CD26/Dipeptidyl-Peptidase IV Down-Regulates the Eosinophil Chemotactic Potency, But Not the Anti-HIV Activity of Human Eotaxin by Affecting Its Interaction with CC Chemokine Receptor 3

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CD26/Dipeptidyl-Peptidase IV Down-Regulates the Eosinophil Chemotactic Potency, But Not the Anti-HIV Activity of Human Eotaxin by Affecting Its Interaction with CC Chemokine Receptor 3

Sofie Struyf,* Paul Proost,* Dominique Schols,† Erik De Clercq,‡ Ghislain Opdenakker,* Jean-Pierre Lenaerts,* Michel Detheux,§ Marc Parmentier,§ Ingrid De Meester,¶ Simon Scharpé,¶ and Jo Van Damme²*

Chemokines attract and activate distinct sets of leukocytes. The CC chemokine eotaxin has been characterized as an important mediator in allergic reactions because it selectively attracts eosinophils, Th₂ lymphocytes, and basophils. Human eotaxin has a penultimate proline, indicating that it might be a substrate for dipeptidyl-peptidase IV (CD26/DPP IV). In this study we demonstrate that eotaxin is efficiently cleaved by CD26/DPP IV and that the NH₂-terminal truncation affects its biological activity. CD26/DPP IV-truncated eotaxin(3–74) showed reduced chemotactic activity for eosinophils and impaired binding and signaling properties through the CC chemokine receptor 3. Moreover, eotaxin(3–74) desensitized calcium signaling and inhibited chemotaxis toward intact eotaxin. In addition, HIV-2 infection of CC chemokine receptor 3-transfected cells was inhibited to a similar extent by eotaxin and eotaxin(3–74). Thus, CD26/DPP IV differently regulates the chemotactic and antiviral properties of eotaxin by the removal of two NH₂-terminal residues. This physiological processing may be an important down-regulatory mechanism, limiting eotaxin-mediated inflammatory responses. The Journal of Immunology, 1999, 162: 4903–4909.

Chemokines are structurally related, low m.w. proteins that attract and activate subsets of leukocytes. In addition to guiding cells to sites of inflammation, these cytokines are also involved in hemopoiesis, angiogenesis, and homing of immunocompetent cells (reviewed in Refs. 1–4). Their primary structure is characterized by the presence of four conserved cysteine residues that form disulfide bridges essential for chemotactic activity. In the CC chemokine subfamily the first two cysteines are adjacent, whereas in the CXC branch these residues are separated by a single amino acid. Fractalkine is a distinct chemokine type containing a transmembrane domain and a transmembrane domain and a chemokine domain typified by a CX3C motif atop an extracellular mucin stalk. Lymphotactin is a fourth chemokine type, in which only two of the four conserved cysteines are present. Different chemokine classes tend to exhibit different ranges of leukocyte specificity. CXC chemokines predominantly target neutrophils and, to a lesser extent, lymphocytes. CC chemokines mainly attract monocytes, but also lymphocytes, basophils, eosinophils, dendritic cells, and NK cells. These leukocyte subtypes differentially express chemokine receptors, which are G-protein-coupled receptors with seven transmembrane domains. The finding that chemokine receptors act as cofactors for HIV-1 entry into CD4⁺ cells and that their ligands can suppress HIV replication has intensified the interest in these proteins (5–7). The CC chemokine receptor 5 (CCR5),¹ which binds macrophage inflammatory protein-1α, macrophage inflammatory protein-1β, monocyte chemotactic protein-2 (MCP-2), and RANTES, and the CXC chemokine receptor 4 (CXCR4), which recognizes stromal cell-derived factor-1 (SDF-1) are considered to be the major HIV-1 coreceptors. During the first asymptomatic phase of infection, M-tropic strains preferring CCR5 are isolated, while in the later stages of disease, T-tropic viruses emerge. Intermediately the virus expands its coreceptor repertoire to include CCR2b, CCR3, and eventually CXCR4 when the disease progresses (8, 9). Eotaxin can block infection of PBMC by this intermediary viruses (10, 11). The eotaxin receptor (CCR3) has also been implicated in the infection of the central nervous system by HIV-1 (12). In addition, CCR3 is used as coreceptor by some primary HIV-2 isolates, which seem to use a broader range of coreceptor molecules (13).

