The serine protease CD26/dipeptidyl-peptidase IV (CD26/DPP IV) and chemokines are known key players in immunological processes. Surprisingly, CD26/DPP IV not only removes the expected Gly-Pro dipeptide from the NH₂ terminus of macrophage-derived chemokine (MDC) but subsequently also the Tyr₁-Gly₂ dipeptide, generating MDC(5–69). This second cleavage after a Gly residue demonstrated that the substrate specificity of this protease is less restricted than anticipated. The unusual processing of MDC by CD26/DPP IV was confirmed on the synthetic peptides GPYGANMED (MDC(1–9)) and YGANMED (MDC(3–9)). Compared with intact MDC(1–69), CD26/DPP IV-processed MDC(5–69) had reduced chemotactic activity on lymphocytes and monocyte-derived dendritic cells, showed impaired mobilization of intracellular Ca²⁺ through CC chemokine receptor 4 (CCR4), and was unable to desensitize for MDC-induced Ca²⁺-responses in CCR4 transfectants. However, MDC(5–69) remained equally chemotactic as intact MDC(1–69) on monocytes. In contrast to the reduced binding to lymphocytes and CCR4 transfectants, MDC(5–69) retained its binding properties to monocytes and its anti-HIV-1 activity. Thus, NH₂-terminal truncation of MDC by CD26/DPP IV has profound biological consequences and may be an important regulatory mechanism during the migration of Th2 lymphocytes and dendritic cells to germinal centers and to sites of inflammation.

CD26/dipeptidyl-peptidase IV (CD26/DPP IV) is a 110-kDa glycoprotein expressed on the membrane of a variety of cells including epithelial and endothelial cells. Moreover, its expression is up-regulated on activated T cells (1–3). The proteolytic activity of CD26/DPP IV is located in the extracellular domain of the protein, which also occurs in a soluble active form in plasma. CD26/DPP IV has a unique specificity compared with other exopeptidases. It is known to cleave dipeptides from the NH₂ terminus of peptides with a penultimate Pro, Hyp, or Ala residue. The penultimate NH₂-terminal Pro is present in a number of cytokines (e.g. interleukin-1β (IL-1β), IL-2, IL-5, IL-6, and IL-10), growth factors (e.g. insulin-like growth factor 1, granulocyte colony-stimulating factor, and growth hormone), neuro- and vasoactive peptides (e.g. neuropeptide Y, peptide YY, and substance P) and chemokines (e.g. stromal cell-derived factor-1α (SDF-1α) and RANTES). This Pro residue protects these molecules from degradation by most aminopeptidases. Short proline-containing peptides, including substance P and gastrin-releasing peptide have been known for some time as effective substrates for CD26/DPP IV (3). In contrast, none of the intact cytokines with a penultimate Pro has been identified as a CD26/DPP IV substrate although smaller peptides containing their NH₂-terminal sequences were cleaved (5).

A number of observations indicate that CD26/DPP IV plays an important role in immunology, in particular during T cell activation and proliferation (1, 2). The involvement of the enzymatic activity in the immunoregulatory function of CD26/DPP IV is demonstrated under several circumstances. These include the in vitro normalization of impaired responses to recall antigens by the addition of soluble CD26/DPP IV (6, 7) and the in vivo suppression of immune activation upon alloantigen challenge by specific CD26/DPP IV inhibitors (8). Recently, CD26/DPP IV has been shown to process the NH₂ terminus of a number of chemokines including RANTES, granulocyte chemotactic protein-2 (GCP-2), and SDF-1, generating naturally occurring truncated molecules with a significantly altered biological activity (9–12). Indeed, truncation of RANTES by CD26/DPP IV into RANTES(3–68) generated a chemotaxis antagonist with enhanced anti-HIV-1 activity against macrophage-tropic (M-tropic) HIV-1 strains. Incubation of SDF-1 with CD26/DPP IV drastically reduced the chemotactic activity of this chemokine but also reduced its anti-HIV-1 activity against T cell-tropic (T-tropic) HIV-1 strains.

The recently identified CC chemokine macrophage-derived chemokine (MDC), also designated stimulated T cell chemotactic protein-1 (STCP-1), binds to CC chemokine receptor 4 (CCR4). The MDC cDNA was one of the most abundant sequences identified in a macrophage library. MDC is synthe-
sized by macrophages and dendritic cells and is highly expressed in the thymus (13–16). NH2-terminally truncated forms of natural MDC were isolated from a CDS+T cell clone and have been reported to inhibit HIV-1 infection (17). Here, we show that MDC is a CD26/DPP IV substrate. Surprisingly, the enzyme removes an additional dipeptide from the MDC NH2 terminus proving that the substrate specificity of this dipeptidyl-peptidase is less restricted than anticipated. In addition, this double truncation affects the receptor interaction and chemotactic activity of MDC.

