

Eotaxin induces a sustained reduction in the functional adhesive state of very late antigen 4 for the connecting segment 1 region of fibronectin

K.-L. Paul Sung, PhD,^a Li Yang, BS,^a John Kim, BS,^a Derek Ko, BS,^a Gregory Stachnick, BS,^b Diego Castaneda, BS,^b Jyothi Nayar, BS,^b and David H. Broide, MBChB^b La Jolla, Calif

Background: Eosinophils that have bound to extracellular matrix proteins, such as the connecting segment 1 (CS-1) region of fibronectin, need to deadhere before undergoing chemotaxis through the extracellular matrix.

Objective: We have investigated whether eotaxin can regulate the strength of eosinophil adhesion to the CS-1 region of fibronectin.

Methods: We have used a micropipette single-cell adhesion assay to determine the force of eosinophil adhesion to the CS-1 region of fibronectin.

Results: Eosinophils bound to CS-1 with high avidity, and this binding could be inhibited with neutralizing antibodies to $\alpha 4$ integrins expressed by eosinophils or with neutralizing antibodies to CS-1. Eosinophils incubated in the presence of eotaxin demonstrated a transient increase in the force of eosinophil adhesion to CS-1, which was followed by a more sustained reduction in the force of eosinophil adhesion to CS-1, as assessed in the micropipette single-cell adhesion assay. This decreased binding of eosinophils to CS-1 was not due to alterations in very late antigen 4 (VLA-4) receptor number, as assessed with FACS analysis, or alterations in VLA-4 receptor distribution, as assessed with immunofluorescence microscopy. **Conclusions:** These studies suggest that eotaxin can cause a transient increase followed by a more sustained reduction in the functional force of VLA-4 adhesion to CS-1 and thus promote deadhesion of CS-1 adherent eosinophils in the extracellular matrix. (J Allergy Clin Immunol 2000;106:933-40.)

Key words: Eosinophil, adhesion, integrin, eotaxin

Eosinophil recruitment to tissue sites of inflammation is a multistep process during which eosinophils use adhesion receptors to bind to counterreceptors expressed by inflamed vascular endothelium.¹⁻³ Once eosinophils have exited the blood vessel at sites of allergic inflammation, they enter the extracellular matrix where they can bind to

Abbreviations used

CS-1: Connecting segment 1
GTP: Guanosine triphosphate
MAPK: Mitogen-activated protein kinase
MCP-3: Monocyte chemoattractant protein 3
VLA-4: Very late antigen 4

matrix proteins, such as fibronectin. The firm adhesion of eosinophils to fibronectin is mediated through the binding of eosinophil-expressed very late antigen 4 (VLA-4) to the connecting segment 1 (CS-1) region of fibronectin.^{4,5} The locomotion of leukocytes through the extracellular matrix has best been studied in neutrophils, although there are limited studies in eosinophils. Neutrophil chemotaxis is characterized initially by cell polarity, the most conspicuous feature of which is the development of an organelle-free, thin, veil-like structure termed the leading lamellae that extends from the organelle-rich cell body in the direction of movement.⁶ As the lamellae advance, they consolidate their forward progress by transient attachment to the underlying surface.⁶

Although this sequence of leukocyte lamellae locomotion at the leading edge is well studied, there is little information available about the initial step of deadhesion from the extracellular matrix by eosinophils (or other leukocytes), which would be required before locomotion could occur. In order for such a firmly bound eosinophil to undergo chemotaxis through the extracellular matrix in response to a chemotactic signal, it would need to first deadhere before initiating locomotion. Several potential mechanisms could allow for deadhesion, including a redistribution of cell surface adhesion receptors, a downregulation in the number of adhesion receptors, and/or a reduction in binding affinity of the adhesion receptor. Because the eosinophil-expressed integrin VLA-4 can change its conformation to bind with low or high affinity to counterligands, such as the CS-1 region of fibronectin,^{7,8} we have investigated whether an eosinophil lineage-specific chemokine eotaxin could decrease the affinity of VLA-4 for its counterligand CS-1, and we suggest one potential mechanism responsible for the deadhesion of eosinophils from fibronectin before chemotaxis.

From ^aBioengineering and Orthopedics and ^bthe Department of Medicine, University of California San Diego, La Jolla.

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Reprint requests: David Broide, MBChB, University of California San Diego, 9500 Gilman Dr, (MC 0635), La Jolla, CA 92093-0635.

