANTES against M-tropic strains. However, at late stages of HIV-1 infection, when T-tropic viruses are more frequently isolated, CD26/DPP IV activity can promote infection with T-tropic viruses due to the reduced antiviral activity of truncated SDF-1(3-68). These findings may help to explain the conflicting results on the role of CD26/DPP IV in AIDS. However, further studies on other chemokines which may be processed by CD26/DPP IV (e.g. MIP-1β) are necessary to clarify this issue.

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