EXPERIMENTAL PROCEDURES

Reagents and Cells—Recombinant synthetic MDC and 123I-MDC were obtained from PeproTech (Rocky Hill, NJ), Gryphon Sciences (San Francisco, CA), and Amersham Pharmacia Biotech (Little Chalfont, UK), respectively. Synthetic NH2-terminal chemokine fragments (GPYGANMED or MDC(1–9), YGANMED or MDC(3–9), ANMED or MDC(5–9), and SPYSSDTTP or RANTES(1–9)) were synthesized using an automated PS3 solid phase peptide synthesizer (Rainin Instrument Company Inc., Woburn, MA) using Fmoc (N-(9-fluorenylmethoxycarbonyl) chemistry. The peptides were purified by HPLC and analyzed on an electrospray VG Quattro-II triple quadrupole mass detector (VG, Hertfordshire, UK). Recombinant CD26/DPP IV was obtained from Biosource (Camarillo, CA, USA) and was purified to homogeneity by anion exchange followed by affinity chromatography onto immobilized adenosine deaminase (18). Soluble CD26/DPP IV, without membrane anchor and starting at amino acid Gly31, was obtained from total seminal plasma and purified and characterized as described (19).

Fresh peripheral blood-derived mononuclear cells (PBMC) were obtained from healthy donors and isolated by hydroxyethyl starch sedimentation and Ficol-sodium metrizoate centrifugation (20). Monocytes were separated from the mononuclear cell fraction with anti-CD14 antibodies coupled to magnetic microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). Monocyte-derived dendritic cells were purified as described previously (13). Lymphocytic SUP-T1 cells were cultured in RPMI 1640 (BioWhittaker, Walkersville, MD) containing 1 mM EDTA. At indicated time points, 5–106 cells were washed three times with buffer.

Chemokine and Peptide Degradation with CD26/DPP IV—MDC was treated with membrane-bound or soluble CD26/DPP IV and purified by HPLC as described previously (9, 11). NH2-terminal truncation was verified after electrophoretic or HPLC on 0.5–2 μg of MDC by Edman degradation on a 477A/120A protein sequencer (Perkin-Elmer).

Degradation of the small synthetic MDC peptides was analyzed by fluorescamine derivatization. The synthetic peptides (1 μM) were incubated at 37 °C with CD26/DPP IV in phosphate-buffered saline, pH 8.0, containing 1 mM EDTA. At indicated time points, 5–μl samples were diluted in 0.5 ml of 100 mM sodium-borate buffer, pH 8.5. Immediately thereafter, fluorescamine (3.4 mg/ml) was added in dry acetone. The mixture was vortex mixed. Fluorescence measurements were performed on a model RF5000 fluorimeter (Shimadzu, Tokyo, Japan) at excitation and emission wavelengths of 390 and 475 nm, respectively. Linearity of the fluorescence toward the concentration was verified with Phe as a standard and with intact substrates and peptides.

Alternatively, peptide degradation was analyzed by HPLC. The peptides (5 μM) were incubated at 37 °C with CD26/DPP IV in 100 mM Tris-HCl, pH 7.5, containing 1 mM EDTA. At indicated time points, 40-μl samples were taken and diluted in 120 μl of 0.1% trifluoroacetic acid in water. Samples were applied to an Ultrasphere ODS column (4.6 × 250 mm, 5 μm, Beckman, Fullerton, CA) and the peptides were eluted in a linear acetonitrile gradient and monitored at 214 nm. Disappearance curves of MDC analogues were constructed from the integrated peak areas versus time.

To compare the relative affinities of CD26/DPP IV for the various peptides and the synthetic substrate Gly-Pro-p-nitroanilide, IC50 values were determined. CD26/DPP IV enzymatic activity was measured at 37 °C using Gly-Pro-p-nitroanilide as a chromogenic substrate. The reaction was monitored at 405 nm, and the initial rate was determined between 0 and 0.2 absorbance units. The reaction mixture contained 0.5 mM substrate, approximately 1 milliunit of CD26/DPP IV activity, 40 mM Tris-HCl buffer, pH 8.3, and test compounds at final concentrations between 0 and 1.5 mM. Measurements were performed in duplicate.

