Blockade of Focal Clustering and Active Conformation in β2-Integrin-Mediated Adhesion of Eosinophils to Intercellular Adhesion Molecule-1 Caused by Transduction of HIV TAT-Dominant Negative Ras

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Blockade of Focal Clustering and Active Conformation in β2-Integrin-Mediated Adhesion of Eosinophils to Intercellular Adhesion Molecule-1 Caused by Transduction of HIV TAT-Dominant Negative Ras

Shigeharu Myou, Xiangdong Zhu, Evan Boetticher, Saori Myo, Angelo Meliton, Anissa Lambertino, Nilda M. Munoz, and Alan R. Leff

We transduced dominant negative (dn) HIV TAT-Ras protein into mature human eosinophils to determine the signaling pathways and mechanism involved in integrin-mediated adhesion caused by cytokine, chemokine, and chemotractant stimulation. Transduction of TAT-dnRas into nondividing eosinophils inhibited endogenous Ras activation and extracellular signal-regulated kinase (ERK) phosphorylation caused by IL-5, eotaxin-1, and fMLP. IL-5, eotaxin-1, or fMLP caused 1) change of Mac-1 to its active conformation and 2) focal clustering of Mac-1 on the eosinophil surface. TAT-dnRas or PD98059, a pharmacological mitogen-activated protein/ERK kinase inhibitor, blocked both focal surface clustering of Mac-1 and the change to active conformational structure of this integrin assessed by the mAb CBRM1/5, which binds the activation epitope. Eosinophil adhesion to the endothelial ligand ICAM-1 was correspondingly blocked by TAT-dnRas and PD98059. As a further control, we used PMA, which activates ERK phosphorylation by postmembrane receptor induction of protein kinase C, a mechanism which bypasses Ras. Neither TAT-dnRas nor PD98059 blocked eosinophil adhesion to ICAM-1, up-regulation of CBRM1/5, or focal surface clustering of Mac-1 caused by PMA. In contrast to β2-integrin adhesion, neither TAT-dnRas nor PD98059 blocked the eosinophil adhesion to VCAM-1. Thus, a substantially different signaling mechanism was identified for β1-integrin adhesion. We conclude that H-Ras-mediated activation of ERK is critical for β2-integrin adhesion and that Ras-protein functions as the common regulator for cytokine-, chemokine-, and G-protein-coupled receptors in human eosinophils. The Journal of Immunology, 2002, 169: 2670–2676.

Traffic of leukocytes from the vasculature to target tissues during inflammation occurs by a multistep process consisting of rolling, firm adhesion, and diapedesis. These steps are regulated by the sequential activation of adhesive proteins and their ligands on both eosinophils and endothelial cells (1–4). Although leukocytes share a number of recruitment pathways, their responses to chemotactic and inflammatory signals are affected by their qualitative and quantitative expression of adhesion molecules (5, 6).

Integrins are a diverse family of αβ heterodimetric transmembrane adhesion receptors that are important for firm adhesion and subsequent transendothelial migration of leukocytes (5, 7). The β2-integrin subfamily consists of four integrins, CD11a (LFA-1), CD11b (Mac-1), CD11c, and CD11d, which share a common β2 subunit (CD18) and are exclusively expressed on leukocytes (8). The Mac-1 (CD11b/CD18) molecule as well as the very late antigen-4 (VLA-4) (CD49d/CD29) molecule of the β1-integrin subfamily contribute to eosinophil adherence to endothelium through binding to the endothelial ligands ICAM-1 and VCAM-1 (9, 10), in which bidirectional (inside-out and outside-in) transmembrane signal transduction is involved.

Recent studies indicate that cytosolic phospholipase A2 (cPLA2) phosphorylation is essential for integrin-mediated eosinophil adhesion (11, 12). We also have reported previously that pharmacological inhibition of cPLA2 prevents Ag and IL-5-induced eosinophil migration and airway hyperresponsiveness in immune-stimulated guinea pigs (13). However, the process by which cPLA2 is activated to cause integrin-mediated adhesion remains unknown. H-Ras has been reported to cause attenuation of IL-5-mediated survival of eosinophils in vitro at 16 h (14). H-Ras also has been reported to enhance both β1- and β2-integrin-mediated adhesion in nondifferentiated cell systems (15, 16). By contrast, H-Ras has been reported to dowregulate β1-, β2-, and β3-integrin adhesion (17, 18).