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Eotaxin, an 8-kd protein that belongs to the C-C chemokine family,^{9,10} regulates eosinophil trafficking by binding to a specific 7-transmembrane-spanning, G protein-linked, CCR-3 receptor¹¹⁻¹³ present on eosinophils. The potential for eotaxin to interact with eosinophils in the extracellular matrix is suggested from studies demonstrating eotaxin messenger (m)RNA expression at sites of eosinophilic inflammation after allergen challenge.^{14,15} Eosinophil recruitment is significantly inhibited in eotaxin-deficient mice¹⁴ or in mice treated with neutralizing antibodies to eotaxin.¹⁵ Although the ability of eotaxin to act as a chemoattractant *in vitro*^{9,10} and *in vivo*^{14,15} is well recognized, this study demonstrates that eotaxin may also act to assist chemotaxis in the extracellular matrix by promoting deadhesion of eosinophils.

METHODS

Reagents

The 25 amino acid sequence of CS-1 was synthesized by Cytel Corporation (San Diego, Calif) on the basis of published sequences of CS-1.¹⁶ Neutralizing antibodies to CS-1 (mAb 90.45) and to $\alpha 4$ integrin (mAb P4C2) were obtained from Dr Mariano Elices (Cytel).⁴ Eotaxin was purchased from R&D (Minneapolis, Minn).

Eosinophils

Eosinophils were purified from the peripheral blood of 8 patients who had mild allergic rhinitis in a protocol approved by the University of California San Diego Human Subjects Committee. Eosinophils with greater than 95% purity and greater than 95% viability were recovered by means of negative selection with a Magnetic Assembly Cell Separator (Miltenyi Biotec, Auburn, Calif) and magnetized anti-CD16 antibody, as previously described in this laboratory.¹

Eosinophil adhesion to CS-1

A saturating concentration of CS-1 (5 $\mu\text{g/mL}$) was coated onto a glass coverslip precoated with 5 $\mu\text{g/mL}$ polylysine, as previously described in this laboratory.⁴ In brief, the saturating concentration of CS-1 was determined in a 30-minute static adhesion assay in which the number of eosinophils adherent to varying concentrations of CS-1 was assessed by light microscopy. A CS-1 dose-response curve demonstrated that increasing numbers of eosinophils bound to CS-1 until a saturating concentration of CS-1 (5 $\mu\text{g/mL}$) was attained, above which there was no increase in eosinophil binding to CS-1.⁴ Nonspecific binding on CS-1-coated coverslips was reduced by blocking noncoated sites on the coverslips with 0.5% BSA and by using neutralizing antibodies to CS-1 and $\alpha 4$ to demonstrate binding specificity.

Single eosinophil micropipette adhesion assay

The procedure of micropipette aspiration has been described previously.^{4,17,18} In brief, a micropipette with an internal radius of 1.6 to 3.3 μm is manipulated by using a hydraulic micromanipulator (Narishige Scientific Instrument Laboratory, Tokyo, Japan) mounted on the stage of an inverted microscope. The tip of the pipette is positioned in the cell chamber, and the wide end of the pipette is connected to a pressure regulation system. Eosinophils adhering to CS-1 are randomly chosen and held at the tip of the pipette by the application of an initial aspiration pressure. The aspiration pressure in the pipette is increased stepwise with increments of 100 to 500 dyne/cm². At each pressure level, the pipette is pulled away gradu-

ally by means of micromanipulation. For low pressures, such pulling causes the eosinophil to slip out of the pipette while remaining adherent to CS-1. At a sufficiently high pressure level, the eosinophil becomes completely separated from CS-1. The minimum aspiration pressure that leads to the total separation of the eosinophil from CS-1 is referred to as the critical separation force (F_c). The critical separation force is calculated as follows: $F_c = (P_c)(\pi R_p^2)$, where P_c is the critical separation pressure, and R_p is the radius of the pipette.^{17,18} These studies are performed at room temperature. The adhesion force between eosinophil and CS-1 is measured at 1-minute intervals by means of micromanipulation over a period of up to 105 minutes. The experiment is recorded on videotape, and the time course of adhesion is obtained by analyzing the recorded video images.

Eotaxin stimulation of eosinophils

Eosinophils (1×10^6) were incubated in the presence or absence of 1 to 10 ng/mL eotaxin for 10 to 30 minutes at 37°C before the micropipette adhesion assay.

Immunofluorescence detection of VLA-4 expression by eosinophils

The distribution of eosinophil $\alpha 4$ integrin receptors after incubation with eotaxin was determined by using immunofluorescence microscopy, as previously described.¹ Eosinophils incubated for 30 minutes in the presence or absence of 10 ng/mL eotaxin were adhered on CS-1-coated slides. After blocking nonspecific binding sites with 10% BSA for 15 minutes, the slides were incubated for 1 hour with 5 $\mu\text{g/mL}$ of an FITC-labeled mouse anti-human $\alpha 4$ mAb (Gibco BRL, Gaithersburg, Md) or a species and isotype-matched IgG mouse antibody. The slides were washed, mounted in PBS glycerol, and stored in the dark until viewed by using a Nikon Diaphot-TMD fluorescence microscope (Nikon, Melville, NY) with a 100 $\times$ magnification objective.

