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IL-16 Activates Plasminogen-Plasmin System and Promotes Human Eosinophil Migration into Extracellular Matrix via CCR3-Chemokine-Mediated Signaling and by Modulating CD4 Eosinophil Expression

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Increased eosinophil counts are a major feature of asthmatic airways. Eosinophil recruitment requires migration through epithelium and tissue extracellular matrix by activation of proteases. We assessed the capacity of IL-16, a CD4+ cell chemotactic factor, to induce migration of eosinophils through a reconstituted basement membrane and evaluated the proteases, mediators, and receptors involved in this migration. IL-16 added to lower chambers of Invasion Chambers elicited eosinophil migration through Matrigel. This effect was decreased by inhibition of the plasminogen-plasmin system (Abs against urokinase plasminogen activator receptor or plasminogen depletion), but not by anti-matrix metalloproteinase-9 Abs. Abs against CD4 also inhibited IL-16-induced eosinophil migration. At the baseline level, few eosinophils (4.6% positive cells with a mean fluorescence of 0.9) expressed surface membrane CD4, while most permeabilized eosinophils (68% positive cells with a mean fluorescence of 18) express the CD4 Ag. TNF-pretreatment increased surface membrane CD4 expression by 6-fold as previously described, and increased IL-16-induced cell migration by 2.2-fold. Incubation of eosinophils with IL-16 also increased surface membrane CD4 expression by 5.4-fold, supporting the role of CD4 as receptor for IL-16. Abs against CCR3, eotaxin, or RANTES blocked IL-16-induced migration. In conclusion, IL-16 promotes eosinophil migration in vitro, by activating the plasminogen-plasmin system and increasing the membrane expression of its receptor. This effect is initiated via CD4 and mediated via the release of CCR3 ligand chemokines. Interestingly, most eosinophils express intracellular CD4. Hence, IL-16 may play an important role in the recruitment of blood eosinophils to the bronchial mucosa of asthmatics. The Journal of Immunology, 2004, 173: 4417–4424.
release of bioactive IL-16 in bronchoalveolar lavage fluid (15). CD4 serves as a signal-transducing receptor for IL-16, and its expression is required for mediating IL-16 functions for lymphocytes (10, 16). However, a recent study on CD4 knockout mice suggests that CD4 is not required for all IL-16 functions (17). In this study, we evaluated the capacity of IL-16 to induce eosinophil migration through a reconstituted basement membrane, and we investigated the role of uPAR, MMP-9, and different receptors and mediators in IL-16-induced eosinophil migration and the modulation of eosinophil CD4 expression by IL-16.

Materials and Methods

Reagents

Recombinant human (rh)-IL-5, rh-eotaxin, rh-IL-16 and rh-TNF were purchased from PeproTech (Rocky Hill, NJ); plasmogen (proflibromycin from human serum) from Roche Diagnostics (Laval, Quebec, Canada); FITC-conjugated mouse IgG1, FITC-conjugated anti-human CD4, and BD Cytofix/Cytoperm kit from BD Biosciences (Mississauga, Ontario, Canada); 5-oxo-ETE from Cayman Chemical (Ann Arbor, MI); mAb to CD87 (uPAR) (clone 3936) from American Diagnostica (Greenwich, CT); monoclonal mouse IgG2a (isotype control), and mAb to CD4 (clone RPA-T4) from BD Biosciences; mAb to CCR3 (clone 61828.111) and monoclonal rat IgG2a (isotype control, clone 54447.11) from R&D Systems (Minneapolis, MN); mAb to RANTES (clone VL1) from Serotec (Oxford, U.K.); polyclonal Ab to eotaxin from Biogenesis (Kingston, NH); and mAb to CD16, conjugated to magnetic beads, from Milenyi Biotec (Bergisch Gladbach, Germany). BSA (fraction V) and EDTA were purchased from Sigma-Aldrich (Oakville, Ontario, Canada); Dextran T-500 and Ficoll-Paque from Amersham Biosciences (Baie d’Urfée, Quebec, Canada); and RPMI 1640 medium, HBSS without calcium/magnesium, penicillin/streptomycin, and FBS from Invitrogen Life Technologies (Burlington, Ontario, Canada).