To assess the mechanism of β2-integrin-mediated integrin adhesion in human eosinophils, we transduced dominant negative (dn) H-Ras protein using an HIV-TAT protein vector into fully differentiated human eosinophils and examined the mechanism of chemokine,
cytokine, and chemotactic peptides in the induction of adhe-

sion. To examine the role of Ras in eosinophil adhesion, we con-
structed a dnRas plasmid (pTAT-dnRas) containing six His resi-
dues, 11 amino acids of TAT, an N17 dn H-Ras, and purified TAT-dnRas fusion protein (Fig. 1A). The role of H-Ras in adhe-

sion was then determined in TAT-dnRas-transduced eosinophils

stimulated by the cytokine IL-5, the chemokine eotaxin-1, or the

chemoattractant fMLP. We further determined the involvement of

H-Ras in the regulation of Mac-1 expression, conformational

change to the activated state, and lateral clustering of integrin on

the eosinophil surface. Our data demonstrate that Ras is a common

regulator of extracellular signal-regulated kinase (ERK) phosfor-

ylation, which changes β2-integrin into its active conformation and

activates lateral movement of integrin to achieve critical clustering

for adhesion mediated by cytokines, chemokines, and chemotac-

tantic substances.

Materials and Methods

Materials

IL-5, eotaxin-1, soluble human ICAM-1, and soluble human VCAM-1

were purchased from R&D Systems (Minneapolis, MN). Eosinophil iso-

lation materials were purchased from Miltenyi Biotec (Sunnyvale, CA).

fMLP was purchased from Sigma-Aldrich (St. Louis, MO). Polyethylene

96-well microtiter plates were purchased from Costar (Cambridge, MA).

H-Ras cDNA (17N mutant) in pUSEamp, GST-Ras binding domain

(RBD), and anti-Ras mAb (clone RAS10) were obtained from Upstate

Biotechnology (Lake Placid, NY). pcDNA3.1/CT-GFP-TOPO vector was

obtained, as assessed by Wright-Giemsa staining. Cells were kept on ice

until use.

Isolation of human eosinophils

Eosinophils were isolated by a method modified from Hansel et al. (20).

The method is based on Percoll centrifugation (density 1.089 g/ml) to

isolate granulocytes, hypertonic lysis of RBCs, and, finally, immunomag-

netic depletion of neutrophils by a magnetic cell separation system using

anti-CD16-coated MACS particles. Eosinophil purity of 99% was routinely

obtained, as assessed by Wright-Giemsa staining. Cells were kept on ice

for 4 h.

Immunoblot analysis of ERK1/2

Eosinophils (2 × 106/group) were stimulated with 10 ng/ml IL-5 for 10

min, 100 ng/ml eotaxin-1 for 1 min, 1 nM fMLP for 1 min, or 1 nM PMA

for 5 min, and the reaction was stopped by centrifugation at 12,000 × g for

30 s. The pellets were then lysed in 80 μl of lysis buffer (20 mM Tris-HCl

(Ph 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton

X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM

Na3VO4, 1 μg/ml leupeptin, and 1 mM PMSF). After 20 min on ice, the

samples were centrifuged at 12,000 × g for 2 min to remove nuclear and

cellular debris. The supernatants were then mixed with 14 μl of 6× sample

buffer and boiled for 5 min. The samples were collected and kept at

−70°C.

Samples were subjected to SDS-PAGE, using 10% acrylamide gels

downstream (70 g/l) or 10% polyacrylamide gels (5× acrylamide/gel). The

gels were stained with Coomassie blue, destained, and photographed by

using a Transilluminator (Gel Doc II; Bio-Rad, Hercules, CA). The

fluorescence intensity was determined on at least 5000 cells from each

analysis. Aliquots of 5 × 104 cells were analyzed. Flow cytometry was

performed by FACScan (BD Biosciences).

Analysis of surface integrin expression by immunofluorescence

flow cytometry and confocal microscopy

Eosinophils were preincubated with TAT-dnRas or PD98059 for 30 min at

37°C and then stimulated by 10 ng/ml IL-5, 100 ng/ml eotaxin-1, 1 μg/ml

fMLP, or 1 nM PMA for 30 min. Thereafter, cells were centrifuged at

400 × g for 10 min, and the pellets were resuspended in PBS with 1% BSA

for FACS analysis or in PBS with 0.1% BSA for confocal microscope analysis.