FACS analysis of $\alpha 4$ integrin expression

Expression of the $\alpha 4$ subunit of VLA-4 on eosinophils was assessed on a FACStar flow cytometer (Becton Dickinson, San Jose, Calif). An FITC-labeled anti-VLA-4 mAb P4G9 and an irrelevant, nonbinding, isotype-matched IgG2b control mAb were used for flow cytometric determination of cell surface expression, as described previously.¹ A saturating concentration of fluorescent mAb P4G9 or control antibody was added to 50 μL of eosinophils (5×10^5 cells) and incubated for 30 minutes. In some experiments eosinophils were incubated in the presence or the absence of varying concentrations (0-10 ng/mL) of eotaxin for 30 minutes at room temperature before incubation with FITC-labeled mAbs. Surface expression was determined after gating for eosinophils by using characteristic forward and side light scatter. Results are expressed as the median fluorescence channel numbers.

Statistical analysis

ANOVA single-factor analysis was used to determine whether there were statistically significant differences between groups. A P value of less than .05 was considered statistically significant. All results are reported as means $\pm$ SEM unless otherwise indicated.

RESULTS

Eosinophil adhesion to CS-1

The adhesion force exerted between each eosinophil and CS-1 was measured by using the separation force. The separation force is calculated by stepwise increases

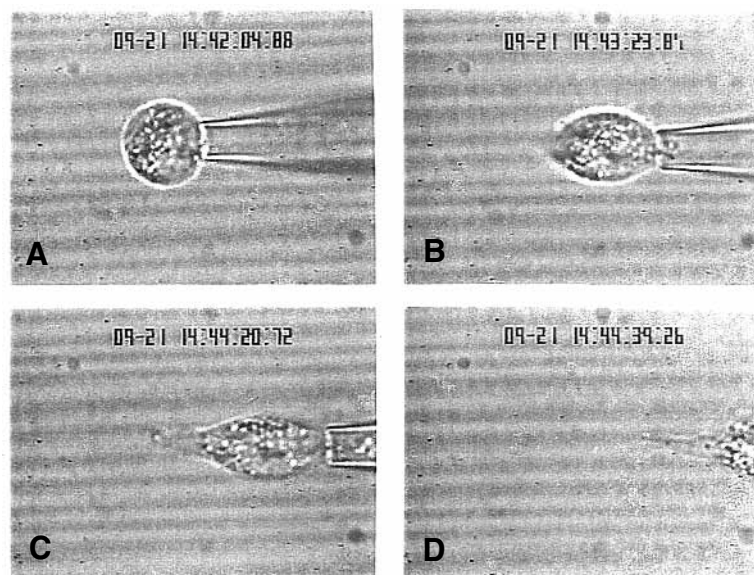


FIG 1. Single-cell micropipette adhesion assay. In the single-cell micropipette adhesion assay eosinophils are allowed to adhere to CS-1-coated coverslips for 30 minutes. The micropipette is manipulated to capture a randomly adherent eosinophil. The aspiration pressure in the pipette is increased stepwise with increments of 100 to 500 dyne/cm². At each pressure level, the pipette is pulled away gradually by means of micromanipulation (**A**, **B**, and **C**) until at sufficiently high pressure, the eosinophil adherent to CS-1 becomes detached (**D**). The minimum aspiration pressure that leads to the total separation of the eosinophil from CS-1 is referred to as the critical separation pressure from which the critical separation force is calculated.

in the aspiration pressure of the micropipette positioned on the eosinophil cell surface (Fig 1). At each pressure level, the pipette holding the eosinophil is pulled away gradually (at a rate of approximately 0.25 mm/s). For low pressures, such pulling causes the eosinophil to slip out of its pipette while remaining conjugated with CS-1. With a sufficiently high pressure (critical pressure) in the pipette attached to the eosinophil, the eosinophil will completely detach from CS-1 (Fig 1).

In preliminary studies examining the interaction of eosinophils with CS-1, the mean adhesion force of eosinophils and CS-1 was 0.18 ± 0.01 mdynes ($n = 145$ eosinophils), which was significantly greater than the strength of adhesion to control non-CS-1 coated coverslips (0.01 ± 0.01 mdynes, $P = .0001$). The eosinophil CS-1 adhesion force (0.18 ± 0.01 mdynes) was inhibitable by neutralizing antibodies to CS-1 (mean adhesion force, 0.03 ± 0.005 mdynes; $P = .0001$) or by neutralizing antibodies to $\alpha 4$ (mean adhesion force, 0.10 ± 0.01 mdynes; $P = .0001$).

