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CD26-processed RANTES(3–68), but not intact RANTES, has potent anti-HIV-1 activity

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Abstract

The natural CC-chemokine RANTES(3–68), missing two NH_2-terminal residues, has been isolated from leukocytes and tumor cells. The highly specific aminopeptidase dipeptidyl peptidase IV (DPP IV), also called CD26, was shown to be responsible for this NH_2-terminal truncation of RANTES. Here it is reported that CD26/DPP IV treatment of RANTES enhances its anti-HIV-1 activity. RANTES(3–68) inhibited infection of PBMC by M-tropic HIV-1 strains ten-fold more efficiently than intact RANTES. This difference in antiviral potency between intact and truncated RANTES was even more pronounced (at least 25-fold) in CCR5-transfected cell lines. In HOS.CD4.CCR5 transfected cells, RANTES(1–68) had virtually no anti-HIV-1 activity ($IC_{50} > 130$ nM), whereas RANTES(3–68) was a potent inhibitor of HIV-1 replication (IC_{50}: 5.5 nM). The anti-HIV-1 activity of RANTES(1–68) in the different cell types correlated with the expression of CD26. Moreover, the addition of soluble CD26 together with RANTES(1–68) significantly enhanced the antiviral activity of RANTES in HOS.CD4.CCR5 cells (IC_{50}: 13 nM). These observations point to an important role of CD26-mediated processing of RANTES in inhibiting the replication of CCR5-binding HIV strains in HIV-infected persons and in preventing the development of AIDS.

Keywords: CD26; RANTES; Anti-HIV-1 activity; CCR5

1. Introduction

Chemotactic cytokines or chemokines selectively activate leucocyte subpopulations by bind-

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ing to distinct receptors on their surface. Before its identity was known as a CXC-chemokine receptor, fusin, now called CXCR4, was shown as the main coreceptor used by T cell line-tropic (T-tropic) HIV-1 strains to enter the target cells (Berson et al., 1996; Feng et al., 1996), whereas the CC-chemokine receptor, CCR5, and, to a lesser extent CCR2b and CCR3 allow entry of macrophage-tropic (M-tropic) HIV-1 strains (Alkhatib et al., 1996; Choe et al., 1996; Deng et al., 1996; Doranz et al., 1996; Dragic et al., 1996). Stromal cell-derived factor-1α (SDF-1α), the natural ligand for CXCR4, has been shown to inhibit T-tropic (but not M-tropic) viruses and primary HIV isolates (Bleul et al., 1996; Oberlin et al., 1996), whereas the CC-chemokines interacting with CCR5 such as regulated on activation normal T cell expressed and secreted (RANTES), macrophage inflammatory protein (MIP)-1α and MIP-1β are inhibitors of M-tropic viruses (Cocchi et al., 1995). The importance of chemokine receptors for HIV entry and AIDS pathogenesis has been illustrated in numerous studies over the last 2 years. Moreover, CCR5-deficient individuals have been described which are relatively resistant to infection with HIV-1 (Dean et al., 1996; Liu et al., 1996; Samson et al., 1996; Quillent et al., 1998) and derivatives of the CC-chemokine RANTES have been described as CCR5 antagonists with activity against M-tropic HIV-1 strains (Arenzana-Seisdedos et al., 1996; Simmons et al., 1997). These observations have initiated worldwide efforts to develop CCR5 and/or CXCR4 antagonists.

A natural form of RANTES missing the first two amino acids i.e. RANTES(3–68), was isolated from stimulated sarcoma cells, fibroblasts and peripheral blood mononuclear cells (PBMC) (Struyf et al., 1998). The NH_2-terminal truncation of RANTES reduced its ability to mobilize intracellular Ca^{2+} in mononuclear cells. RANTES(3–68) failed to chemoattract monocytes and functioned as an inhibitor of monocyte chemotaxis (Proost et al., 1998a). However, RANTES(3–68) induced Ca^{2+} fluxes equally well as intact RANTES in CCR5 transfectant cells, but was ineffective on CCR1- and CCR3-transfectant cells (Struyf et al., 1998). The serine protease CD26/dipeptidyl peptidase IV (CD26/DPP IV) was shown to be able to cleave the first two amino acids from peptides such as RANTES containing a penultimate proline, alanine or hydroxyproline residue (Walter et al., 1980). Recently, two reports demonstrated that CD26 cleaves the two NH_2-terminal amino acids from granulocyte chemotactic protein 2 (GCP-2), interferon inducible protein 10 (IP-10), eotaxin, and RANTES, but not from the monocyte chemotactic proteins MCP-1, MCP-2 and MCP-3 (Oravecz et al., 1997; Proost et al., 1998a).

Here, we describe the potent antiviral activity of a natural form of RANTES(3–68), but not of intact RANTES, against two different M-tropic HIV-1 strains in phytohemagglutinin (PHA)-stimulated PBMC, and in two human cell lines transfected with CD4 and CCR5. The activity of RANTES(1–68) was directly correlated with the presence of CD26 expressed on the cell membrane of the target cells. Moreover, intact RANTES became antivirally active in CD26-negative CCR5-transfected cells following the addition of natural soluble CD26 (sCD26).