Aliquots of 5 × 104 cells were incubated with 10 μg/ml of mAb
directed against Mac-1, CBMR1/5, or isotype-matched control Ab for 30

min at 4°C. After two washes, the cells were incubated with an excess of

FITC-conjugated goat anti-Ig mouse Ab for 20 min at 4°C. The cells were

washed twice, resuspended in 1% paraformaldehyde, and kept at 4°C until

analyzed. Flow cytometry was performed by FACSscan (BD Biosciences).

Fluorescence intensity was determined on at least 5000 cells from each

sample. The results were expressed as the specific mean fluorescence

intensity (MFI) (control Ab fluorescence subtracted).

To confirm the distribution of Mac-1, Mac-1 staining was also per-

formed on cytospin preparations (Cytospin 2; Shandon, Pittsburgh, PA),

and fluorescence was analyzed using an Axiovert confocal microscope

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Ras activation assay

The activated Ras was affinity precipitated following the manufacturer’s instructions (Upstate Biotechnology). Next, the eosinophils (5 × 10^6/group) were incubated with 100 nM TAT-dnRas for 30 min and then stimulated with 10 ng/ml IL-5 for 10 min, 100 ng/ml eotaxin-1 for 1 min, 1 nM fMLP for 1 min, or 1 nM PMA for 1 min. The reaction was stopped by centrifuging at 12,000 × g for 30 s, and the cell pellets were immediately lysed in Ras affinity precipitation lysis buffer (25 mM HEPES, pH 7.5, 150 mM NaCl, 10 mM MgCl_2, 2 mM EGTA, 10% glycerol, 1% Nonidet P-40, 5 μg/ml aprotinin, 10 μg/ml leupeptin, 5 μg/ml pepstatin, 50 mM NaF, 2 mM Na_3VO_4, and 1 mM PMSF). After 20 min on ice, the sample was centrifuged at 16,000 × g for 10 min at 4°C to remove nuclear and cellular debris. The supernatants were then incubated with GST-RBD for 30 min at 4°C with rocking. The GST-RBD beads were washed three times with lysis buffer. Bound proteins were eluted by boiling in 2× Laemmli sample buffer, separated by SDS-PAGE, and processed for immunoblot analysis using an anti-Ras mAb (clone RAS10).

Statistical analysis

All measurements were expressed as mean ± SEM. Variation between two groups was tested using Student’s t test. Variation among more than two groups was tested using ANOVA followed by Fisher’s protected least significant difference. A value of p < 0.05 was accepted as statistically significant.

**Results**

**Transduction of TAT-dnRas into eosinophils**

To access the efficacy of protein transduction, eosinophils were incubated with 100 nM TAT-dnRas (Fig. 1A) for ≤30 min, and samples were analyzed by Western blot. TAT-dnRas in cell lysates was detected within 5 min of incubation; maximal detection occurred within 20–30 min (Fig. 1B). Consequently, a 30-min incubation time was used in the subsequent experiments. Eosinophils were 95% viable during the course of these experiments, as indicated by trypan blue exclusion. TAT-dnRas transduction into eosinophils inhibited IL-5-induced ERK1/2 phosphorylation in a time-dependent fashion. ERK1/2 phosphorylation was reduced at 10 min and blocked completely after 15 min (Fig. 1C, upper panel). TAT-dnRas caused concentration-dependent blockade of IL-5-stimulated ERK1/2 phosphorylation. ERK1/2 phosphorylation was reduced at 30 nM and blocked completely with 100–300 nM (upper panel). TAT vehicle control (Fig. 1D, second from right) did not block ERK phosphorylation. TAT-dnRas alone had no effect on ERK phosphorylation (Fig. 1D, right). These results demonstrated that TAT-dnRas functionally suppressed ERK phosphorylation after transduction into eosinophils using HIV-TAT protein.
Effect of TAT-dnRas on adhesion of eosinophils to ICAM-1
causés by IL-5, eotaxin-1, fMLP, and PMA