Effect of eotaxin on eosinophil adhesion to CS-1

In initial experiments we investigated the kinetics of eosinophil adhesion to CS-1 in the presence or absence of eotaxin. The kinetics of eosinophil adhesion to CS-1 in the absence of eotaxin appeared to follow a curve characterized by an initial increase in force of adhesion from 15 to 30 minutes, a plateau in the force of adhesion from 30 to 60 minutes, and a further increase in the force

of adhesion after 60 minutes of conjugation (Fig 2). We have focused our studies of eotaxin modulation of eosinophil adhesion to CS-1 especially on the plateau phase of stable eosinophil CS-1 adhesion, although eotaxin also reduced the force of eosinophil adhesion to CS-1 at other time points studied. In the absence of eotaxin, the force of eosinophil adhesion to CS-1 was stable between 30 to 60 minutes after eosinophils had been added to CS-1 (Figs 2 and 3).

Eotaxin (10 ng/mL) significantly reduced the force of eosinophil adhesion to CS-1 during the plateau phase of eosinophil adhesion to CS-1 (30-60 minutes, Fig 2), as well as during the subsequent time period (60-105 minutes, Fig 2) when eosinophil adhesion to CS-1 in the absence of eotaxin continued to strengthen. Increasing the concentration of eotaxin above 10 ng/mL did not further reduce the force of eosinophil adhesion to CS-1 (data not shown).

In the aforementioned experiments (Fig 2), eosinophils were incubated with eotaxin for 30 minutes before the CS-1 adhesion assay. To evaluate whether a shorter period of incubation of eosinophils with eotaxin would induce similar changes in eosinophil adhesion to CS-1, eosinophils were incubated in the presence or absence of 10 ng/mL eotaxin for 10 minutes, and the force of eosinophil adhesion to CS-1 was assessed. Fig 3 demonstrates that a 10-minute incubation of eosinophils with eotaxin also reduced the force of eosinophil adhesion to CS-1 as effectively as a 30-minute incubation at time points from 30 to 75 minutes after adhesion.

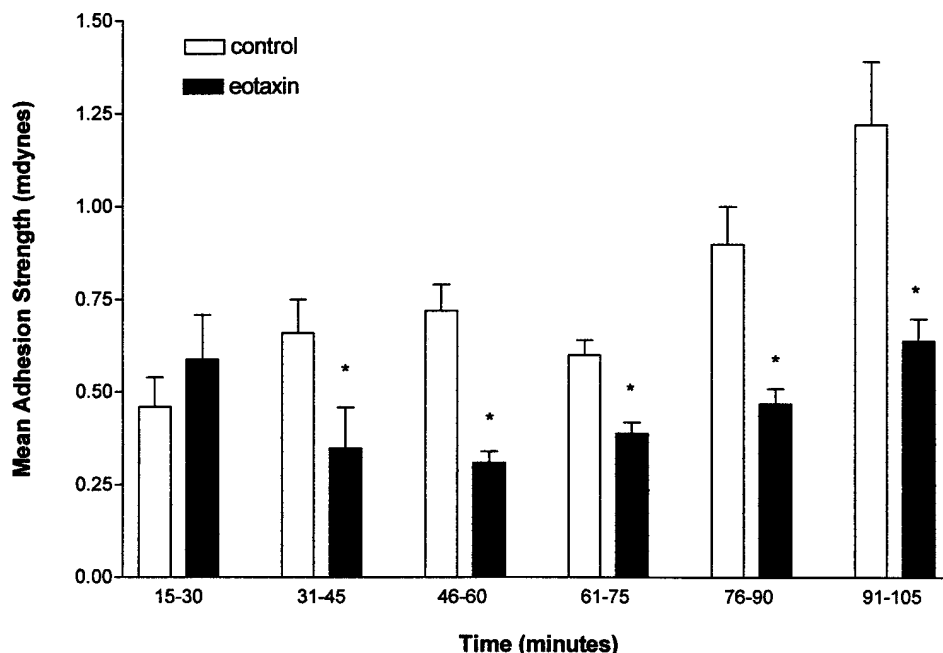


FIG 2. Eotaxin induces a sustained reduction in the force of eosinophil adhesion to CS-1. Eosinophils were incubated in the presence or absence of eotaxin (10 ng/mL) for 30 minutes before adhesion to CS-1. The mean adhesion force of eosinophils to CS-1 was assessed by using the micropipette adhesion assay at varying time points (15-105 minutes after adhesion of eosinophils to CS-1). Compared with control, eotaxin reduced the mean adhesion force of eosinophils to CS-1 at the time points indicated (control, $n = 5$ separate experiments; * $P < .05$).