Allergic reactions are characterized by the accumulation of an abnormally high number of eosinophils at the site of inflammation. One of the responsible, locally produced eosinophil chemoattractants was identified as a new chemokine, named eotaxin (14, 15). This CC chemokine is most closely related to the MCPs, having 66–75% amino acids identical with MCP-1, -2, -3, and -4 (16).

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Abbreviations used in this paper: CCR, CC chemokine receptor; MCP, monocyte chemotactic protein; CXCR4, CXC chemokine receptor 4; SDF, stromal cell-derived factor; DPP IV, dipeptidyl-peptidase IV; TFA, trifluoroacetic acid; RP-HPLC, reverse phase high performance liquid chromatography; [Ca²⁺], intracellular calcium concentration; GCP-2, granulocyte chemotactic protein-2; CI, chemotactic index.

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Based on this high sequence homology, eotaxin and the MCPs form a separate branch in the CC chemokine subfamily. More recently, eotaxin-2, a rather distantly related chemokine (only 40% homology to eotaxin), also named myocardial progenitor inhibitor-factor-2, has been cloned (17–19). All members of the MCP subfamily recognize CCR3, which is the unique receptor for eotaxin and eotaxin-2. The other CCR3 ligands, however, recognize additional CCRs, thus broadening their spectrum of target cells. Indeed, eotaxin is only chemotactic for eosinophils, basophils, and Th2 lymphocytes, expressing CCR3 (20–24). In contrast, MCP-3 binds to CCR1, CCR2, CCR3, and CCR10 and is the most pluri-potent chemokine, acting on most leukocytic cell types. The CC chemokine RANTES, which also binds to CCR3, is functionally related to eotaxin, since both chemokines are reported to be involved in allergic reactions. However, RANTES additionally signals through CCR1 and CCR5, which is preferentially expressed on Th1, but not on Th2 cells (24).

Recently, RANTES was characterized as a substrate for the T cell activation Ag CD26/dipeptidyl-peptidase IV (DPP IV) (25, 26). This membrane-bound protease cleaves dipeptides from peptides with a penultimate proline residue, hydroxyproline, or alanine residue (27–29). The truncation of RANTES by CD26 has far-reaching consequences, since it reduces its chemotactic potency, but increases its HIV-1-inhibiting capacity. In this study we verified whether eotaxin, which possesses a penultimate proline residue, is cleaved by CD26/DPP IV and whether truncation affects the biological activity of eotaxin. We investigated the influence of NH2-terminal truncation on the interaction of eotaxin with CCR3 (binding and signaling), its chemotactic activity for eosinophils, and its HIV-suppressive capacity.

Materials and Methods

Cell cultures, chemokines, and CD26/DPP IV

The coding sequence of human CCR3 was inserted into a bicistronic expression vector, and a transfected cell line (K562, a myelogenous leukemia cell line) was established as previously described for CCR5 (30). Human K562 cells transfected with CCR3 were cultured in RPMI 1640 (BioWhitaker, Verviers, Belgium) supplemented with 10% FCS, sodium pyruvate, and 2-ME. Geneticin (400 µg/ml; Life Technologies, Paisley, U.K.) was added to the medium as a selection agent. Human glioblastoma astrocytoma U87 cells transfected with CD4 and CCR3 (31) were obtained from Dr. N. Landau. Intact recombinant human RANTES and eotaxin (carrier-free) were purchased from R&D Systems (Abingdon, U.K.) or PeproTech (Rocky Hill, NJ). Soluble CD26/DPP IV was purified to homogeneity from prostatomes (prostate-derived organelles, that occur freely in seminal plasma) by ion exchange chromatography on DEAE-Sepharose and affinity chromatography on immobilized adenosine deaminase (32).