Selection of subjects

Eight normal subjects (7 females and 1 male, age 34.0 ± 4.5 years) without a history of allergy or asthma, and 24 asthmatic subjects (11 females and 13 males, age 26.2 ± 1.5 years) meeting the criteria of the American Thoracic Society for the diagnosis of asthma (18) were recruited for this study. The asthmatics had mild asthma defined by a morning prebronchodilator-forced expiratory volume in 1 s (FEV1) > 85% of predicted, and the requirement of only short-acting β2-agonist on demand. The inclusion criteria were stable asthma for >3 mo, no inhaled corticosteroids or asthma medication other than β2-agonist over the 3 mo preceding the study, the use of no other drugs, and no disease other than asthma. Approval from the local Ethics Committee was obtained and subjects signed informed consent forms. All subjects underwent blood sampling early in the morning. FEV1 values was measured in the morning at least 8 h after any β2-agonist inhalation with a PFT II Vitalograph Spirometer (Vitalograph, Buckingham, U.K.) (19). The mean FEV1 values of the normal and asthmatic subjects were 109.8 ± 5.3 and 96.6 ± 1.9% of the predicted values, respectively. Mean blood eosinophil counts measured with a Coulter STKS (Model 809; Beckman Coulter, Miami, FL) were 0.16 ± 0.4 and 0.28 ± 0.03 × 109/liter for normal and asthmatic subjects, respectively.

Blood cell processing and eosinophil purification

Blood eosinophils were purified as previously described (9, 20). Briefly, venous blood (150 ml) was centrifuged to remove platelet-rich plasma, and the leukocytes were sedimentated from the buffy coat on Dextran 6%. The leukocytes were resuspended and centrifuged on Ficoll-Paque (1.077 g/ml) for 20 min at 700 × g. No eosinophils were observed in the lymphocyte layer. The granulocyte layer was resuspended and red cells lysed with distilled water. Eosinophils were purified from neutrophils by negative selection using bead-conjugated anti-CD16 (FcγR II) mAb and a magnetic cell sorter (epics). Total cell count (hemacytometer) and cell viability (trypan blue exclusion) were determined on the resulting cell suspensions and differential cell counts (Diff-Quik; Dade Behring, Newark, CA) were evaluated on cyto spun preparations. The purity of the eosinophil preparations was always >98%, the few contaminating cells were neutrophils and/or lymphocytes. Eosinophil viability was always >99%. Eosinophils were resuspended in culture medium (RPMI 1640 with 10% FBS and 1% penicillin/streptomycin).

Migration assay and protease inhibition

The migration of eosinophils through the basement membrane components was evaluated in 24-well Matrigel Invasion Chambers (BD Biosciences) as previously described (9). Briefly, eosinophils (0.5 × 109 in 0.5 ml) were incubated with or without IL-5 (10 ng/ml) for 30 min at 37°C in 5% CO2, placed in the upper chamber of the Matrigel Invasion Chamber, and incubated at 37°C in 5% CO2 for 18 h, except for kinetic study where cells were incubated for 1–24 h. IL-16 or eotaxin were used as chemotactic factors and added separately in the lower chambers to reach different concentrations: IL-16, 0.001–1 nM; and eotaxin, 10 nM. Eotaxin concentration was chosen from our previous report (9). At the end of the incubation, cells in both the upper and lower chambers were removed by aspiration. The remaining cells on both sides of the membranes were gently washed and harvested twice with cold RPMI 1640 plus 5 mM EDTA without FBS. Cells of each chamber were counted on a hemacytometer. For each concentration, the percentage of migration was calculated from the number of cells in the lower chamber of the Matrigel Invasion Chamber divided by the number of cells in the lower chamber of a control invasion chamber without the Matrigel membrane and multiplied by 100. Very few cells moved through the insert in the absence of chemotactic factors (7, 9). To study the role of the various proteases, receptors, and mediators on migration, eosinophil suspensions (0.5 × 109 cells/0.5 ml in complete RPMI 1640) were preincubated with IL-5 with or without monoclonal anti-CD87 Ab (10 μg/ml), monoclonal anti-MMP-9 Ab (10 μg/ml), monoclonal anti-CD4 Ab (10 μg/ml), monoclonal anti-CCR3 Ab (5 μg/ml), monoclonal anti-eotaxin Ab (10 μg/ml), or monoclonal anti-RANTES Ab (1 μg/ml), or isotype control Ab for 30 min at 37°C in 5% CO2 before incubation in the invasion chamber. To further evaluate the role of plasmigen present in FBS on IL-16-induced eosinophil migration, migration was measured in the presence of low concentration of FBS (0.5%) supplemented or not with plasminogen (2 U/ml) as previously described (9). To further evaluate the role of CD4 on IL-16-induced eosinophil migration, cells were pre-treated or not with TNF (10 ng/ml) for 18 h at 37°C in 5% CO2 to increase CD4 expression, as previously reported (21), before migration assay was performed. To evaluate possible modulation of CD4 expression by IL-16, eosinophil CD4 expression was measured in nonpermeabilized and permeabilized cells after incubation with IL-5 alone or IL-5 and IL-16 for 18 h. Eosinophils from asthmatic subjects were used for all these experiments. Due to a smaller number of cells, eosinophils from normal subjects were used to evaluate the inhibitory effect of anti-CD87, anti-CD4, and anti-CCR-3 Abs on IL-16-induced eosinophil migration, and to measure the baseline CD4 expression. Eosinophil viability measured at the end of all these assays was >95%.