Eotaxin induces an initial transient increase in eosinophil adhesion to CS-1

Our initial experiments (Fig 2) demonstrated that eotaxin induced a sustained reduction in eosinophil adhesion for at least 75 minutes (from 31-105 minutes, Fig 2). Because we also noted that eotaxin induced a transient slight increase in the strength of eosinophil adhesion to CS-1 at 15 to 30 minutes ($P =$ not significant, Fig 2), we evaluated earlier time points (0-10 minutes, 10-20 minutes, and 20-30 minutes). These studies demonstrated that eotaxin induced a transient increase in the strength of eosinophil adhesion to CS-1, which was most prominent at 0 to 10 minutes ($P = .05$, Fig 4). Although eotaxin induced a slight increase in the strength of eosinophil adhesion to CS-1 at later time points studied (ie, 11-20 minutes and 21-30 minutes), this was not statistically significant compared with control eosinophils (Fig 4).

Effect of eotaxin on the distribution and level of $\alpha 4$ expression by eosinophils

To determine whether eotaxin modulated the number or distribution of $\alpha 4$ integrin receptors, eosinophils incubated in the presence of eotaxin were processed for FACS analysis and immunofluorescence microscopy. FACS analysis (Fig 5) demonstrated no difference in the level of $\alpha 4$ integrin expression by eosinophils cultured in the presence (mean channel fluorescence, 12.0) or absence (mean channel fluorescence, 12.2) of eotaxin. Immunofluorescence microscopy (Fig 6) did not reveal

any change in the distribution of $\alpha 4$ integrins in eotaxin-treated compared with control eosinophils.

DISCUSSION

In this study we have demonstrated that eotaxin induces a sustained reduction in the force of eosinophil adhesion to the CS-1 region of fibronectin. In addition, we have demonstrated that eotaxin induces a transient increase in adhesion of eosinophils to CS-1 when eosinophils initially adhere to CS-1. Because eosinophils use $\alpha 4$ integrins to bind to the CS-1 region of fibronectin, these studies suggest that eotaxin modulates the $\alpha 4$ integrin CS-1 interaction. At sites of allergic inflammation, eotaxin may therefore not only be acting as an eosinophil chemoattractant by inducing directed migration of eosinophils along a chemotactic gradient but also acting indirectly to assist chemotaxis by promoting the deadhesion of eosinophils from extracellular matrix proteins, such as the CS-1 region of fibronectin.

The ability of $\beta 1$ integrins, such as VLA-4 ($\alpha 4\beta 1$), to modulate their binding affinity has previously been demonstrated by using both biophysical measurements,⁴ as used in this study, and immunologic approaches in which the expression of new $\beta 1$ integrin antigenic epitopes associated with changes in $\beta 1$ integrin-binding affinity have been demonstrated.^{19,20} Upregulation of $\beta 1$ integrin binding in eosinophils can be stimulated by extracellular ions (Mg^{2+} , Ca^{2+} , and Mn^{2+}),²¹ certain anti-integrin activating antibodies,²² and cytokines, such as

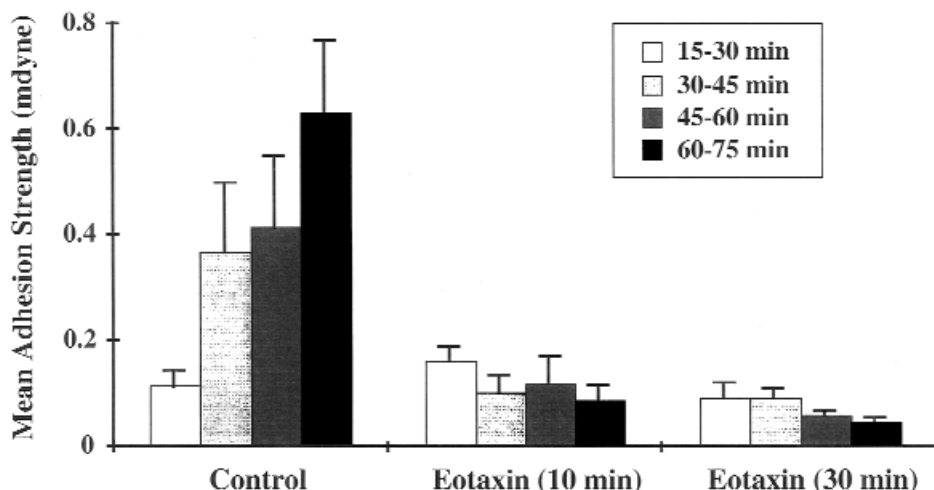


FIG 3. Effect of the duration of eosinophil incubation with eotaxin on eosinophil adhesion to CS-1. Eosinophils were incubated in the presence or absence of eotaxin (10 ng/mL) for either a brief 10-minute period or a 30-minute period before adhesion to CS-1. The mean adhesion force of eosinophils to CS-1 was assessed by using the micropipette adhesion assay at varying time points (15-75 minutes after adhesion of eosinophils and CS-1). Compared with control, eotaxin (10- or 30-minute incubation with eosinophils) did not reduce the mean adhesion force of eosinophils to CS-1 at 15 to 30 minutes after adhesion ($P = \text{not significant}$). However, from 30 to 75 minutes, both a 10-minute and 30-minute incubation of eosinophils with eotaxin reduced the force of eosinophil adhesion to CS-1 ($P = .05$; control, $n = 31$ eosinophils/time point; eotaxin 10 minutes, $n = 30$ eosinophils/time point; eotaxin 30 minutes, $n = 32$ eosinophils/time point).