The IC50 value is defined as the concentration of test peptide required to reduce CD26/DPP IV activity to 50% of the control. To verify the specificity of the observed reaction, the enzyme was inactivated by the specific irreversible CD26 inhibitor Pro-Pro-diphenylphosphonate (22) before the start of the experiment.

Detection of Chemotactic Activity, Intracellular Ca2+ Concentrations and Competition for MDC Binding—The chemotactic activity of chemokines for lymphocytic SUP-T1 cells, monocytes, or monocyte-derived dendritic cells was determined by Boyden chemotaxis chambers as described previously (11, 13). Briefly, SUP-T1 cells (5 × 106 cells/ml) were allowed to migrate at 37 °C for 4 h through 5-μm pore-size fbronectin-coated polycarbonate filters (11). With monocytes and monocyte-derived dendritic cells (106 cells/ml), chemotaxis through polyvinylpyrrolidone-treated polycarbonate filters was stopped after 2 h (13). Filters were removed, cells were fixed and stained and counted microscopically. Results are expressed as chemotactic index corresponding to the number of cells migrated to the sample over the number of cells that migrated to control medium.

The intracellular Ca2+ concentrations ([Ca2+]i) were determined spectrofluorometrically using the fluorescent dye fura-2 (11) and were calculated from the Gryniewicz equation (23). For desensitization experiments, cells were stimulated first with intact or CD26/DPP IV-truncated MDC and 100 s later with intact MDC at a concentration (3 nM) that induced a significant increase in [Ca2+]i, after pretreatment with buffer.

Competitive binding was measured on purified PBMC, monocytes (>95% pure) or lymphocytes (>95% lymphocytes) or CCR4-transfected cells as described (24). Briefly, five (other cell types) million cells were incubated for 2 h at 4 °C with 0.06 nm 123I-MDC and varying concentrations of unlabeled chemokine. Cells were centrifuged and washed three times with 2 ml of phosphate-buffered saline supplemented with 2% (w/v) bovine serum albumin, and the radioactivity present on the cells was measured in a gamma counter.

Detection of Anti viral Activity—Purified PBMC from healthy donors were stimulated with phytohemagglutinin in 1 μg/ml for 3 days before infection with the T-tropic HIV-1 strain NL4.3 (obtained through the National Institute of Allergy and Infectious Diseases, AIDS reagent program, Bethesda, MD). The activated cells were washed three times with phosphate-buffered saline to remove nonadsorbed virus and varying concentrations of intact or CD26/DPP IV-truncated MDC were added (11). Cells were cultured in the presence of IL-2 (25 units/ml), and after 8 days, cell supernatants were collected. HIV-1 core antigen was analyzed by a p24 antigen enzyme-linked immunosorbent assay kit (NEN Life Science Products).

RESULTS

Proteolytic Cleavage of MDC by CD26/DPP IV—Recombinant and synthetic MDC(1–69) were treated with purified CD26/DPP IV. Incubation of synthetic MDC(1–69) with natural intact membrane-bound CD26/DPP IV for 48 h resulted in the removal of four NH2-terminal residues (Gly-Pro-Tyr-Gly) yielding MDC(5–69) (Table I). This finding was confirmed with recombinant MDC(1–69) incubated with the soluble form of the protease. However, a small amount of MDC(3–69) was also recovered. Prolonged treatment of MDC(3–69) with either membrane-bound or soluble CD26/DPP IV also resulted in complete conversion into MDC(5–69). Specific inactivation of CD26 with Pro-Pro-diphenylphosphonate prevented the formation of MDC(5–69) leaving ±85% of the MDC(3–69) intact (Table I).

To investigate whether the nature of the amino acids in the immediate proximity of the scissile bond exerts an important influence on the cleavage, the NH2-terminal peptides MDC(1–9), MDC(3–9), and RANTES(1–9) were synthesized. The inference of these peptides with the hydrolysis of a commonly used chromogenic CD26/DPP IV substrate, i.e. Gly-Pro-p-nitroanilide, was determined, and the IC50 values of competition between this and the peptide-substrate were calculated. RANTES(1–9) competed most efficiently (IC50: 0.15 ± 0.02 mM) for cleavage of Gly-Pro-p-nitroanilide, compared with MDC(1–9) (IC50: 0.94 ± 0.09 mM). In addition, MDC(3–9) (IC50: > 1.5 mM) was a less efficient competitor for CD26/DPP IV cleavage compared with MDC(1–9).