2. Materials and methods

2.1. Virus stocks, cell lines and cell cultures

The HIV-1 M-tropic strains BaL and SF162 were obtained through the MRC AIDS reagent project program (Herts, UK). CXCR1-, CCR1-, CCR3- or CCR5-transfected human embryonic kidney (HEK) 293 cells (Ben-Baruch et al., 1995) were kindly provided by Dr J.-M. Wang (NCI-FCRDC, Frederick, MD). The cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS; Gibco BRL/Life Technologies, Paisley, UK) and 800 μg/ml of geneticin. Human osteosarcoma cells that express human CD4 and the chemokine receptor CCR5 (HOS.CD4.CCR5; Connor et al., 1997) were obtained from the NIAID AIDS reagent program. Astroglioma U87.CD4 cells transfected with CCR5 (U87.CD4.CCR5; Deng et al., 1997) were kindly provided by Dr D.R. Littman (New York University Medical Center, NY). The transfectant cell lines were infected with 500 pg/ml of virus and the supernatant was collected 6–10 days after infec-

tion and stored at −20°C. Human monocytic THP-1 cells (ATCC, Rockville, MD) were grown in RPMI 1640 medium with 10% FBS.

PBMC from healthy donors were isolated by density gradient centrifugation (Blood transfusion centers of Antwerp and Leuven) and stimulated with PHA at 1 μg/ml (Sigma, Bornem, Belgium) for 3 days at 37°C. The activated cells (PHA-stimulated blasts) were washed three times with phosphate-buffered saline (PBS), and viral infections were done as described previously (Schols et al., 1997a,b). HIV-1-infected or mock-infected PHA-stimulated blasts were cultured in the presence of 25 U/ml of IL-2 and varying concentrations of the chemokines were added at the start of the experiment. The cell supernatant was collected at days 6 and 10, and HIV-1 core antigen in the culture supernatant was analyzed by the p24 Ag ELISA kit from DuPont (NEN Life Science Products, Brussels, Belgium).

2.2. Human CD26 and chemokines

Human CD26/DPP IV was obtained from prostasomes (prostate-derived organelles which occur freely in seminal plasma). The enzyme was purified to homogeneity using anion exchange followed by affinity chromatography onto immobilized adenosine deaminase (De Meester et al., 1996). sCD26, starting at amino acid Gly-31, was obtained from total seminal plasma and was characterized as described (Lambeir et al., 1997). The enzymatic activity was determined using the fluorogenic substrate Gly-Pro-4-methoxy-2-naphthylamide (De Meester et al., 1996). Truncated MIP-1α were produced by cultured human monocytic THP-1 cells or freshly isolated PBMC and purified as previously described (Proost et al., 1998b). The intact recombinant CC-chemokines, RANTES and MIP-1α were obtained from Peprotech (Rocky Hill, NJ) or R&D Systems (Europe Ltd, Abingdon, UK). ^{125}I-RANTES was purchased from Amersham Pharmacia Biotech (Rainham, UK). RANTES(3–68) was obtained by CD26/DPP IV treatment of intact RANTES followed by C-8 RP-HPLC purification (Proost et al., 1998a). The purity and concentration of the chemokines was determined by SDS-PAGE and automatic Edman degradation on a pulsed liquid phase 477A/120A protein sequencer (Perkin Elmer, Foster City, CA; Proost et al., 1998b).

2.3. Chemotaxis assay

Chemokines were tested for their chemotactic potency in the Boyden microchamber using THP-1 cells (Proost et al., 1998b). The cells that migrated through the 5 μm pore-size polycarbonate membranes were counted microscopically in ten oil immersion fields. The chemotactic index (C.I.) of a sample (triplicates in each chamber) was calculated as the number of cells that migrated to the test sample divided by the number of cells that migrated to control medium.

2.4. Calcium fluorimetry

Intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) were measured using the fluorescent indicator fura-2 as previously described (Wuyts et al., 1997). Fura-2 fluorescence was measured in an LS50B luminescence spectrophotometer (Perkin Elmer) and the $[Ca^{2+}]_i$ was calculated from the Grynkiewicz equation (Grynkiewicz et al., 1985). In order to determine R_{max}, the cells were lysed with 50 μM digitonin. Subsequently, the pH was adjusted to 8.5 with 20 mM Tris and R_{min} was obtained by addition of 10 mM EGTA to the lysed cells. The K_d used for calibration was 224 nM.

2.5. Flow cytometric analysis

The transfectant cell lines were first trypsinized, washed once in medium with 10% FBS and left at room temperature for 2 h before the start of the staining protocol. The cells were washed once in PBS and anti-CD26 mAb (clone BA5; Immunotech, Marseille, France) directly labeled with phycoerythrin (PE), was added for 30 min at 4°C. Then the cells were washed twice with PBS and fixed in 1% formaldehyde in PBS and analyzed by a FACScan (Becton Dickinson, San Jose, CA) flow cytometer.