Proteolytic processing of eotaxin by CD26/DPP IV

Eotaxin was incubated for 48 h at 37°C with or without soluble CD26/DPP IV in 100 mM Tris-HCl, pH 7.7, at an enzyme/substrate ratio of 1:1000. The processed chemokine was acidified with 0.1% trifluoroacetic acid (TFA) and was separated from CD26/DPP IV by Ca2+ reverse phase HPLC (RP-HPLC). Briefly, the chemokine/protease solution was loaded onto an Aquapore C-8 RP-300 column (Applied Biosystems/Perkin-Elmer, Foster City, CA) equilibrated with 0.1% TFA, and proteins were eluted in a gradient of acetonitrile (0–80%) in 0.1% TFA. To check that no intact eotaxin was left in the preparation, the CD26-treated eotaxin was sequenced by Edman degradation on a pulsed liquid amino acid sequencer (477A/120A, Applied Biosystems/Perkin-Elmer). About 40% of eotaxin (3–74) was recovered after purification, and the conversions were 96 and >99% for the eotaxins from R&D Systems and PeproTech, respectively.

Immunoblotting

After separation of the proteins by SDS-PAGE under reducing conditions in Tris-tricine gels (33), the proteins were transferred to a Problot membrane (Applied Biosystems). The pretransfected M2 markers (Bio-Rad, Richmond, CA) used for immunoblotting were OVA (M2 = 49,200), carbonic anhydrase (M2 = 34,500), soybean trypsin inhibitor (M2 = 28,800), lysozyme (M2 = 20,500), and aprotinin (M2 = 7,400). The membrane was incubated overnight with a rabbit polyclonal anti-human eotaxin antisem (PeproTech) and was subsequently treated for 1 h with alkaline phosphatase-conjugated anti-rabbit IgG (Jackson Immunoresearch Laboratories, West Grove, PA). Immunoreactive proteins were visualized by color reaction with nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate.

Isolation of eosinophilic granulocytes

Granulocytes were isolated from single blood donations of healthy donors. Mononuclear and polymorphonuclear cells were separated by density gradient centrifugation on Ficoll-sodium metrizoate (Lymphoprep, Life Technologies). Afterward, the cell pellet containing granulocytes and erythrocytes was suspended in hydroxyethyl starch (Plasmastar, Fresenius, Bad Homburg, Germany) and placed at 37°C for 30 min to remove erythrocytes by sedimentation. Residual erythrocytes were lysed by hypotonic shock (30 s) in bidistilled water. Finally, after labeling of the neutrophilic granulocytes with anti-CD16-coated microbeads, eosinophilic granulocytes were isolated by magnetic cell sorting (VarioMACS, Miltenyi Biotec, Bergisch Gladbach, Germany) as the negatively selected cell fraction (>95% purity).

Chemotaxis assay

Chemotactic activity for eosinophils was determined in the Boyden microchamber assay (Neuroprobe, Cabin John, MD). Samples were diluted in HBSS (Life Technologies) supplemented with 1 mg/ml of human serum albumin (Belgian Red Cross) and were tested in duplicate. Migration of eosinophils (1 × 106/ml, upper wells) through 5-µm pore size polynyl pyrrolidone-free polycarbonate filters (Nuclepore, Pleasanton, CA) to the chemoattractant (lower wells) was allowed for 1 h at 37°C. Migated cells were fixed and visualized using Hemacolor staining solutions (Merck, Darmstadt, Germany) and were counted microscopically in 10 oil immersion fields at ×500 magnification. Chemotactic indexes were calculated by dividing the number of migrated cells toward the chemokine by the number of cells migrated toward the dilution buffer. For checkerboard analysis to measure chemokinesis, various concentrations of intact or truncated eotaxin were added to the cells at the time of transfer to the wells of the microchamber. In desensitization experiments, cells were preincubated with buffer or with 150 ng/ml of truncated eotaxin for 10 min at 37°C before transfer to the upper wells. For antagonization, 150 ng/ml of truncated eotaxin was added together with the agonist to the lower wells.