Peptide hydrolysis by purified CD26/DPP IV was also monitored by fluorescamine derivatization of free NH2 termini of
the peptides resulting from incubation with CD26/DPP IV. The NH₂-terminal nonapeptides of RANTES and MDC were efficiently cleaved by CD26/DPP IV into RANTES(3–9) and MDC(5–9), respectively. To prove that CD26/DPP IV is responsible for the observed truncations of the nonapeptides, EDTA was included in all incubation buffers to inhibit metallopeptidases. In a control experiment in the presence of the CD26 specific inhibitor Pro-Pro-diphenylphosphonate, the hydrolysis of MDC(1–9) was prevented. CD26/DPP IV was found to metabolize RANTES(1–9) and MDC(1–9) more quickly, generating more free NH₂ termini during the same time interval, compared with MDC(3–9) (results not shown). To obtain more information on the difference in kinetics between both cleavage steps at the NH₂ terminus, MDC(1–9) and MDC(3–9) were incubated with CD26/DPP IV and the resulting peptides, collected at different time points, were separated by HPLC. Under the experimental conditions used, almost all MDC(1–9) was cleaved within 30 min. Most of the generated MDC(3–9) is further converted into MDC(5–9) within the following hours (Fig. 1A). Using the same incubation conditions and starting with MDC(3–9), 50% of this peptide is processed after about 100 min (Fig. 1B). In addition, 2 μg of MDC(1–9) and MDC(3–69) were incubated with CD26/DPP IV at 37 °C for 1 h, blotted on polyvinylidene difluoride membranes, and the relative amounts of the different forms of MDC were determined by Edman degradation (Table I). The result of this short term incubation indicates that both dipeptides are efficiently and sequen- tially removed from the MDC NH₂ terminus and that incubation indicates that both dipeptides are efficiently and Edman degradation (Table I). The result of this short term amounts of the different forms of MDC were determined by.

### Table I

<table>
<thead>
<tr>
<th>MDC Source</th>
<th>Incubation</th>
<th>Sequence</th>
<th>Amount of Protein</th>
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<tr>
<td>syntMDC(1–69)</td>
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<td>100</td>
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<td>YGAMED ...</td>
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<td>ANMED ...</td>
<td>94</td>
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<td>YGAMED ...</td>
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<td>ANMED ...</td>
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*Synthetic (synt) MDC or recombinant (rec) MDC.

**Amount of protein in percentage of the by sequence analysis-detected proteins.**

**Incubation with membrane-bound CD26/DPP IV of which the biological activity was inhibited with the selective inhibitor Pro-Pro-diphenylphosphonate. Remaining free inhibitor was removed by gel filtration before the incubation.

**Incubation with membrane bound CD26/DPP IV without inhibitor but including the gel filtration step (positive control).**

The peptides resulting from incubation with CD26/DPP IV. The NH₂-terminal nonapeptides of RANTES and MDC were efficiently cleaved by CD26/DPP IV into RANTES(3–9) and MDC(5–9), respectively. To prove that CD26/DPP IV is responsible for the observed truncations of the nonapeptides, EDTA was included in all incubation buffers to inhibit metallopeptidases. In a control experiment in the presence of the CD26 specific inhibitor Pro-Pro-diphenylphosphonate, the hydrolysis of MDC(1–9) was prevented. CD26/DPP IV was found to metabolize RANTES(1–9) and MDC(1–9) more quickly, generating more free NH₂ termini during the same time interval, compared with MDC(3–9) (results not shown). To obtain more information on the difference in kinetics between both cleavage steps at the NH₂ terminus, MDC(1–9) and MDC(3–9) were incubated with CD26/DPP IV and the resulting peptides, collected at different time points, were separated by HPLC. Under the experimental conditions used, almost all MDC(1–9) was cleaved within 30 min. Most of the generated MDC(3–9) is further converted into MDC(5–9) within the following hours (Fig. 1A). Using the same incubation conditions and starting with MDC(3–9), 50% of this peptide is processed after about 100 min (Fig. 1B). In addition, 2 μg of MDC(1–9) and MDC(3–69) were incubated with CD26/DPP IV at 37 °C for 1 h, blotted on polyvinylidene difluoride membranes, and the relative amounts of the different forms of MDC were determined by Edman degradation (Table I). The result of this short term incubation indicates that both dipeptides are efficiently and sequentially removed from the MDC NH₂ terminus and that the first truncation, resulting in MDC(3–69), is more rapid compared with the second cleavage.