We then examined the role of H-Ras in IL-5-, eotaxin-1-, or fMLP-
induced eosinophil adhesion to plated ICAM-1. Adhesion of eosi-

nophils to ICAM-1 was previously confirmed to be mediated by

$\beta_2$-integrin (Mac-1) in our system (12). To examine the potential
role of ERK in eosinophil adhesion, we first used a pharmacolog-
ical inhibitor of mitogen-activated protein/ERK kinase (MEK),
PD98059. This compound inhibits ERK1/2 phosphorylation and acti-

vation (21, 22). In preliminary studies, we determined the con-

centrations of IL-5, eotaxin-1, fMLP, and PMA causing maximal
adhesion of eosinophils to ICAM-1 (see Materials and Methods).
PD98059 blocked adhesion to eosinophils activated by IL-5, eotaxin-1, and fMLP at their most efficacious concentrations in a

concentration-dependent manner (Fig. 2A).

Having demonstrated that 1) ERK activation mediates eosino-

phil adhesion to ICAM-1 (Fig. 2A) and 2) dnRas prevents ERK

phosphorylation (Fig. 1, C and D), we next tested the hypothesis
that TAT-dnRas would block eosinophil adhesion to ICAM-1. Ad-

hesion of nonstimulated eosinophils to ICAM-1 was 3.0 ± 0.8%.
Adhesion caused by 10 ng/ml IL-5, 100 ng/ml eotaxin-1, and 1

$\mu$M fMLP (as above) was blocked by transduction of 100 nM
TAT-dnRas from 30.6 ± 4.5 to 14.1 ± 1.7% ($p < 0.01$), from

28.7 ± 1.6 to 5.7 ± 1.8% ($p < 0.01$), and from 24.4 ± 2.1 to

12.6 ± 1.7% ($p < 0.01$), respectively (Fig. 2B). As a further con-

trol, eosinophils also were pretreated with PMA, which activates
ERK phosphorylation by postmembrane receptor induction of pro-

tein kinase C, a mechanism which bypasses Ras. PD98059

inhibited eosinophil adhesion to ICAM-1, which is not H-Ras mediated, was

not blocked by PD98059 or TAT-dnRas (Fig. 2).

In contrast to $\beta_2$-integrin adhesion, which requires integrin ac-

tivation, $\beta_1$-integrin (VLA-4) adheres to VCAM-1 in its constitu-

tive state (12). Eosinophil adhesion was 24.0 ± 4.3% for VCAM-

1-coated wells vs 2.4 ± 0.5% for buffer-coated control wells ($p <

0.01$) (Fig. 3A). PD98059 had no inhibitory effect on eosinophil

adhesion to VCAM-1 (Fig. 3A). Accordingly, pretreatment with
TAT-dnRas also did not prevent spontaneous eosinophil adhesion
to VCAM-1 (Fig. 3), suggesting that H-Ras is not involved in
VLA-4-mediated eosinophil adhesion (see Discussion).

Effect of TAT-dnRas on endogenous Ras and ERK activation
causés by IL-5, eotaxin-1, or fMLP

We next examined whether TAT-dnRas suppressed endogenous
Ras and ERK activation caused by IL-5, eotaxin-1, or fMLP. Eos-

inophils were pretreated with or without TAT-dnRas for 30 min
at 37°C and then stimulated with 10 ng/ml IL-5, 100 ng/ml
eotaxin-1, 1 $\mu$M fMLP, or 1 nM PMA. Cell lysates were precipi-
tated by a GST fusion protein containing RBD of Raf-1 (amino
acids 1–149 of Raf-1), which binds only to GTP-bound (activated)
Ras, and were probed by a mAb to Ras. IL-5, eotaxin-1, or fMLP
all caused Ras activation (Fig. 4A). Maximum Ras activation oc-
turred at 10 min for IL-5 and at 1 min for eotaxin-1, fMLP, and
PMA. PMA, which caused adhesion that was not blocked by TAT-
dnRas, caused no activation of Ras (Fig. 4A). Ras activation
causés by IL-5, eotaxin-1, or fMLP caused ERK1/2 phosphoryla-
tion in eosinophils, which was blocked by TAT-dnRas (Fig. 4B).