Calcium assay

The increase in the intracellular calcium concentration ([Ca2+]i) induced by chemokines was monitored by fluorescence spectrophotometry. Freshly isolated eosinophils or K562 cells transfected with CCR3 (105 cells/ml) were loaded with 2.5 µM fura-2 (Molecular Probes Europe, Leiden, The Netherlands) in Eagle’s MEM supplemented with 2% FCS or in culture medium for eosinophils and K562 cells, respectively. After incubation at 37°C for 30 min, cells were washed twice and resuspended in calcium buffer (HBSS containing 1 mM Ca2+ and 0.1% FCS and buffered with 10 mM Hepes at pH 7.4) to a final concentration of 106 cells/ml. The cell preparations were placed at 4°C, and just before measuring fura-2 fluorescence in an LS50B luminescence spectrophotometer (Perkin-Elmer) they were equilibrated at 37°C for 10 min. The excitation wavelengths used were 340 and 380 nm; emission was measured at 510 nm. [Ca2+]i was calculated from the Grynkiewicz equation (34). The K d was 224 nM; [Ca2+]i was determined by addition of 10 mM EGTA, after adjustment of the pH with 20 mM Tris. For desensitization experiments, cells were first stimulated with buffer or chemokine. As a second stimulus, intact eotaxin was added at 10 ng/ml, a concentration that induces a significant increase in [Ca2+]i after preincubation with buffer. The percent inhibition of the response to the second stimulus was calculated using the increase in [Ca2+]i after preincubation with buffer as the 100% value.

Receptor binding assay

125I-labeled eotaxin was purchased from Amersham (Aylesbury, U.K.). Membrane extracts from CCR3-transfected K562 cells were prepared for binding experiments as follows. The cells were centrifuged for 3 min at 1500 × g, and the pellets were suspended in buffer A (15 mM Tris-HCl (pH 7.5), 2 mM MgCl2, 0.3 mM EDTA, and 1 mM EGTA) and homogenized. The crude membrane fraction was collected by two consecutive centrifugation steps at 40,000 × g for 25 min, with an intermediate washing step in buffer A. The final pellet was resuspended in 500 µl of buffer B (7.5 mM Tris-HCl (pH 7.5), 12.5 mM MgCl2, 0.3 mM EDTA, 1 mM
EGTA, and 250 mM sucrose) and flash-frozen in liquid nitrogen. The protein content was assayed by the Folin method (35). Binding experiments were performed in duplicate in minisorp tubes in a final volume of 0.1 ml containing binding buffer (25 mM HEPES (pH 7.6), 5 mM MgCl₂, 1 mM CaCl₂, 0.1% NaN₃, and 0.1% BSA), K562/CCR3 membrane extracts (15 μg/tube), and 0.1 nM [¹²⁵I]eotaxin. The samples were incubated for 90 min at 25°C and then filtered on GF/B filters (Whatman, Maidstone, U.K.) presoaked in 0.5% PEI (polyethylenimine), using a multiple membrane filter (Lincar Lamon Instrumentation, Tel Aviv, Israel). Filters were washed three times with 4 ml of cold binding buffer containing 0.5 M NaCl, and bound [¹²⁵I]eotaxin was determined by gamma scintillation counting.

**HIV infection assay**

U87 cells transfected with CD4 and CCR3 were treated with varying concentrations of intact or truncated eotaxin at the time of infection with the HIV-2 ROD strain (36). The virus was obtained through the Medical Research Council AIDS Reagent Project, National Institute for Biological Standards and Control (Herts, U.K.). The coreceptors used by this HIV-2 strain are CCR3, CCR5, and CXCR4 (37). On day 7 cell supernatants were collected and stored at −20°C. HIV-2 titers were determined in the culture supernatant with a commercial p27 Ag ELISA (Innogenetics, Zwijnaarde, Belgium).