**Comparison of the Chemotactic Activity of Intact and CD26/DPP IV-cleaved MDC—**Intact MDC was incubated at 37 °C for 1, 24, or 48 h with natural soluble (s) or membrane-bound (m) CD26/DPP IV purified to homogeneity. Detected NH₂-terminal sequences after purification by HPLC or after electrophoretic transfer to nitrocellulose or polyvinylidene difluoride membranes, and the relative amounts of the different forms of MDC were determined by HPLC at different time intervals. Fast cleavage of MDC(1–9) into RANTES(3–9) and MDC(5–9) (Fig. 1A) was followed by slower conversion of MDC(3–9) into RANTES(3–9) (Fig. 1B). The y-axis depicts the integrated peak areas (as a value for the amount of peptide) and the x-axis the incubation time. Linear analysis based on first order kinetics of the disappearance of MDC(1–9) (panel A) and MDC(3–9) (panel B) reveals a half-life of 8.6 min and 108 min, respectively.

**Signaling and Receptor Binding Properties of Intact and Truncated MDC—**To explain the altered chemotactic responses, it was verified whether MDC signaling through and binding to CCR4, the only known MDC receptor, was affected by treatment of MDC with CD26/DPP IV. Indeed, intact MDC(1–69) dose-dependently induced a [Ca²⁺] rise in CCR4-transfected HOS cells (minimal effective concentration of 0.3 nM), whereas MDC(5–69) remained inactive at concentrations up to 10 nM.

**MDC(5–69) Inhibit Infection of PBMC with HIV-1—**Intact MDC has been reported to have antiviral activity...

**Fig. 1. Kinetics of the NH₂-terminal truncation of MDC(1–9) and MDC(3–9) by CD26/DPP IV.** MDC(1–9) (panel A) and MDC(3–9) (panel B) were incubated with CD26/DPP IV, and cleavage was monitored using HPLC at different time intervals. Fast cleavage of MDC(1–9) (○) to MDC(3–9) (□) was followed by slower conversion of MDC(3–9) to MDC(5–9) (×). The y-axis depicts the integrated peak areas (as a value for the amount of peptide) and the x-axis the incubation time. Linear analysis based on first order kinetics of the disappearance of MDC(1–9) (panel A) and MDC(3–9) (panel B) reveals a half-life of 8.6 min and 108 min, respectively.
against T- and M-tropic HIV-1 strains (17). CD26/DPP IV-truncated MDC and intact MDC had comparable anti-HIV-1 activity against infection of PBMC with the T-tropic strain NL4.3 (Fig. 5). Although 50% inhibition of HIV-1 infection was not obtained, the inhibition was significant in all four experiments (11 to 28% at 50 nM of intact or CD26/DPP IV-truncated MDC).

**DISCUSSION**

CD26/DPP IV is involved in the in vivo metabolism of a number of relatively small natural peptides containing a penultimate Pro (e.g. pancreatic polypeptide family) or Ala (e.g. secretins) (4, 25). Only recently, and in contrast to a number of cytokines, which have a penultimate Pro at their NH₂ terminus, some chemokines have been identified as selective substrates for this protease (9–12). Other chemokines, i.e. the monocyte chemotactic protein-1, -2, and -3 were found to be protected from CD26/DPP IV-processing by an NH₂-terminal pyroglutamic acid (9, 26). Based on the MDC sequence, containing a penultimate Pro, and on the observation that different natural NH₂-terminally truncated MDC forms have been isolated (17), the CC chemokine MDC was investigated as a
candidate substrate for CD26/DPP IV.

Recombinant and synthetic MDC(1–69) were treated with the purified CD26/DPP IV to verify whether MDC was cleaved after the penultimate Pro as previously shown for the chemokines RANTES, GCP-2 and SDF-1α (9–12). It was observed that incubation of MDC(1–69) with CD26/DPP IV resulted in the unexpected removal of four NH2-terminal residues (Gly-Pro-Tyr-Gly) yielding MDC(5–69) instead of the predicted MDC(3–69). A small amount of MDC(3–69) was also recovered indicating that this form is generated as an intermediate. The conversion of MDC(3–69) into MDC(5–69) was prevented by the specific CD26/DPP IV inhibitor Pro-Pro-diphenylphosphonate. This confirmed that the generation of MDC(5–69) was specific for CD26/DPP IV and that the cleavage involved the sequential removal of the two NH2-terminal dipeptides Gly2-Pro3 and Tyr9-Gly4. The efficient cleavage after Gly4 is unexpected based on previous reports on the specificity of this peptidase (1–4, 27). Interference by other aminopeptidases in the purified natural membrane-bound or soluble CD26/DPP IV preparations was found to be unlikely, because no further truncation of RANTES, GCP-2, or SDF-1α was obtained with the same CD26/DPP IV preparations using identical incubation conditions (9, 11). Investigation of the kinetics of the cleavage of intact MDC and of NH2-terminal MDC-fragments by CD26/DPP IV confirmed that both dipeptides were subsequently removed and that the first hydrolysis proceeds more rapidly compared with the second one.