Effect of Ras inhibition on surface Mac-1 (CD11b/CD18)
expression and active conformational change

We next examined the role of three previously postulated mech-

anisms of integrin-mediated adhesion: 1) up-regulation of surface
integrin molecules (23), 2) the affinity/conformational changes of
the $\beta_2$-integrin (10), and 3) the avidity/clustering of surface inte-

grins (24). Accordingly, we first determined the role of H-Ras in

regulating Mac-1 (CD11b/CD18) expression by flow cytometry.
Stimulation of eosinophils with 10 ng/ml IL-5, 100 ng/ml
eotaxin-1, 1 $\mu$M fMLP, or 1 nM PMA all caused increased ex-
pression of the CD11b $\alpha$-chain of Mac-1 on the eosinophil surface
from 38.6 ± 7.9 to 79.0 ± 13.4 sMFI ($p < 0.01$), 61.1 ± 13.4
sMFI ($p < 0.01$), 67.0 ± 11.0 sMFI ($p < 0.01$), and 98.2 ± 13.3
sMFI ($p < 0.01$), respectively (Fig. 5A). Pretreatment of eosi-
nophils with a concentration of 50 $\mu$M PD98059 (Fig. 5A) or 100 nM
TAT-dnRas (Fig. 5B), concentrations which blocked eosinophil
adhesion to ICAM-1 (Fig. 2), did not block up-regulation of
Mac-1.

We next determined whether H-Ras regulates the change to ac-

tive conformation for Mac-1 (CD11b/CD18) caused by IL-5,
eotaxin-1, or fMLP. The activated conformation of Mac-1, as iden-
tified by the mAb CBRM1/5 (10), was induced after treatment with
IL-5, eotaxin-1, fMLP, or PMA (Fig. 5C). Both TAT-dnRas and
PD98059 prevented the active conformation of Mac-1 caused by
PMA as measured by CB1R1/5 expression was not attenuated
by either TAT-dnRas or PD98059 (Fig. 5C).

Involvement of H-Ras in Mac-1 clustering

We further determined the mechanism of the Mac-1 clustering in
eosinophil adhesion using TAT-dnRas. Confocal immunofluores-
cence microscopy demonstrated that IL-5, eotaxin-1, and fMLP all
caused focal clustering of Mac-1 on the eosinophil surface, which
was blocked by pretreatment with TAT-dnRas (Fig. 6) or PD98059
(data not shown). In contrast, PMA caused Mac-1 clustering and
subsequent adhesion to ICAM-1 that was not inhibited by either

FIGURE 3. Effect of PD98059 and TAT-dnRas on spontaneous eosin-

ophil adhesion to VCAM-1. A, Eosinophils were pretreated with the MEK
inhibitor PD98059 for 30 min at 37°C, and then cells were added into
VCAM-1-coated wells. B, Eosinophils were pretreated with 100 nM TAT-
dnRas or 100 nM TAT-vehicle for 30 min at 37°C, and then cells were
added into wells coated with (filled bars) or without VCAM-1. Eosinophil
adhesion was measured by residual eosinophil peroxidase activity as de-

scribed in Materials and Methods. NC, Buffer-coated negative controls;
PC, VCAM-1-coated positive controls. Each point represents the mean ±
SEM of four separate experiments.

FIGURE 4. Effect of TAT-dnRas on Ras and ERK activation
causés by IL-5, eotaxin-1, or fMLP.

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TAT-dnRas or PD98059. These data indicate that Mac-1 up-regulation is not the mechanism by which H-Ras causes adhesion of eosinophils to ICAM-1. Rather, Ras-mediated adhesion corresponds to active conformational change and focal clustering of β2-integrin on the eosinophil surface.

Discussion
In this study, we used HIV-TAT transduction of dnRas into fully mature human eosinophils to determine the role and mechanism by which Ras protein regulates β2-integrin adhesion to the endothelial counterligand ICAM-1. By transducing dnRas directly into fully mature human eosinophils using an HIV-TAT fusion protein, we were able to determine the common pathway for induction of eosinophil adhesion to Mac-1 caused by cytokine (IL-5), chemokine (eotaxin-1), and chemoattractant (fMLP) stimulation. TAT-dnRas blocked endogenous Ras activation and subsequent ERK phosphorylation, as predicted from pharmacological studies using an inhibitor of MEK (Fig. 4). For IL-5, eotaxin-1, and fMLP, this substantially attenuated Mac-1 adhesion to ICAM-1 (Fig. 2). These results indicate that Ras may play a critical role in eosinophil adhesion caused by inside-out signaling from cytokine, chemokine, and chemoattractant.