**Results**

*Human eotaxin is NH₂-terminally cleaved by CD26/DPP IV*

The amino acid sequence of mature eotaxin is characterized by a penultimate proline residue at the NH₂-terminus (14, 15). Effective cleavage by CD26/DPP IV of the chemokines SDF-1 (38, 39) and RANTES (25, 26) after this residue has been reported. Therefore, intact recombinant eotaxin from two different commercial sources (10 μg from PeproTech and 30 μg from R&D Systems) was incubated for 48 h with CD26/DPP IV to verify that eotaxin is indeed a CD26/DPP IV substrate. After incubation, the processed chemokine was separated from CD26/DPP IV by RP-HPLC, before determination of the NH₂-terminal sequence by Edman degradation. Fig. 1A shows the purification by RP-HPLC of eotaxin, intact eotaxin incubated without addition of CD26/DPP IV, and eotaxin incubated with CD26/DPP IV. NH₂-terminal sequencing of the eotaxin confirmed that CD26/DPP IV specifically removed the first two amino acids from the intact chemokine, thereby generating eotaxin(3–74). The removal of the two NH₂-terminal residues (glycine and proline) of eotaxin caused a minimal shift in the elution position of eotaxin, which could be expected based on the rather hydrophobic nature of these residues (Fig 1A). The incubation (2 days at 37°C) of eotaxin by itself had no impact on its elution pattern on RP-HPLC. Its unaltered biochemical properties were verified by SDS-PAGE, immunoblotting, and NH₂-terminal sequence analysis (data not shown). In contrast, eotaxin(3–74), truncated by CD26/DPP IV had a reduced Mᵦ compared with that of intact or mock-incubated eotaxin (Fig. 1B). It was also confirmed that the incubation and purification procedure had no effect on the potency of intact eotaxin in either the chemotaxis or calcium signaling assay (data not shown). Since for RANTES and SDF-1 it was found that truncation by CD26/DPP IV alters their biological potency, eotaxin(3–74) was functionally characterized in parallel with intact eotaxin.

**CD26/DPP IV processing of eotaxin impairs its interaction with CCR3**

First, it was verified whether NH₂-terminal processing of eotaxin affects the interaction between the chemokine and its unique receptor, CCR3. Fig. 2 shows the binding of [¹²⁵I]eotaxin to CCR3-transfected K562 cells in the presence of increasing concentrations of unlabeled intact and truncated eotaxin. On the average, the concentration of intact eotaxin displacing 50% of the bound [¹²⁵I]eotaxin was 1.2 ± 0.3 nM, whereas 50% displacement was only achieved with 7.5 ± 1 nM of eotaxin(3–74). Thus, eotaxin(3–74) competed sixfold less efficiently with [¹²⁵I]eotaxin binding than did intact eotaxin.

In K562/CCR3 cells, intact recombinant eotaxin induced a rise in [Ca²⁺]ᵢ in a dose-dependent manner; the minimal effective dose was 3 ng/ml. In contrast, 100 ng/ml of recombinant truncated eotaxin(3–74) was required to achieve a comparable response (Fig. 2). These results indicate that CD26/DPP IV processing of eotaxin impairs its interaction with CCR3.

**FIGURE 1. Biochemical characterization of eotaxin, NH₂-terminally cleaved by CD26/DPP IV.** A, RP-HPLC elution pattern of fresh intact eotaxin(1–74), intact eotaxin after incubation (2 days at 37°C) without CD26/DPP IV (eotaxin(1–74)inc.), and eotaxin incubated with CD26/DPP IV (eotaxin(1–74)) cleaved by CD26/DPP IV, and eotaxin incubated with CD26/DPP IV. NH₂-terminal sequencing of the eotaxin confirmed that CD26/DPP IV specifically removed the first two amino acids from the intact chemokine, thereby generating eotaxin(3–74). The removal of the two NH₂-terminal residues (glycine and proline) of eotaxin caused a minimal shift in the elution position of eotaxin, which could be expected based on the rather hydrophobic nature of these residues (Fig 1A). The incubation (2 days at 37°C) of eotaxin by itself had no impact on its elution pattern on RP-HPLC. Its unaltered biochemical properties were verified by SDS-PAGE, immunoblotting, and NH₂-terminal sequence analysis (data not shown). In contrast, eotaxin(3–74), truncated by CD26/DPP IV had a reduced Mᵦ compared with that of intact or mock-incubated eotaxin (Fig. 1B). It was also confirmed that the incubation and purification procedure had no effect on the potency of intact eotaxin in either the chemotaxis or calcium signaling assay (data not shown). Since for RANTES and SDF-1 it was found that truncation by CD26/DPP IV alters their biological potency, eotaxin(3–74) was functionally characterized in parallel with intact eotaxin.