Flow cytometry analysis

To measure CD4 cell surface expression, eosinophils (350,000 cells/100 μl) obtained from the above experiments were washed once with PBS 1× supplemented with 1% BSA, and incubated 45 min at 4°C with 1 μg/ml FITC-conjugated mouse monoclonal anti-human CD4 Ab or mouse IgG2a (isotype control Ab). After incubation, cells were washed once with PBS and fixed in 1% paraformaldehyde (15 min at 4°C). To measure intracellular CD4, eosinophils were permeabilized using BD Cytofix/Cytoperm kit for 20 min (BD Biosciences). The cells were washed with PBS 1×, supplemented with 1% BSA and 0.1% saponin, incubated 45 min at 4°C with 1 μg/ml FITC-conjugated mouse monoclonal anti-human CD4 Ab or mouse IgG2a (isotype control Ab). Flow cytometry analysis was performed using an EPICS XL–MCL flow cytometer (Beckman Coulter), equipped with an air-cooled 15-mw argon laser as a light source and an EXPO 32 acquisition software (version 1.1c). Acquisition of fluorescence data was gated by forward angle light scatter and side scatter and the data rate was set at <500 events per second. Results were expressed as a percentage of CD4-positive cells and as mean fluorescence (MF) intensities.

Binding of IL-16 to CCR3

Receptor binding assays using 125I-eotaxin (sp act, 2000 Ci/mmole) from Amersham Biosciences were performed with eosinophils isolated as described above. Chemokine binding to target cells was conducted using a modified Scatchard method previously described (22). Cells were labeled in HBSS and resuspended in binding buffer (50 mM HEPES, pH 7.5, 1 mM CaCl2, 5 mM MgCl2, 0.5% azide) at a concentration of 1 × 107/ml. Aliquots of 50 μl (5 × 105 cells) were dispensed into microfuge tube, followed by the addition of various concentrations of competitor (eotaxin or IL-16) and radiolabeled eotaxin (0.6 mM). The final reaction volume was 500 μl. After 60-min incubation at room temperature, the cells were washed twice with assay buffer. Cell pellets were then analyzed by a gamma-well scintillation
counter (Cobra II; Canberra Packard Instruments, Concord, Ontario, Canada) with the results expressed in cpm. The specific binding was determined by subtracting the background cpm obtained without 125I-eotaxin. The competition was presented as the percentage of specific binding calculated as: IL-16 or eotaxin (0.1–100 nM) cpm plus 125I-eotaxin (0.6 nM) cpm /125I-eotaxin (0.6 nM) cpm × 100.