2.6. Receptor binding assay

CCR1-, CCR3- and CCR5-transfected HEK293 cells were incubated for 2 h with 0.6 nM ^{125}I-RANTES with or without varying concentrations of intact RANTES or RANTES(3–68). Cells were centrifuged and washed three times with 2 ml of PBS supplemented with 2% (w/v) BSA and the radioactivity present on the HEK293 cells was counted in a γ counter. Total binding for CCR1-, CCR3- and CCR5-transfected HEK293 cells was 5300–6100, 4000–6100 and 12500–16500 cpm, respectively. Non-specific binding of ^{125}I-RANTES to CXCR1-transfected HEK293 cells was 2000–3000 cpm. Non-specific binding was subtracted from the total binding to obtain the specific binding.

3. Results

3.1. Intact RANTES and CD26-processed RANTES(3–68) compete in a different manner with ^{125}I-RANTES binding to CCR1, CCR3 and CCR5

In a previous study it was shown that natural RANTES(3–68) has impaired chemotactic activity compared to intact RANTES (Proost et al., 1998a). In order to verify altered receptor recognition, competition for binding to CCR1, CCR3 and CCR5 was verified. Intact recombinant RANTES competed with the binding of ^{125}I-RANTES to CCR1- and CCR3-transfected HEK293 cells from 0.13 nM onwards (Fig. 1). At least a ten-fold higher amount of CD26-truncated recombinant RANTES(3–68) was required to obtain a comparable competition. The IC_{50} for RANTES(1–68) was 1.1 nM as compared to 31 nM for RANTES(3–68) in the CCR1 transfected cells and in the CCR3 transfected cells the IC_{50} for RANTES(1–68) was 0.83 nM compared to greater than 38 nM for RANTES(3–68). On CCR5-transfected HEK293 cells, however, RANTES(3–68) (IC_{50}: 23 nM) competed more efficiently with ^{125}I-RANTES binding than intact RANTES (IC_{50}: 35 nM; Fig. 1). Thus, CD26/DPP IV-mediated processing of RANTES weakens RANTES(3–68) binding to CCR1 and CCR3 but significantly improves the interaction with CCR5.

3.2. CD26-specific truncation of RANTES is necessary for its antiviral activity

The effects of the different forms of RANTES were first evaluated against two different M-tropic HIV-1 strains (BaL and SF162) in human PBMC derived from healthy blood donors. The IC_{50} of the intact RANTES(1–68) against the BaL strain was 3.4 nM and for RANTES(3–68) the IC_{50} was 0.39 nM. Also the IC_{90} value for RANTES(1–68) was ten-fold higher than the IC_{90} value for RANTES(3–68). Against the SF162 strain when evaluated in PBMC, RANTES(1–68) was more than ten-fold less active than RANTES(3–68) (Table 1). The concentration-dependent effects of both chemokines at concentrations ranging from 130 down to 0.21 nM against HIV-1 SF162 replication in PBMC are shown in Fig. 2. A concentration of 5.2 nM RANTES(3–68) was clearly effective in reducing virus replication, whereas RANTES(1–68) was inactive at this concentration. No difference in antiviral activity was noticed between intact RANTES obtained from PeproTech or R&D Systems.

The striking difference in antiviral activity between the two forms of RANTES became even more apparent when tested in the human CCR5 transfected cells. In U87.CD4.CCR5 cells, the IC_{50} for RANTES(1–68) and RANTES(3–68) against the BaL strain was 21 and 0.65 nM, respectively. Also in Table 1, it is shown that RANTES(1–68) was virtually inactive in the HOS.CD4.CCR5 cells whereas RANTES(3–68) is a potent inhibitor of HIV-1 BaL replication in these cells (IC_{50}: 5.5 nM). However, no IC_{90} values were reached for both forms of RANTES in these cells.