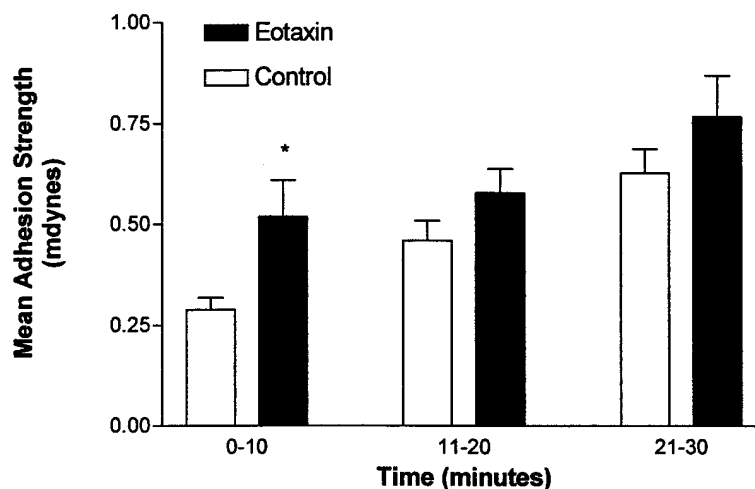


FIG 4. Eotaxin induces a transient increase in the force of eosinophil adhesion to CS-1. Eosinophils were incubated in the presence or absence of eotaxin (10 ng/mL) for 30 minutes before adhesion to CS-1. The mean adhesion force of eosinophils to CS-1 was assessed by using the micropipette adhesion assay at varying time points (0-10 minutes, 10-20 minutes, and 20-30 minutes after adhesion of eosinophils to CS-1). Compared with control, eotaxin increased the mean adhesion force of eosinophils to CS-1 at 0 to 10 minutes (control, $n = 5$ separate experiments; * $P < .05$).

GM-CSF.⁴ Freezing VLA-4 in a high-affinity state with an activating $\beta 1$ mAb has been shown to block eosinophil migration across fibronectin or endothelium.²² We have also previously demonstrated that GM-CSF upregulates the binding of eosinophils to vascular cell adhesion molecule 1 or CS-1 by upregulating the binding affinity of VLA-4 for vascular cell adhesion molecule 1 or CS-1.⁴ This GM-CSF-induced increase in VLA-4-binding affinity is not associated with any

changes in the number of VLA-4 receptors expressed or VLA-4 receptor distribution, suggesting that a conformational change in the VLA-4 receptor may be responsible for the change in binding affinity. Thus GM-CSF and eotaxin are examples of cytokines and chemokines that have differing effects in regulating the strength of VLA-4 adhesion to CS-1.

Eotaxin binds to a 7-transmembrane G protein-coupled CCR-3 receptor expressed by eosinophils.