Statistical analysis
Means and SEM were determined for continuous variables. Kinetic and dose-response data were evaluated by repeated-measures ANOVA using a spatial covariance structure with an exponential distribution. The effects of IL-16, IL-16 with IL-5, and eotaxin on eosinophil migration and effects of TNF on CD4 expression and migration were compared using the Student paired t test. The effects of different mAbs on migration were compared by ANOVA (randomized block design). The effect of plasminogen on eosinophil migration was analyzed with a three-way ANOVA using the subject as the blocking factor. At posteriori comparisons were performed using Tukey’s method. For all analyzes, normality and variance assumptions based on tests and graphical representations were fulfilled. The results were considered significant if p values were <0.05. The data were analyzed using the statistical package program SAS (8.2 version; SAS Institute, Cary, NC).

Results
IL-16 promotes eosinophil migration through Matrigel
Because IL-16 is a chemotactic factor for CD4-bearing eosinophils, we evaluated its capacity to induce migration of blood eosinophils through a basal membrane using Matrigel Invasion Chambers. Dose-response experiments showed that, in the presence of IL-5, the IL-16 maximal effect was obtained with a concentration of 0.7 nM (data not shown), thus this concentration was used in subsequent experiments. To determine the time needed to increase eosinophil migration, eosinophils from asthmatics were treated with IL-16 and the migration assay was stopped at different times (Fig. 1A). IL-16-elicited migration increased significantly up to 18 h (p = 0.003).

Because IL-5 has been shown to increase 5-oxo-ETE and eotaxin-induced migration through the Matrigel (7, 9), eosinophils from asthmatics were incubated with IL-16 in the absence or presence of IL-5. The addition of IL-5 significantly increased the IL-16-induced cell migration from 12.1 ± 4.9% to 37.9 ± 10.4% (Fig. 1B) (p = 0.01). Fig. 1C compares eosinophil migration obtained with IL-16 (0.7 nM) and eotaxin (at previously determined optimal concentration, 10 nM) (9) in the presence of IL-5. As previously shown (9), IL-5 alone did not induce eosinophil migration (mean, 1.4%). IL-16 mediated a lower eosinophil migration than eotaxin; 31.8 ± 4.6% and 42.1 ± 7.9%, respectively (p = 0.04).

IL-16-induced migration is mediated by the plasmin-plasminogen system
To determine the role of various proteases in IL-16-induced migration, different inhibition procedures were used. Anti-CD87 mAbs used to block the plasminogen activator receptor (CD87) significantly decreased the IL-16-induced migration of eosinophils from asthmatics by 51.4 ± 6.8% (p < 0.0001 compared with control condition, Fig. 2A). Isotype IgG control Abs were used in all experiments implicating mAbs and had no significant effect on IL-16-induced eosinophil migration (migration with and without isotype control Ab; 38.3 ± 4.8% and 45.1 ± 5.5%, respectively, p = 0.3, randomized block design, ANOVA). Compared with 10% FBS that contains plasminogen, the incubation of eosinophils from asthmatics in low FBS concentration (0.5%), consequently, in a low concentration of plasminogen, decreased IL-16-induced migration by 89%; migration of 30.6 ± 8.3% and 3.7 ± 1.4%, respectively (p < 0.0001, Fig. 2B). The addition of plasminogen to 0.5% FBS restored the IL-16-induced migration rate to the level observed with 10% FBS; migration rate of 25.4 ± 5.2%. However,
mAbs against MMP-9 that block the activity of this protease had no effect on eosinophil migration (27.6 ± 2.0% compared with control condition; 30.6 ± 4.0%, n = 4). These data show that the IL-16-induced migration through Matrigel is largely mediated by the plasmin-plasminogen system as previously reported with eotaxin (9).

**IL-16-induced eosinophil migration is mediated via CD4 whose expression is up-regulated by IL-16**

Anti-CD4 mAbs and preincubation with TNF that increases eosinophil CD4 expression (21) were used to evaluate the role of this receptor in IL-16-mediated migration. Anti-CD4 mAbs significantly decreased the IL-16-induced migration of eosinophils from asthmatics by 63.5 ± 6.6% (p < 0.0001 compared with control condition, Fig. 3A). These data demonstrate that CD4 mediates IL-16 effect on eosinophil migration as previously reported (23). Incubation of eosinophils from asthmatics with TNF increased counts of CD4+ eosinophils by 8.7-fold from 4.6 ± 0.9% to 40.1 ± 8.3% positive cells (p = 0.001, Fig. 3B) and CD4 expression measured as MF by 6-fold from 0.9 ± 0.4 to 5.6 ± 1.1, p = 0.002). The resulting eosinophil population with a higher expression of CD4 presented a 2.2-fold increase in migration rate in response to IL-16 compared with the eosinophils with a lower CD4 expression; 43.7 ± 11.1 and 19.6 ± 4.9, respectively (p = 0.04).