3.3. The antiviral activity of RANTES is dependent on the presence of membrane bound or soluble CD26 (sCD26)

The CD26 expression on the two different

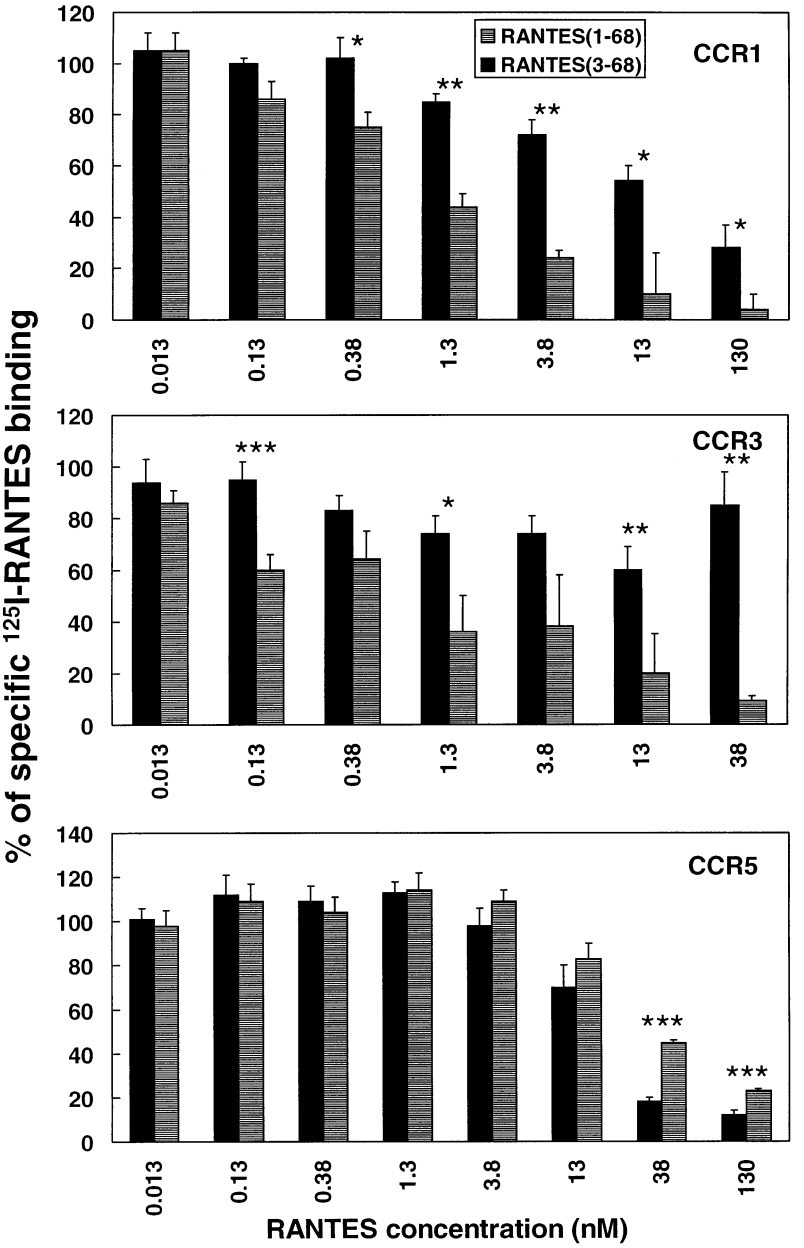

Fig. 1. The % of specific binding of ^{125}I-RANTES to CCR1-, CCR3- or CCR5-transfected HEK293 cells in the presence of varying concentrations of RANTES(1–68) or RANTES(3–68). The results are mean ± SEM of three to six independent experiments. Student's *t*-test: RANTES(1–68) compared to RANTES(3–68): $*P < 0.1$, $**P < 0.05$ and $***P < 0.01$.

CCR5-transfected cell lines and on freshly isolated PBMC was evaluated. HOS transfectants were negative for CD26 expression as determined by flow cytometric analysis, whereas U87 transfectants stained weakly, but significantly positive with the anti-CD26 mAb (Fig. 3). In addition, a subpopulation of freshly isolated PBMC was found to be strongly positive for CD26 expression (Fig. 3).