**FIGURE 2. Competition by eotaxin(3–74) for [¹²⁵I]eotaxin binding to CCR3-transfected cells.** Increasing concentrations of unlabeled intact eotaxin(1–74) and truncated eotaxin(3–74) were added together with 0.1 nM [¹²⁵I]eotaxin(1–74) to K562 cells transfected with CCR3. Results are expressed as the percentage of residual specific binding. One experiment of two, each performed in duplicate, is shown.
eotaxin(3–74) elicited only a weak calcium rise (Fig. 3A). In addition, 100 ng/ml of eotaxin(3–74) added as the first stimulus to CCR3 transfectants partially desensitized (~40%) the response to 10 ng/ml of intact eotaxin. Intact eotaxin, however, added at 100 ng/ml as the first stimulus, could fully desensitize a second stimulation by 10 ng/ml intact eotaxin (Fig. 3B). Similar results were obtained on eosinophils purified from human peripheral blood (Fig. 4). Eotaxin(3–74) at 100 ng/ml induced a 60-nM increase in $[Ca^{2+}]_i$, whereas with a threefold lower dose of intact eotaxin an augmentation of 160 nM was obtained. Furthermore, the calcium response to 30 ng/ml of intact eotaxin was partially (72%) inhibited in eosinophils by eotaxin(3–74) at 100 ng/ml. Thus, truncation of eotaxin by CD26/DPP IV diminished the calcium-inducing capacity of eotaxin in both normal eosinophils and CCR3-transfected K562 cells.

**Truncated eotaxin(3–74) has strongly reduced chemotactic activity for eosinophils and inhibits eosinophil chemotaxis toward intact eotaxin**

Next, it was examined whether removal of two NH$_2$-terminal residues affects the chemotactic potency of eotaxin. Intact eotaxin induced migration of freshly isolated peripheral blood eosinophils from 15 ng/ml onward. Its chemotactic potency was similar to that obtained with 50 ng/ml of intact RANTES (Fig. 5). Truncated eotaxin(3–74), however, was still inactive at the highest concentration tested (150 ng/ml). Thus, CD26/DPP IV processing of eotaxin resulted in at least a 30-fold reduction in chemotactic potency.

Since truncated eotaxin(3–74) had impaired chemotactic activity, but still interacted with its receptor, chemotaxis desensitization and antagonization experiments were performed. It was first demonstrated by checkerboard analysis that truncated eotaxin(3–74) did not induce chemokinesis when added with the eosinophils to the top wells of the microchamber (Table I). In addition, chemotaxis toward intact eotaxin in the lower compartment was prevented when an equimolar concentration of intact or a threefold higher concentration of inactive truncated eotaxin(3–74) was added to the cells in the upper compartment (Table I). Furthermore, preincubation of eosinophils for 10 min at 37°C with 150 ng/ml of eotaxin(3–74) completely (98–100%) desensitized migration toward equimolar (and lower) concentrations of intact eotaxin in the lower chamber compartment (Fig. 6). CD26/DPP IV-processed eotaxin(3–74) also partially antagonized eotaxin-induced migration of eosinophils when added together with intact