**FIGURE 2.** Role of the plasminogen-plasmin system in IL-16-induced eosinophil migration through Matrigel basement membrane. A, Eosinophils were preincubated with or without anti-CD87 or anti-IgG2a mAbs for 30 min in the presence of IL-5. The cells were added to the upper chamber and incubated for 18 h at 37°C, IL-16 (0.7 nM) being added to the lower chamber. Anti-CD87 mAbs significantly decreased IL-16-induced eosinophil migration (n = 13, p < 0.0001, randomized block design, ANOVA). B, Eosinophils were preincubated with IL-5 and added to the upper chamber in the presence of 10% FBS (control condition) or 0.5% FBS with or without human plasminogen (2 U/ml) and incubated for 6 h at 37°C, IL-16 (0.7 nM) being added to the lower chamber. Serum depletion (0.5% FBS) almost abrogated IL-16-induced eosinophil migration, which was restored by addition of plasminogen (Plg) (n = 5, p < 0.0001, a > b, three-way ANOVA).

**FIGURE 3.** Role of eosinophil CD4 expression on IL-16-induced eosinophil migration through the Matrigel basement membrane. A, Eosinophils were preincubated with or without anti-CD4 or anti-IgG2a mAbs for 30 min in the presence of IL-5. The cells were added to the upper chamber and incubated for 18 h at 37°C, IL-16 (0.7 nM) being added to the lower chamber. Anti-CD4 mAbs decreased IL-16-induced eosinophil migration (n = 13, p < 0.0001, randomized block design, ANOVA). B, Eosinophils were preincubated for 18 h with or without TNF (10 ng/ml) in the presence of IL-5. CD4 expression was measured by flow cytometry and migration of eosinophils through Matrigel was evaluated after a 6-h incubation. TNF up-regulated eosinophil CD4 expression (n = 12, p = 0.001) and TNF-pretreated cells had an increased IL-16-induced migration (n = 6, p = 0.04, Student’s paired t test).
Although very low levels of CD4 may mediate IL-16-induced migration, the high migration rate observed with IL-16 in the presence of a low baseline CD4 surface expression raises the possibility that IL-16 by itself up-regulates eosinophil CD4 expression. To evaluate this possibility, CD4 expression was evaluated after incubation with IL-5 (10 ng/ml) or IL-5 and IL-16 (0.7 nM). A kinetic study in the presence of IL-16 showed that no increase of CD4 expression was observed at 2, 4, and 6 h (MF at baseline, 1.0 ± 0.3; 2 h, 0.6 ± 0.1; 4 h, 1.1 ± 0.5; 6 h, 1.1 ± 0.4). However, a significant increase was found at 18 h (MF, 4.8 ± 1.3; p < 0.0001, n = 6–17, ANOVA) compared with all the preceding values. At 18 h, in the presence of IL-5 alone, CD4 expression of nonpermeabilized cells was low (2.7 ± 1.6% positive cells, Fig. 4C, with a MF of 1.0 ± 0.3, n = 17, typical cytometry log-scaled plots shown in Fig. 4A), whereas most permeabilized eosinophils presented high CD4 expression (68.9 ± 11.5% positive cells with a MF of 18.1 ± 4.6, n = 6, typical cytometry log-scaled plots shown in Fig. 4B). The incubation with IL-16 increased cell surface CD4 expression to 10.5 ± 3.9% positive cells with a MF of 4.8 ± 1.3 (p = 0.01, compared with IL-5 alone, Fig. 4C). TNF was used as positive control and also increased membrane CD4 expression (data not shown). Consequently, these data support the hypothesis of the modulation of CD4 expression by IL-16 raised to explain the high migration rate observed with IL-16 in the presence of a low baseline cell surface CD4 expression.