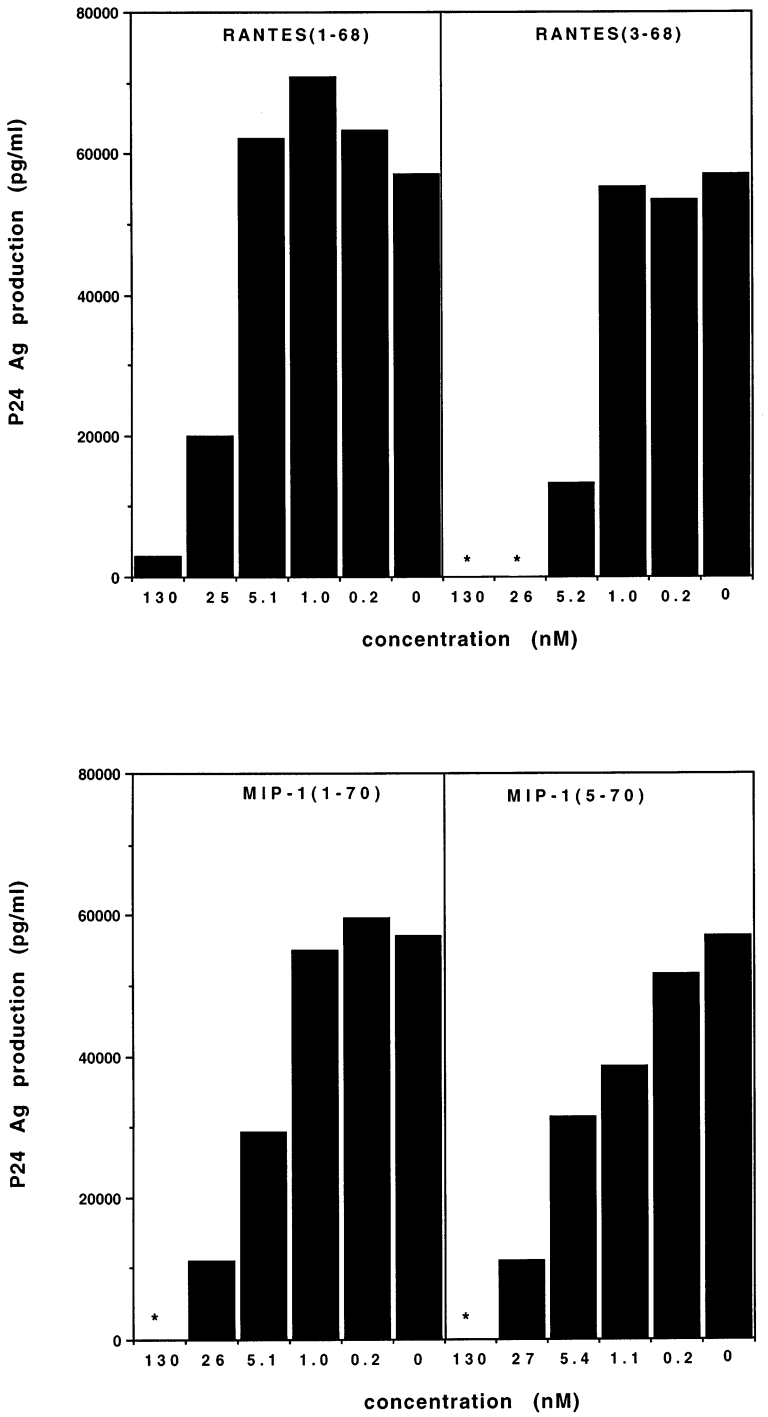

Fig. 2. Effects of RANTES and MIP-1α on infection by the HIV-1 SF162 strain in PHA-activated PBMC. Virus yields were monitored 10 days after infection by a p24 Ag ELISA on the cell supernatant. Results of a representative experiment out of three are shown. * Under the detection limit of the p24 Ag ELISA (< 5 pg/ml).

Table 1
Anti-HIV-1 activity of RANTES and MIP-1α in PHA-stimulated PBMC, U87.CD4.CCR5 cells and HOS.CD4.CCR5 cells

	RANTES(1–68)		RANTES(3–68)		MIP-1α(1–70)		MIP-1α(5–70)	
	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}
PBMC								
BaL	3.4	71	0.39	6.9	1.9	62	1.6	13
SF162	23	95	2.0	8.2	3.1	30	3.6	32
U87.CD4.CCR5								
BaL	21	>130	0.65	63	ND	ND	ND	ND
HOS.CD4.CCR5								
BaL	>130	>130	5.5	>130	32	>130	21	>130

Virus yield was monitored in the cell-free supernatant 8–12 days after infection by viral p24 Ag ELISA. The mean IC_{50}s and IC_{90}s (in nM) are shown. The data represent the means of two to four independent experiments. The value marked by '>130' indicates that 50 or 90% inhibition is not achieved at 130 nM. ND, not done.

The concentration-dependent effect of RANTES(1–68) and RANTES(3–68) on viral p24 Ag production by the BaL strain in HOS.CD4.CCR5 transfected cells in the presence of sCD26 is shown in Fig. 4. Addition of sCD26, together with RANTES(1–68) at the start of the HIV infection, significantly enhanced the antiviral activity of intact RANTES in HOS.CD4.CCR5 cells. When sCD26 at 50 U/l was added together with RANTES, an IC_{50} of 13 nM of RANTES was obtained. The addition of sCD26 alone had no effect on virus replication. The addition of sCD26 to RANTES(3–68) did also not change the antiviral activity of RANTES(3–68) (data not shown). Thus, the presence of CD26 is essential for intact RANTES to become antivirally active.

3.4. Amino terminal truncation of natural MIP-1α does not affect its anti-HIV-1, chemotactic and Ca^{2+}-mobilizing activity

Since the majority of natural MIP-1α is NH_2-terminally truncated (four amino acids), we investigated whether this truncated MIP-1α(5–70) had an altered HIV-1 inhibitory capacity. In contrast with the results obtained for RANTES, no significant differences were detected for the IC_{50} values of intact MIP-1α and MIP-1α(5–70) in PBMC or CCR5-transfected cells (Table 1 and Fig. 2). In addition, intact MIP-1α and truncated MIP-1α(5–70) were compared in chemotaxis and intracellular Ca^{2+}-mobilization assays on THP-1 monocytic cells. Table 2 demonstrates that the minimal effective dose of MIP-1α(5–70) inducing a rise in the $[Ca^{2+}]_i$ was only slightly lower than for intact MIP-1α. Furthermore, although maximal migration obtained with 0.13 nM in the chemotaxis assay was higher for intact MIP-1α, the minimal effective concentrations of both MIP-1α isoforms were rather similar. Taken together, it must be concluded that NH_2-terminal processing of MIP-1α in contrast to RANTES, only minimally weakens its inflammatory and anti-HIV-1 activity.