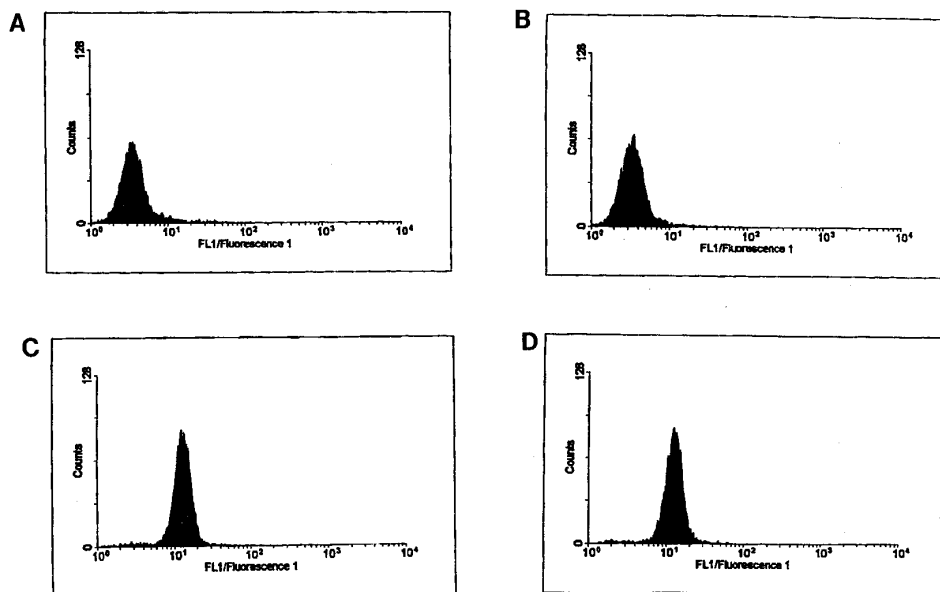


FIG 5. Effect of eotaxin on $\alpha 4$ integrin expression by eosinophils, as assessed by FACS. Eosinophils were incubated in the presence or absence of 10 ng/mL eotaxin for 30 minutes before determination of $\alpha 4$ integrin expression by using FACS analysis with an FITC-labeled mouse anti-human $\alpha 4$ integrin mAb. Eosinophils incubated with control IgG antibody and without eotaxin (**A**) and eosinophils incubated with control IgG antibody and with eotaxin (**B**) demonstrate minimal background mean channel fluorescence of 4.1 and 3.7, respectively. There was no significant difference in $\alpha 4$ integrin expression in eosinophils cultured without eotaxin (mean channel fluorescence 12.2, **C**) compared with eosinophils cultured with eotaxin (mean channel fluorescence 12.0, **D**).

Human eosinophils have been shown to express high levels of the CCR-3 receptor (40,000-400,000 receptors per cell).^{11,12} Activation of the eosinophil CCR-3 receptor by eotaxin results in Gi protein coupling, calcium transients, activation of protein kinase C and mitogen-activated protein kinase (MAPK), and downregulation of the number of CCR-3 receptors.²³⁻²⁵ Eotaxin reduces surface expression of the CCR-3 receptor on eosinophils to 43% at 15 to 60 minutes after eotaxin exposure, with CCR-3 receptor levels returning to 76% at 3 hours after eotaxin exposure.²⁵ CCR-3 receptor internalization induced by eotaxin is mediated by an endocytic pathway shared with the transferrin receptor.²⁵ CCR-3 receptor internalization is accompanied by partial degradation of CCR-3, and re-expression of CCR-3 is dependent in part on de novo protein synthesis.²⁵ Eotaxin induces rapid activation of several MAPKs, including extracellular signal-regulated kinase 2, p38, p42, and p44.^{23,24} Because the inhibition of MAPKs may modulate cytoskeletal rearrangements necessary for chemotaxis, these MAPKs may be required for eotaxin-stimulated directed locomotion in eosinophils.²⁴

The mechanism by which eotaxin signals to the $\alpha 4$ integrin receptor is not known. Insights into potential signal transduction pathways from the CCR-3 receptor to the $\alpha 4$ integrin cytoplasmic domain (inside-out signaling, Fig 7) are suggested from studies of integrin activation with R-ras.²⁶ R-ras is a guanosine triphosphate (GTP)-binding protein, which enhances integrin ligand

binding activity to extracellular matrix substrates. Blocking endogenous R-ras by expression of dominant negative mutants in a mouse myeloid cell line decreases integrin-mediated adhesion to fibronectin.²⁶ In addition, integrins can be activated by treating cells with nonhydrolyzable GTP analogs that activate GTP-binding proteins,²⁷ further suggesting that GTP might activate the GTP-binding protein R-ras to modulate integrin function. Thus R-ras provides a potential mechanism for signals arising at a cell surface receptor (? CCR-3 receptor) to convert R-ras from the inactive GDP-bound form to the active GTP-bound form, which then modulates integrin ligand binding activity. Further studies are needed to determine whether eosinophils like mouse myeloid cell lines use R-ras to regulate integrin affinity of binding to fibronectin. However, at this time the signal transduction pathway from the eosinophil CCR-3 receptor to the $\alpha 4$ integrin cytoplasmic domain in eosinophils is not known.

Previous studies have demonstrated that RANTES, monocyte chemoattractant protein 3 (MCP-3), and C5a can regulate eosinophil $\beta 1$ integrin avidity.^{28,29} RANTES, MCP-3, and C5a all bind to 7-transmembrane G protein-coupled receptors. RANTES binds to CCR-1, CCR-3, and CCR-5; MCP-3 binds to CCR-1, CCR-2, CCR-3, and CCR-10; and C5a binds to the C5a receptor.³⁰⁻³⁵ This study demonstrates that eotaxin, which binds only to the CCR-3 receptor (and not to alternate CC chemokine or complement receptors, as do