To our knowledge, this is the first report on the CD26/DPP IV-mediated release of an NH2-terminal Xaa-Gly dipeptide from a natural peptide. A study by Bongers et al. (27) on growth hormone-releasing hormone and synthetic analogs revealed that the introduction of a Gly or Ser as the penultimate residue still allowed degradation by CD26/DPP IV although at a slower rate compared with the natural hormone containing a penultimate Ala. In contrast, the synthetic glucagon-like peptide 2 analog with an Ala to Gly substitution at position 2 was resistant to cleavage by CD26/DPP IV (28). Along the same line, we did not observe the generation of RANTES(5–68) by CD26/DPP IV, which means that Tyr9-Ser24 is not released from RANTES(3–68), although the same dipeptide was released from the synthetic growth hormone-releasing hormone analog with a penultimate Ser (27). It was observed for other peptides that the less the residue subject to cleavage is preferred, the more the extended substrate recognition influences the hydrolysis. The nature of the surrounding amino acids clearly influences the efficiency with which CD26/DPP IV removes dipeptides from substrates with an unusual penultimate residue (29).

The CC chemokine MDC is chemotactic for Th2 lymphocytes, monocytes, dendritic cells, and natural killer cells (13, 15, 30). Both intact MDC and MDC(5–69) were equally potent monocyte chemotactic proteins. In contrast, cleavage of MDC into MDC(5–69) resulted in reduced chemotactic activity on lymphocytic and dendritic cells. A similar reduction of the chemotactic activity has been observed for the chemokines RANTES and SDF-1α, whereas CD26/DPP IV processing had no effect on the chemotactic activity of GCP-2. Removal of the Xaa-Pro dipeptide from the NH2 terminus of SDF-1α and RANTES caused the loss of chemotactic activity on lymphocytes (11, 12) and monocytes or eosinophils (31), respectively. The reduced binding of MDC(5–69) to CCR4 and the reduced signaling of CD26/DPP IV-truncated MDC through this receptor explained the lower chemotactic activity on lymphocytes and dendritic cells. Moreover, MDC(5–69) did not desensitize for a subsequent [Ca2+]i increase induced by the intact chemokine. In concert with the chemotaxis data on monocytes, CD26/DPP IV processing had no influence on the binding properties of MDC to monocytes. The weak antiviral activity of MDC against HIV-1 infection of PBMC with a T-tropic strain also remained essentially unaltered. In this respect, it is interesting to note that MDC(5–69) and MDC(3–69) (the intermediate reaction product of MDC cleavage by CD26/DPP IV) are the two most abundant MDC forms that were purified from CD8+ T cells based on their antiviral activity against the T-tropic HIV-1 strain IIIB (17). However, our results with the CD26/DPP IV-truncated MDC indicate that NH2-terminal truncation of MDC cannot explain the conflicting results between several laboratories concerning the absence or presence of anti-HIV-1 activity for this chemokine. For SDF-1α(3–68), binding to and signaling through its receptor were also strongly reduced. However, this SDF-1α(3–68), in contrast to MDC, lost most of its antiviral activity against T-tropic HIV-1 strains (11, 12, 32).

In conclusion, MDC is a CD26/DPP IV substrate with an unexpected second cleavage site resulting in the generation of MDC(3–69) and MDC(5–69). Processing of MDC by this dipeptidyl-peptidase results in reduced biological activity on lymphocytes and dendritic cells, but not on monocytes. Thus, in addition to the regulation of chemokine and chemokine receptor expression by endogenous and exogenous immunomodulation, the NH2-terminal processing of MDC by CD26/DPP IV may have an important down-regulatory function in Th2 lymphocyte and dendritic cell trafficking without affecting its monocyte chemotactic and antiviral activity.

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Truncation of Macrophage-derived Chemokine by CD26/ Dipeptidyl-Peptidase IV beyond Its Predicted Cleavage Site Affects Chemotactic Activity and CC Chemokine Receptor 4 Interaction

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