4. Discussion

Post-translational modifications such as proteolytic cleavage at the NH_2-terminus have been observed for both CXC- and CC-chemokines. Although this proteolytic process has not been unequivocally demonstrated at the molecular level, there is evidence that proteolysis increases chemotactic activity (CXC-chemokines such as IL-8 (Van Damme et al., 1990)) or decreases chemotactic activity (CC-chemokines such as MCP-2 (Proost et al., 1998b)). Recently, the CC-chemokines RANTES and eotaxin and the CXC-chemokines IP-10 and GCP-2 have been described as substrates for the serine protease CD26/DPP IV (Oravecz et al., 1997; Proost et al., 1998a).

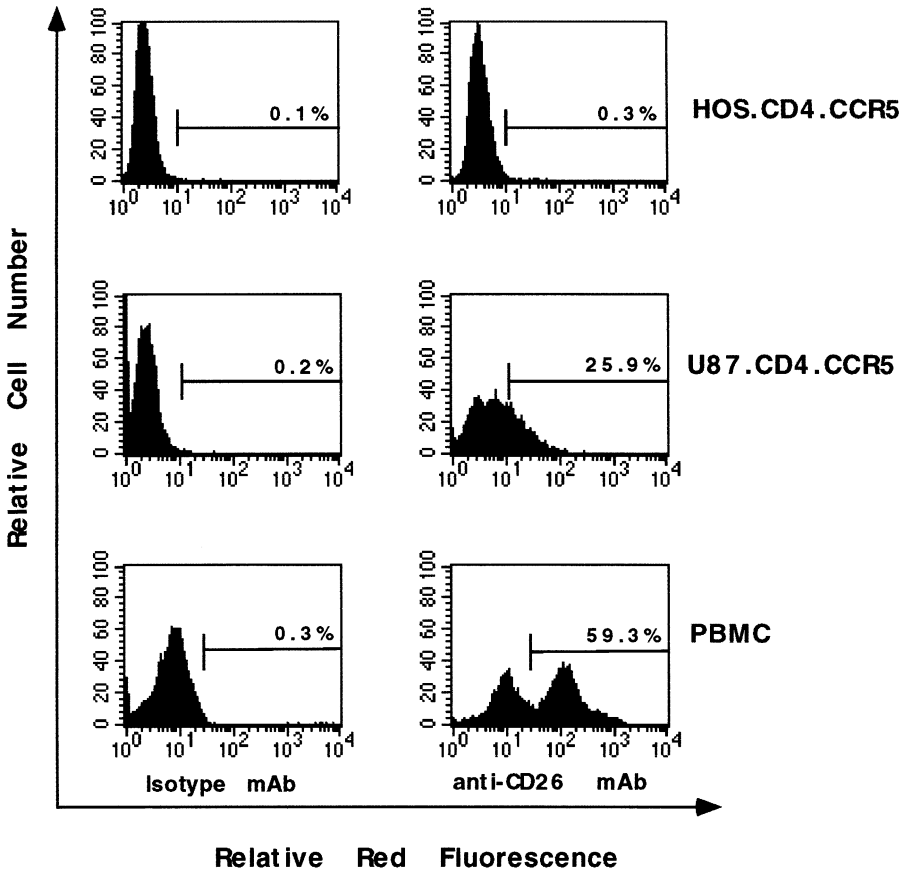

Fig. 3. Expression of CD26 on HOS.CD4.CCR5 cells, U87.CD4.CCR5 cells and freshly-isolated PBMC. The percentage (%) of CD26 positive cells is indicated in each histogram.

Although the consensus sequence for CD26/DPP IV-cleavage is present in the MCPs sequence, no CD26/DPP IV-processing of natural MCP-1, -2 and -3 was observed (Proost et al., 1998a). CD26/DPP IV-mediated truncated forms of RANTES and GCP-2, but not of MCPs were recovered in substantial amounts from natural sources, including peripheral blood leukocytes and tumor cell lines (Proost et al., 1998a; Struyf et al., 1998).

NH_2-terminal cleavage of RANTES at the penultimate position by CD26/DPP IV, resulting in the generation of RANTES(3–68), weakened its Ca^{2+}-mobilizing and chemotactic activity in monocytic cells (Proost et al., 1998a). Compared to intact RANTES, RANTES(3–68) was a weaker Ca^{2+}-mobilizing agent in CCR1- and CCR3-transfected cells, but RANTES(3–68) was equally active as RANTES(1–68) in CCR5-transfected cells (Struyf et al., 1998). More directly, binding studies with CCR1, CCR3 and CCR5 (Fig. 1) revealed that CD26/DPP IV-mediated cleavage of RANTES weakens the interaction with CCR1 and CCR3, but not with CCR5. In contrast to its Ca^{2+}-mobilizing effect, RANTES(3–68) showed even enhanced binding to CCR5. Thus, Pro at position two is not essential for CCR5-binding of RANTES but may be important to displace the NH_2-terminal dipeptide (Ser-Pro) and improve the interaction of the NH_2-terminal loop of RANTES (residues 12–20) with the CCR5 receptor. In contrast, a mutated form of RANTES with a Pro to Ala mutation at position two failed to show receptor binding capacity and signaling properties via CCR5, which