Blockade of ERK phosphorylation caused by dnRas had no effect on up-regulation of Mac-1 after cellular activation. However, dnRas blocked both the induction of active conformation of Mac-1 and the focal lateral clustering of Mac-1 (Fig. 6) as assessed by confocal microscopy and immunofluorescent staining after activation by IL-5, eotaxin-1, and fMLP. This is the first demonstration of a common activation pathway for both induction of the active conformation of Mac-1 and the clustering distribution of adhesion molecules in eosinophils. The relative importance of each of these two mechanisms was not defined in this investigation. It is important to note, however, that the binding of mature human peripheral
blood eosinophils by VLA-4 to VCAM-1 is regulated independently of Ras (Fig. 3) and that our data demonstrate unique and separate mechanisms for cell membrane adhesion of $\beta_2$- and $\beta_1$-integrin to their respective counterligands, ICAM-1 and VCAM-1.

We have shown previously that both $\beta_2$- and $\beta_1$-integrin-mediated adhesion depend critically upon the phosphorylation of the 85-kDa cPLA$_2$ (12) and that eosinophil-mediated adhesion to fibronectin is critically dependent upon ERK phosphorylation (11). However, at the cell membrane, neither ERK inhibition nor dnRas caused any blockade of adhesion of mature human eosinophils to VCAM-1. Thus, a separate mechanism exists at the cell surface for regulation adhesion of VLA-4, which is constitutively expressed in its active state, and for Mac-1 adhesion, which must be activated from its dormant state. Although the mechanism potentially regulating Mac-1 adhesion appears to involve Ras activation as a critical pathway, these studies do not elucidate the mechanism by which VLA-4 adhesion to VCAM-1 is regulated.

It is important to recognize some limitations of these findings. Adhesion in vivo occurs during a state of constant flow and is a multistep process. The extent to which investigations using plated ligands replicate events in the living state, under conditions of constant flow and shear stress, cannot be assessed for individually plated ligands. However, we have shown previously that inhibition of cPLA$_2$ activity, which is activated by ERK1/2, prevents eosinophil migration and airway hyperresponsiveness in guinea pigs (13). We also note that blockade back to baseline control levels of $\beta_2$-integrin-

**FIGURE 6.** Distribution of Mac-1 expressed on the eosinophil surface. Aliquots of eosinophils were preincubated with TAT-dnRas or TAT-vehicle control for 30 min and then were stimulated by IL-5, eotaxin-1, fMLP, or PMA for 30 min. Eosinophils were incubated with anti-CD11b mAb followed by FITC-labeled goat anti-mouse IgG. Mac-1 clustering was assessed by confocal microscopy. Images show cell populations (A) and individual representative cells having optimal confocal imaging (B). Mac-1 is localized in clusters (capping) in IL-5-, eotaxin-1-, or fMLP-treated cells, whereas it is dispersed on nonstimulated control cells (data not shown) or cells pretreated with 100 nM TAT-dnRas. Unlike IL-5, eotaxin-1, or fMLP, PMA caused Mac-1 clustering in the presence of TAT-dnRas. The results shown are representative of three different experiments.
ICAM-1 adhesion was complete only for eotaxin-1 (Fig. 2). This may be a consequence of necessity for examining singly the binding of individual integrin/Ig-supergene reactions, or it may imply that Ras mediation of Mac-1 adhesion to ICAM-1 has additional regulatory components.

The sequential events of eosinophil migration and, particularly, the regulation of integrin avidity still are not fully elucidated, and our study has not outlined each step of Ras-mediated Mac-1 adhesion. However, we have demonstrated for the first time two potentially different mechanisms for regulation of eosinophil adhesion by $\beta_2$- and $\beta_3$-integrin in fully mature human eosinophils by HIV-TAT transduction of a dnRas into primary isolates of peripheral blood eosinophils. We demonstrate that Ras-induced adhesion is highly specific for Mac-1 and that VLA-4-mediated adhesion is regulated in a substantially different manner at the cell surface. Our data demonstrate that receptor-mediated activation of $\beta_3$-integrin may be regulated substantially and uniquely by Ras protein and that integrin surface clustering and conformational change rather than increased surface expression of Mac-1 are the critical steps for adhesion of activated eosinophils. Our findings suggest that Ras may be the common regulator for Mac-1-mediated adhesion of eosinophils induced by cytokines, chemokines, and membrane-activating chemotactic factors.

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