**FIGURE 4.** Modulation of cell surface CD4 expression by IL-16. Representative histograms of CD4 expression by nonpermeabilized (A, black histogram) and permeabilized (B, gray histogram) eosinophils and isotype control Ab (dotted line) after incubation with IL-5 (10 ng/ml) for 18 h at 37°C. CD4 expression of nonpermeabilized cells was low, whereas most permeabilized eosinophils expressed CD4. C, Eosinophils were preincubated for 18 h with or without IL-16 (0.7 nM). Cell surface CD4 expression was significantly increased by IL-16 (n = 17, p = 0.01, Student’s paired t test).

**IL-16-induced eosinophil migration depends on eotaxin/RANTES release and CCR3 activation**

To evaluate the role of eotaxin and RANTES on IL-16 effects as previously reported for IL-4 and leukotriene C4 (LTC4) release (23), Abs against CCR3, eotaxin, and RANTES were used to block this receptor and its ligands. Anti-CCR3 mAbs decreased the IL-16-induced migration of eosinophils from asthmatics by 78.5% (p < 0.0001 compared with control condition, Fig. 5A). Anti-eotaxin mAbs inhibited similarly both IL-16 and eotaxin-induced eosinophil migration by 57.7 and 56.7%, respectively (p = 0.0001 compared with control condition, Fig. 5B). In addition, anti-RANTES mAbs inhibited also IL-16-induced eosinophil migration by 57.1% (n = 4, data not shown). Finally, binding experiments were also performed to rule out the possible interaction between IL-16 and CCR3. In these binding assays, IL-16, as opposed to eotaxin, did not compete with 125I-eotaxin on human eosinophils (data not shown). Altogether, these experiments demonstrate the importance of endogenous chemokines and CCR3 for the IL-16-mediated migration of eosinophils.

Eosinophils from normal subjects present similar migration rate toward IL-16 and similar baseline membrane expression of CD4

To verify whether cells from asthmatics present an activated phenotype compared with the ones of normal subjects, eosinophils from normal subjects were evaluated for the measured functions.
Nonpermeabilized eosinophils from normal subjects had a low eotaxin-induced eosinophil migration (p > 0.05). Anti-eotaxin mAbs decreased both IL-16- and IL-5-induced migration of eosinophils from normal subjects by 39.8 ± 4.6% and 56.8 ± 6.3%, respectively (n = 8, p < 0.0001 compared with control conditions); these inhibitory effects were similar to the ones observed with eosinophils from asthmatics (p = 0.2). These data show that eosinophils from normal and asthmatic subjects behave similarly in terms of baseline CD4 expression and migratory response to IL-16.

**Discussion**

Increased eosinophil count is an important feature of allergic and asthmatic inflammatory process. The recruitment of eosinophils to tissue is a complex process mediated at different steps by numerous mediators and molecules. The capacity of various chemotactic factors to elicit migration of eosinophils into tissue extracellular matrix varies from one to the other and depends notably on their capacity to activate cell proteases (8, 9). Moreover, we previously showed that chemotactic factors also differ in the nature of the proteases they activate (7, 9). This study evaluated the means by which IL-16, an eosinophil chemotactic factor, may promote eosinophil recruitment in tissue.

It shows that IL-16 is active in inducing eosinophil migration of eosinophils from both normal and asthmatic subjects through Matrigel basement membrane at low concentration and that this effect depends predominantly on activation of the plasminogen-plasmin system. As described with other chemotactic factors (7, 9, 24), although IL-16 alone could induce eosinophil migration, its effect was increased by IL-5. Because IL-5 by itself has no effect on cell migration, it likely amplifies the effect of IL-16 by priming eosinophils to have enhanced responsiveness to this chemokine as already proposed (25, 26). CD87 blocking and incubation in medium poorly supplemented with serum, and consequently poor in plasminogen, almost abrogated IL-16-induced migration. Similarly to the previous report on eotaxin (9), the addition of plasminogen restored IL-16-induced eosinophil migration in vitro. The maximal effect of IL-16 on eosinophil migration through Matrigel basement membrane was lower than the one observed with eotaxin, but it occurred at lower concentration, 0.7 vs 10 nM, respectively (9). These data strongly suggest that, as eotaxin, IL-16 promotes eosinophil migration through Matrigel basement membrane by activating predominantly the plasminogen-plasmin system. Other molecules, notably MMP-17 (27) or the family of membrane proteins containing a disintegrin and metalloproteinase domain, could also be involved in cell-mediated extracellular matrix digestion and migration. To our knowledge, no disintegrin and metalloproteinase domain has been observed on eosinophils, and the role of MMP-17 on extracellular matrix digestion remains undefined (27).