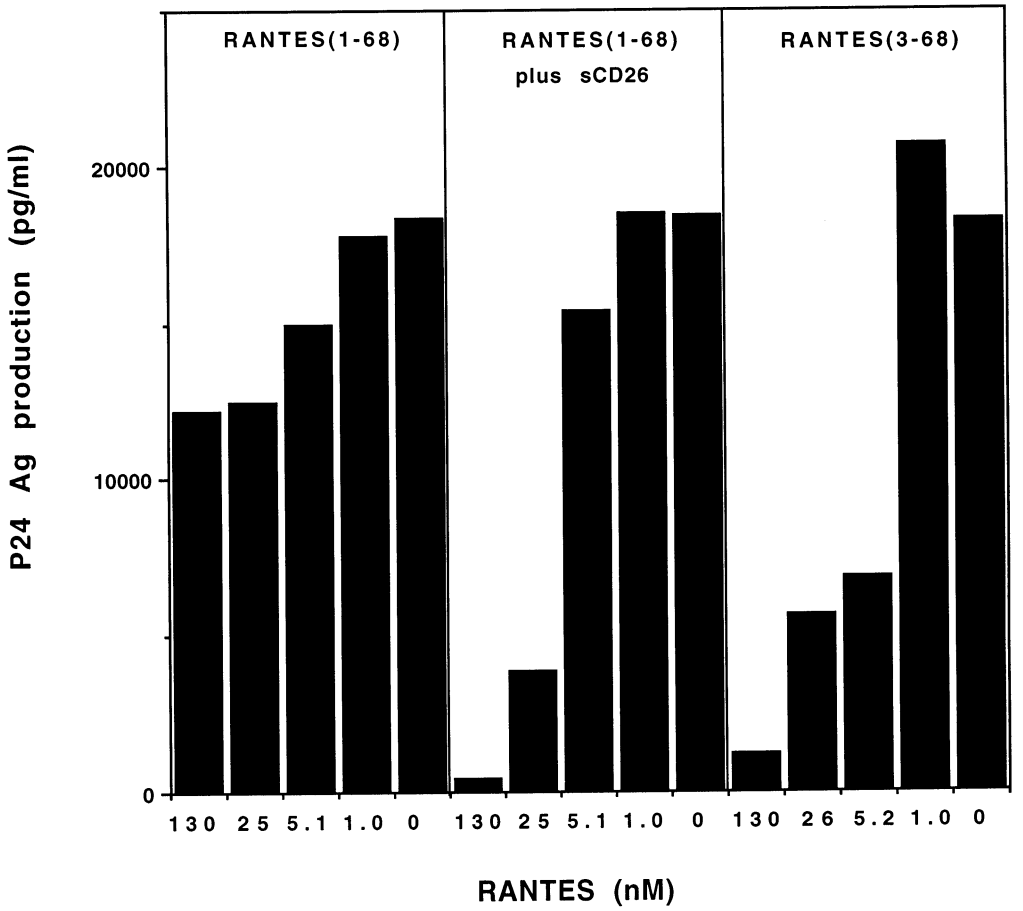

Fig. 4. Effects of RANTES(1–68), RANTES(1–68) plus sCD26 (50 U/l), and RANTES(3–68) on infection of HOS.CD4.CCR5 cells by the HIV-1 BaL strain. Virus yields were monitored in the cell supernatant 8 days after infection by a p24 Ag ELISA. Results of a representative experiment out of three are shown.

led to the speculation that Pro at position two is required for RANTES binding to CCR5 (Pakianathan et al., 1997).

Although the V3 loop of HIV-1 gp120 contains important determinants that are involved in the interaction with CCR5 (Trkola et al., 1996; Wu et al., 1996), the mechanism of blocking HIV infection by chemokines is not yet completely resolved. The gp120 of HIV-1 and the chemokine can be in direct competition for binding to the receptor or the coreceptor. Subsequent receptor internalization can contribute to the antiviral activity. Signal transduction via CCR5 is not required for CCR5 to act as a coreceptor for HIV-1 entry (Atchison et al., 1996; Gosling et al., 1997).

Previous studies with a panel of CCR5/CCR2 chimeras showed that the NH_2-terminus of CCR5 as well as the first, but not the second, extracellular loop of CCR5, was important for HIV-1 entry (Rucker et al., 1996). However, a specific anti-CCR5 mAb, which mapped to the second extracellular loop of CCR5, efficiently blocked the infectivity of M-tropic HIV-1 strains (Wu et al., 1997). Other studies with human/mouse CCR5 chimeras showed that the second extracellular loop was important (Bieniasz et al., 1997) or that multiple extracellular loops were involved (Atchison et al., 1996; Edinger et al., 1997; Picard et al., 1997). Thus, although it is important to understand the interaction between gp120 and

CCR5, especially for developing specific antagonists, results from several studies so far have not reached a consensus on the relative relevance of the various domains of CCR5. Recently, RANTES has been shown to interact mainly with the second extracellular loop of CCR5 (Samson et al., 1997).

From our earlier results it was not clear why intact RANTES was virtually inactive against the BaL strain in the HOS.CD4.CCR5 cell line. A possible explanation was the high level of CCR5 expression on these cells, resulting in incomplete inhibition of all CCR5 receptors by RANTES. However, RANTES(3–68) is very active against the BaL strain in the HOS.CD4.CCR5 cells, thus excluding the high-level receptor expression as an explanation for the inactivity of intact RANTES.