**FIGURE 3.** Calcium mobilization in CCR3-transfected cells by intact and CD26/DPP IV-truncated eotaxin. A, Intact eotaxin(1–74) and truncated eotaxin(3–74) were compared for their ability to induce an increase in the $[Ca^{2+}]_i$ in CCR3-transfected K562 cells. The results represent the mean increase (±SEM) in $[Ca^{2+}]_i$ in three independent experiments. The limit for a significant increase (20 nM) is indicated by the dashed line. B, Desensitization of calcium mobilization by eotaxin in CCR3 transfectants. K562/CCR3 cells were first stimulated with varying concentrations of intact or truncated eotaxin, followed by stimulation with 10 ng/ml of intact eotaxin. The percent inhibition of the response to the second stimulus is shown. Results represent the mean (±SEM) percent inhibition of four independent experiments. Significant calcium increases and percent inhibition (compared with baseline level), as determined by the Mann-Whitney U test, are indicated by asterisks (⁎⁎, $p < 0.05$).

**FIGURE 4.** Desensitization of calcium mobilization by eotaxin(3–74) in eosinophils. Freshly isolated peripheral blood eosinophils were first stimulated with 30 ng/ml of intact eotaxin (upper spectrum) or 100 ng/ml of truncated eotaxin(3–74) (lower spectrum), followed by stimulation with 30 ng/ml of intact eotaxin. The time points of chemokine addition to the cells are indicated by arrowheads.

**FIGURE 5.** Comparison of the chemotactic potencies of intact eotaxin and truncated eotaxin(3–74) and intact RANTES for eosinophils. The chemotactic activity was determined in the microchamber assay using freshly isolated peripheral blood eosinophils. Results represent the mean (±SEM) chemotactic index of three independent experiments.
eotaxin to the lower wells of the microchamber (Fig. 6). Thus, eotaxin, a strong agonist for eosinophils, is processed by CD26/DPP IV into a potent chemotaxis inhibitor.

Both intact and CD26/DPP IV-processed eotaxin(3–74) suppress HIV-2 infection

Finally, intact and truncated eotaxin(3–74) were compared for their abilities to reduce infection of CD4/CCR3-transfected U87 cells with the HIV-2 ROD strain. NH2-terminal processing did not alter the antiviral activity of eotaxin (Table II). Truncated eotaxin as well as intact eotaxin had an IC50 value of 1500 ng/ml. The minimal dose required for a significant inhibition (~20%) of viral replication was 500 ng/ml. At this concentration RANTES caused a 33% decrease in viral p27 production. It can be concluded that the conversion of intact eotaxin to truncated eotaxin(3–74) changed its interaction with its receptor in such a way that a reduction in chemotactic potency occurred, without influencing its ability to suppress CCR3-mediated HIV-2 infection.

Discussion

Allergic reactions are characterized by the accumulation of eosinophils in the inflamed tissue. Eosinophil-derived mediators, such as the major basic protein, are associated with the pathogenesis of allergic inflammation. Several studies that were aimed to identify the molecules responsible for the in vivo eosinophil accumulation have indicated the involvement of chemokines. As a consequence, the CC chemokine eotaxin was originally isolated from bronchoalveolar lavage fluid in a guinea pig model for allergic airway inflammation (40). It was purified from the bronchoalveolar lavage fluid using a skin assay based on the screening for factors inducing eosinophil accumulation after intradermal injection. Since then, evidence that eotaxin has an important role in stimulating the local recruitment of eosinophils from blood microvessels into the tissue at sites of allergic inflammation has accumulated (14, 15, 41–47). Moreover, eotaxin is constitutively expressed in healthy conditions, regulating the physiological trafficking of eosinophils (48, 49). The eotaxin receptor CCR3, originally reported to be selectively expressed on eosinophils, was also detected on basophils and Th2 lymphocytes, other cell types associated with allergic inflammation (20–24). In addition to attracting eosinophils, eotaxin is a potent activator of the respiratory burst (50, 51). Recently, new functional characteristics were reported for eotaxin. First, after i.v. injection, this chemokine mobilizes eosinophils and their progenitors from the bone marrow (52). In this process, eotaxin acts in synergy with IL-5 (53, 54). Furthermore, eotaxin can function as a growth and differentiating factor for myeloid hemopoietic progenitors (55). Since circulating levels of eosinophils are normally low, eotaxin may contribute to the mobilization of progenitors and to their differentiation into mature eosinophils, which are essential for the induction of an allergic reaction.