CD4 is the only described IL-16R, although other mechanisms for IL-16 actions are suspected (17, 28). It has been shown that IL-16-elicted eosinophil chemotaxis was CD4-dependent (11). In this study, we evaluated the means by which IL-16 activates proteases and elicits orientated migration of eosinophils within extracellular matrix component. Treatment of eosinophils with CD4-blocking Abs largely decreased IL-16-elicted migration of eosinophil from both normal and asthmatic subjects. This inhibition was similar to the anti-CD4 Ab-induced decrease in LTC4 and IL-4 release reported by Bandeira-Melo et al. (23). These data show that IL-16-elicted eosinophil migration was mediated at least in large part via CD4 expressed on eosinophils.

Previous studies showed that IL-16-elicted chemotaxis of eosinophils was CD4 dependent (11), and that not only anti-CD4 Fab...
but also recombinant soluble CD4 and an octapeptide (peptide 2) derived from the D4 region of CD4 molecule inhibited the actions of IL-16 on human eosinophils (23). Although these previous data and our observations support a central role for CD4 as receptor for IL-16 on eosinophils, we cannot exclude an alternative receptor for IL-16-induced signaling (17, 28). The increase of cell surface CD4 expression observed with TNF pretreatment was higher (9-fold) than the increase of migration (2-fold). Moreover, the migration rate observed in absence of TNF was greater than it could be expected from the measured baseline eosinophil CD4 expression. These observations suggested that eosinophil CD4 expression increased during the IL-16 elicited migration. Interestingly, analysis of permeabilized eosinophils demonstrated the presence of large intracellular pool of CD4. Specific experiments demonstrated that IL-16 itself was capable of eliciting eosinophil surface CD4 expression likely amplifying the cell response to the cytokine. Note that it is logical to assure that the presence of IL-16, which links to CD4 in culture medium, could decrease surface CD4 detection. However, previous data demonstrating induction of RANTES and IL-4 release by IL-16 within 1 h (23) and our observation of eosinophil migration through Matrigel within 6 h strongly suggests that very low levels of CD4 present on most cells at baseline are capable of mediating the effects of IL-16 on eosinophils. These observations further support the central role of CD4 in the action of IL-16 on eosinophils. Studies are currently under way to investigate furthermore the modulation of cell surface CD4 expression by IL-16.

Bandeira-Melo et al. (23) showed that IL-16 elicits the release of IL-4 via an autocrine effect of eotaxin and RANTES. Herein, IL-16-induced migration was significantly decreased by mAbs against CCR3. We found no binding of IL-16 to this receptor. Moreover, the effect of IL-16 on migration was significantly inhibited by mAbs against eotaxin and RANTES, further demonstrating the role of these chemokines in the IL-16 elicited eosinophil migration through Matrigel. Consequently, these data suggest that eotaxin and RANTES are functioning as autocrine mediators not only for the release of IL-4 and LTC4, as shown by Bandeira-Melo et al. (23), but also for the activation of proteases for migration into extracellular matrix. Based on previous works (11, 23) and our current observations, we propose the diagram presented in Fig. 6 to summarize the documented IL-16 signaling steps.

In conclusion, this study shows that IL-16 is a potent promoter of eosinophil migration through the extracellular matrix components and provides insights into the mechanisms that ended up in the up-regulation protease activities. It demonstrates how IL-16 could play an important role in the recruitment of eosinophils into tissue in eosinophilic diseases such as asthma and allergy as previously suggested (14).

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