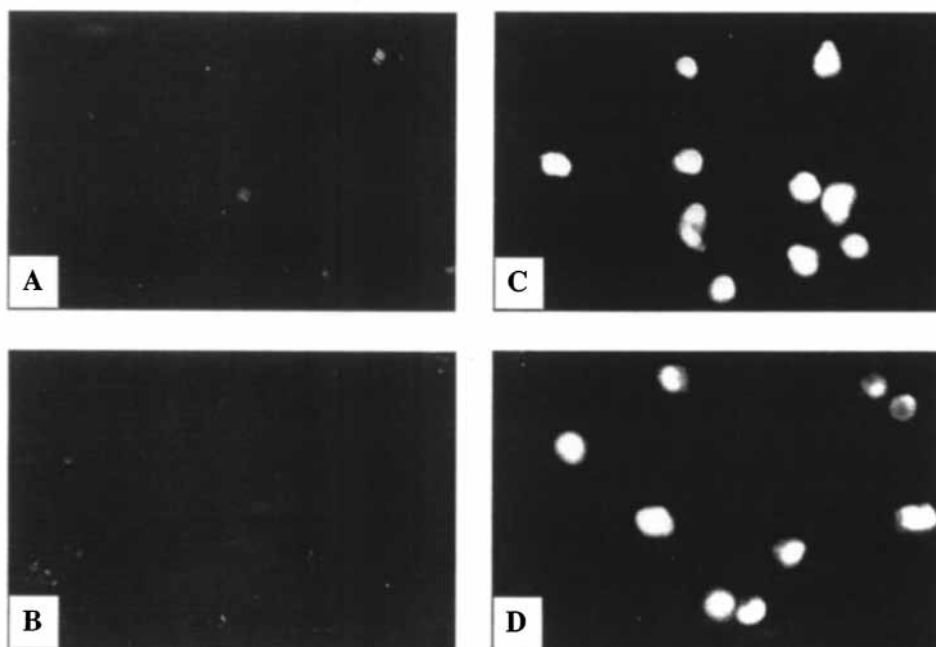


FIG 6. Effect of eotaxin on $\alpha 4$ integrin distribution on eosinophils, as assessed by immunofluorescence microscopy. Eosinophils were cultured in the presence or absence of eotaxin (10 ng/mL) for 30 minutes before incubation with an FITC-labeled mouse anti-human $\alpha 4$ integrin mAb. There was no difference in the level or distribution of expression of $\alpha 4$ integrins on eosinophils cultured without eotaxin (**C**) compared with eosinophils cultured with eotaxin (**D**). In **A** and **B** a control species and isotype-matched antibody has been substituted for the primary anti- $\alpha 4$ mAb, demonstrating the specificity of the $\alpha 4$ staining (magnification 400 $\times$).

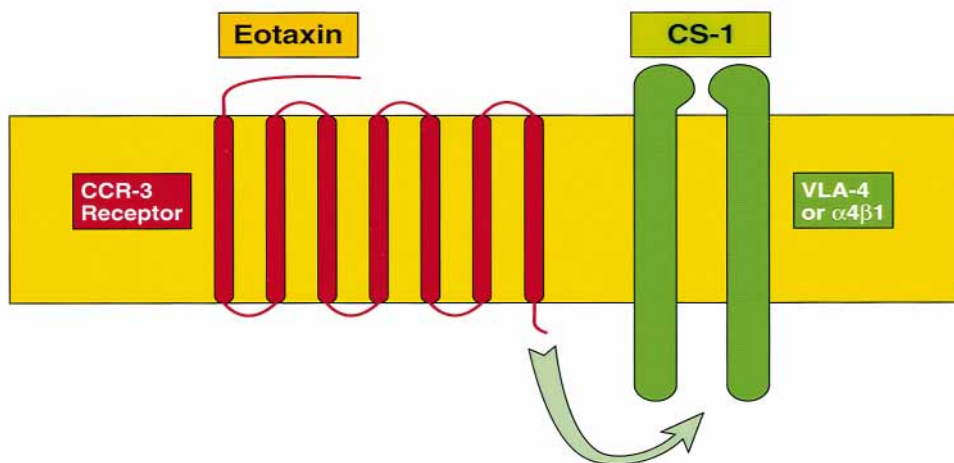


FIG 7. Eotaxin signaling eosinophil VLA-4-mediated adhesion to CS-1. The schematic diagram depicts the potential mechanism by which eotaxin mediates inside-out signaling to the eosinophil VLA-4 receptor. Eotaxin binds to the 7-transmembrane CCR-3 receptor, which mediates inside-out signaling (arrow) to the heterodimeric $\alpha 4\beta 1$ integrin (VLA-4) receptor, resulting in modulation of the binding affinity of VLA-4 for its counterligand the CS-1 region of fibronectin.

RANTES, MCP-3, and C5a), is an additional chemokine that can regulate $\beta 1$ integrin avidity. In addition, this is the first study to demonstrate by direct biophysical measurements that a chemokine such as eotaxin can regulate $\beta 1$ integrin avidity.

In summary, this study demonstrates that eotaxin initially induces a transient increase in the force of adhesion of eosinophil expressed VLA-4 for the CS-1 region of fibronectin, which is followed by a more sustained reduction in the strength of adhesion. This sustained reduced

force of adhesion may provide a mechanism for eosinophils to detach from CS-1 in the extracellular matrix before either initiating or continuing chemotaxis in response to chemokines, such as eotaxin, in the extracellular matrix.

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