Table II. Anti-HIV-2 activity of eotaxin(1–74) and eotaxin(3–74) in CD4/CCR3-transfected U87 cells

<table>
<thead>
<tr>
<th>Chemokine Concentration (ng/ml)</th>
<th>% Inhibition of Viral Production by a</th>
</tr>
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<tbody>
<tr>
<td>Eotaxin(1–74)</td>
<td>Eotaxin(3–74)</td>
</tr>
<tr>
<td>1500</td>
<td>43 ± 8 (2)</td>
</tr>
<tr>
<td>500</td>
<td>22 ± 3 (4)</td>
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<td>&lt;10 (4)</td>
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<tr>
<td>50</td>
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a Viral titers were monitored by a p27 ELISA. 7 days after infection of the CD4/CCR3 U87 cells.
b Mean ± SEM of two to four (n) independent experiments.
c Not determined.
Both intact and truncated (missing two NH2-terminal residues) human eotaxin were isolated from IL-4- or TNF-a-stimulated dermal fibroblasts (56, 57). However, naturally truncated eotaxin(3–74) could not be functionally characterized, since no pure preparation was obtained. In our study, post-translational processing of eotaxin by the serine protease CD26/DPP IV was investigated. DPP IV was originally identified as the activation Ag CD26, a specific aminopeptidase that cleaves NH2-terminal dipeptides from proteins with a proline, hydroxyproline, or alanine residue at the penultimate position (27). Indeed, intact eotaxin, which has a hydrophobic glycine-proline dipeptide at the NH2-terminus, was efficiently cleaved by CD26/DPP IV into eotaxin(3–74). After purification by RP-HPLC, we obtained pure eotaxin(3–74) preparations to be analyzed for receptor recognition, calcium signaling, chemotactic activity, and suppression of HIV-2 infection.

After cleavage of eotaxin by CD26/DPP IV, its chemotactic potency for blood eosinophils and its signaling capacity through CCR3 were 30-fold reduced, whereas its efficacy to displace [125I]eotaxin from CCR3 transfectants was diminished only 6-fold. As a consequence, truncated eotaxin(3–74) was still able to desensitize CCR3 signaling and eosinophil chemotaxis induced by intact eotaxin. Furthermore, eotaxin(3–74) partially antagonized the chemotactic response to intact eotaxin. Effective binding of eotaxin(3–74) to CCR3 was also obvious from its unaltered activity against HIV-2.

Other chemokines recently were shown to be substrates for CD26/DPP IV. The effects of proteolysis on the biological activity of human RANTES, granulocyte chemotactic protein-2 (GCP-2), and SDF-1 were quite diverse. Truncated GCP-2(3–77) was as active on neutrophils as the intact form (25). After cleavage by CD26/DPP IV, SDF-1(3–68) became ineffective in lymphocyte chemotaxis and HIV-1 inhibition assays (38, 39). Processed RANTES(3–68) had reduced chemotactic, but increased HIV-1-inhibiting properties. This is due to a diminished affinity of RANTES(3–68) for CCR1 and CCR3, contrary to a higher affinity of truncated eotaxin. Furthermore, eotaxin(3–74) partially antagonized the chemotactic activity for blood eosinophils and its signaling capacity through CCR3-mediated immunological processes. This is due to a diminished affinity of RANTES(3–68) for CCR1 and CCR3, contrary to a higher affinity of truncated eotaxin. Furthermore, eotaxin(3–74) partially antagonized the chemotactic activity for blood eosinophils and its signaling capacity through CCR3-mediated immunological processes.

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