The present paper describes that CD26 expression on the cell membrane is correlated with the anti-HIV-1 activity of intact RANTES. RANTES(1–68) was inactive in HOS transfectants ($CD26^{negative}$), active in U87 transfectants ($CD26^{low}$) and most active in PBMC ($CD26^{high}$, a subpopulation of PBMC). Moreover, the CD26 enzymatic activity in the supernatant of the different cell types was low (<2 U/l; data not shown). The exogenously truncated RANTES(3–68) was active against M-tropic HIV-1 strains when added to these different cell types. CD26/DPP IV exists in soluble and membrane-expressed forms (Vanhoof et al., 1992; Tanaka et al., 1994). The final CD26/DPP IV activity in the cell cultures in these addition experiments (50 U/l) corresponds to the plasma DPP IV activity in healthy persons (Vanhoof et al., 1992). Thus, we showed that the addition of physiological amounts of sCD26 together with RANTES(1–68) at the time of infection made RANTES(1–68) antivirally active in the HOS.CD4.CCR5 ($CD26^{negative}$) cells.

Table 2
Lack of difference in biological potency between intact and truncated MIP-1α

Chemokine	Chemotaxis[a]		Increase in $[Ca^{2+}]_i$[b]	
	nM	C.I.	nM	nM
MIP-1α(1–70)	1.3	8.5 ± 3.3	3.9	240/195
	0.13	22.2 ± 4.4	0.39	120/130
	0.013	5.7 ± 1.6	0.039	30/14
	0.0013	4.0 ± 3.1		
MIP-1α(5–70)	1.3	14.2 ± 0.4	4.0	196/178
	0.13	11.4 ± 2.9	0.4	71/33
	0.013	3.8 ± 1.4	0.04	10/<10
	0.0013	2.1 ± 0.6		

[a] Migration of monocytic THP-1 cells through 5.0 μm pores in the microchamber. Results are mean $\pm$ SEM of three independent experiments.
[b] Detection of the $[Ca^{2+}]_i$ increase in THP-1 cells. Results of two independent experiments are shown.

This observation can also explain why RANTES, which is active against M-tropic viruses on PM1 cells (a T-cell line) and PBMC (Cocchi et al., 1995), has no or very low anti-HIV activity when evaluated in undifferentiated monocytes (Deng et al., 1996; Schmidtmayerova et al., 1996; Oravecz et al., 1997; Simmons et al., 1997). Indeed, monocytes do not express CD26 or only at very low levels, but start to express CD26 during maturation into macrophages (Naif et al., 1997). In fact, one report showed that RANTES has antiviral activity in macrophages (Verani et al., 1997). The absence or presence of CD26 can explain why intact RANTES is active against M-tropic strains in macrophage cultures (Verani et al., 1997), but not in monocyte cultures. PM1 cells also express high levels of CD26 and this expression correlated with infection of these cells by M-tropic HIV strains (Oravecz et al., 1995). Later on, it was shown that PM1 cells also express the CC-chemokine receptor CCR5 (Wu et al., 1997). CCR5 is expressed by $CD45RO^+$ memory T cells, most of which also expressed high levels of CD26 (Bleul et al., 1997; Wu et al., 1997). This explains why $CD45RO^+$ T cells as well as $CD26^+$ T cells are selectively lost during the first stage of HIV-1 infection (Schnittman et al., 1990; Vanham et al., 1993). As a consequence, it has been suggested that CD26 is a putative coreceptor for HIV (Callebaut et al., 1993).

In contrast to the striking difference in biological potency between intact RANTES and CD26-processed RANTES, intact MIP-1α and truncated MIP-1α had a comparable activity in three differ-

ent assays measuring calcium mobilization, chemotaxis and inhibition of HIV-1 infection, respectively. However, the protease(s) responsible for NH_2-terminal truncation of natural MIP-1α remain unknown. No cleavage site for CD26 is present in the primary MIP-1α sequence. The MIP-1α(5–70) described in this paper is identical to the natural form of MIP-1α isolated by Cocchi et al. (1995) as an inhibitor of HIV-1 infection. Our findings demonstrate that, in contrast to the situation with RANTES, limited NH_2-terminal truncation does not influence the anti-HIV-1 activity of MIP-1α. The MIP-1α data indicate that altered biological activity after cleavage of chemokines by aminopeptidases is not a generalized phenomenon. It can be inferred that CD26 cleavage is restricted to some chemokines and that alternative NH_2-terminal processing of other HIV-1 inhibitory CC-chemokines by different proteases does not necessary enhance the anti-HIV potency of the chemokines. In conclusion, CD26-mediated truncation of the CC-chemokine RANTES has important biological consequences: it antagonizes the inflammatory effects of intact RANTES (reduced activation and inhibition of chemotaxis of mononuclear cells), and it significantly enhances the activity of RANTES against M-tropic HIV-1 strains.

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