Expression of a Functional Laminin Receptor (α6β1, very late activation antigen-6) on Human Eosinophils

By Steve N. Georas, Bradley W. McIntyre, Motohiro Ebisawa, John L. Bednarczyk, Sherry A. Sterbinsky, Robert P. Schleimer, and Bruce S. Bochner

The mechanisms responsible for the accumulation of eosinophils at sites of allergic and other inflammatory reactions are unknown, but recent studies have implicated both eosinophil and endothelial adhesion molecules in this process. However, less well studied have been the adhesive interactions between eosinophils and the subendothelial basement membrane and interstitial connective tissues. To test the hypothesis that eosinophils might interact with extracellular matrix proteins, we analyzed purified human eosinophils for the expression and function of various β1 integrins. Using indirect immunofluorescence and flow cytometry, purified eosinophils from mildly allergic donors were found to consistently express the integrin subunits β1 (CD29), α4 (CD49d, very late activation antigen [VLA]-4a), and α6 (CD49f, VLA-6a). No significant expression of the α1, α2, α3, α5, or β2 subunits was detected. Platelet contamination of the eosinophil preparations was excluded by light microscopy and by the inability to detect expression of platelet glycoproteins αIIb, CD41b, and CD42b. Immunoprecipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of eosinophils confirmed the expression of cell-surface β1, α4, and α6. Furthermore, eosinophils purified from allergic donors were shown to adhere to plate-bound laminin, but not to type 1 or type 4 collagen. Adhesion to laminin was concentration-dependent, required divalent cations, and was completely and specifically inhibited by the anti-α6 monoclonal antibody (MoAb) GoH3 and by the anti-β1 MoAb 33B6. Interestingly, the anti-β1 MoAb 18D3 (which like 33B6 blocks eosinophil binding to VCAM-1) did not inhibit eosinophil adhesion to laminin, suggesting that there are functionally distinct epitopes on the β1 subunit. Eosinophils purified from 4 healthy, nonallergic donors also showed α6-dependent adhesion to laminin, although these cells adhered less well. These studies establish the expression of α6β1 on human eosinophils and document its function as a laminin receptor. Interaction of eosinophil α6β1, with laminin, eg, in basement membranes, may contribute to the localization of these cells at inflammatory sites in vivo.

© 1993 by The American Society of Hematology.

From the Department of Medicine, Divisions of Clinical Immunology and Pulmonary and Critical Care Medicine, Johns Hopkins University School of Medicine, Johns Hopkins Asthma and Allergy Center, Baltimore, MD; and the Department of Immunology, University of Texas M.D. Anderson Cancer Center, Houston, TX.

Submitted October 20, 1992; accepted July 12, 1993.

Supported in part by National Institutes of Health Grants No. AI27429, AR31891, and AI21136, and an Allen & Hanburys Pulmonary Fellowship Award (S.G.). B.S.B. is also supported in part by a Developing Investigator Award from the Asthma & Allergy Foundation of America.

Address reprint requests to Bruce S. Bochner, MD, Assistant Professor of Medicine, Johns Hopkins Asthma and Allergy Center, 5501 Hopkins Bayview Circle, Baltimore, MD 21224-6801.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1993 by The American Society of Hematology.

0006-4971/93/8209-0018$3.00/0

Blood. Vol 82, No 9 (November 1), 1993: pp 2872-2879

OSINOPHILS are now recognized as important effector cells in a variety of immune reactions. In chronic allergic diseases such as asthma, tissue-infiltrating eosinophils are known to contribute to local inflammation through release of granular proteins and bioactive mediators. Recent interest has also focused on the ability of these cells to synthesize proinflammatory cytokines. The mechanisms by which eosinophils emigrate to and accumulate at sites of inflammation are unknown, but mounting evidence supports a role for both eosinophil and endothelial cell (EC) adhesion molecules in these processes. Eosinophils display increased adherence to cytokine-activated EC in vitro, an event now known to be mediated by the endothelial adhesion molecules E-selectin (formerly ELAM-1), intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1). The observation that eosinophils (unlike neutrophils) express the surface molecule α4β1 (very late activation antigen-4 [VLA-4]) suggested that a VLA-4/VCAM-1 pathway might be operational for eosinophil adhesion to activated EC. This hypothesis is supported by the observation that interleukin-4 (IL-4) treatment of EC (a condition known to preferentially induce VCAM-1 expression) results in enhanced adhesion of eosinophils but not of neutrophils, and suggests that this pathway may contribute to the enhanced recruitment of eosinophils to sites of allergic inflammation.
MATERIALS AND METHODS

Monoclonal antibodies. The following monoclonal antibodies (MoAbs) were prepared as described previously.18,19,20,18D3 (IgG1) and 33B6 (both recognizing the β1 subunit), 19H8 and L25 (both IgG1, recognizing the α4 subunit), and P3 (IgG1, an irrelevant control MoAb).19 The following MoAbs were generously provided: H52 (IgG1, recognizing the β2 [CD18] subunit); Dr James Hildreth, Johns Hopkins University, Baltimore, MD);21 and 439-9B (IgG2b, recognizing the β4 subunit); Dr Stephen Kennel, Oak Ridge National Laboratories, Oak Ridge, TN.22 The following MoAbs were purchased: Act-T-Set VLA-1 (mouse IgGl, anti-α1; T Cell Diagnostics, Cambridge, MA); P185 (mouse IgG1, anti-α3; Telios Pharmaceuticals, San Diego, CA); G9 (mouse IgG1, anti-α2), SAM 1 (mouse IgG1, anti-α5), GoH3 (rat IgG2a, anti-α6), AMF/7 (mouse IgG1, anti-αv [CD51]), SZ.21 (mouse IgG1, anti-β3 [CD61]), SZ.22 (mouse IgG1, antiplatelet gpIIb [CD41b]), and SZ.2 (mouse IgG1, antiplatelet gpIIb [CD42b]; all from AMAC Inc, Westbrook, ME). Additional isotype-matched mouse and rat MoAbs (Coulter Immunology, Hialeah, FL, and Pharmingen, San Diego, CA) with irrelevant specificities were used in certain experiments as indicated.

Cell purification. For most experiments, eosinophils were purified from the peripheral blood of allergic donors (n = 12) using density-gradient centrifugation and a negative-selection immunomagnetic bead technique.23 In certain experiments (see below), eosinophils were also purified from healthy, nonallergic donors without peripheral eosinophilia (n = 4) by similar techniques. Purity and viability (determined by Diff-Quik staining of cytospin preparations [Shandon, Pittsburgh, PA] and Erythrosin B exclusion [Sigma Chemical Co, St Louis, MO], respectively) were consistently greater than 95% for these experiments. For adherence assays (see below), eosinophils were labeled with 51Cr sodium chromate (New England Nuclear, Boston, MA) for 30 minutes at 37°C, washed, and resuspended in phosphate-buffered saline containing 130 mg/L CaCl2 and 100 mg/L MgCl2 (PBS-CM: Quality Biologicals Inc, Gaithersburg, MD).

Indirect immunofluorescence and flow cytometry. Purified eosinophils were analyzed for integrin surface expression using indirect immunofluorescence and flow cytometry as previously described.9 Labeled cells were analyzed using an EPICS Profile flow cytometer (Coulter Corp, Hialeah, FL). For each sample at least 5,000 events were collected.

Immunoprecipitation and gel electrophoresis. Eosinophils and cells from the T-lymphocyte cell line HBP-ALL9 were evaluated for surface expression of various integrins. The HPB-ALL cells were maintained in RPMI-1640 medium (Sigma) supplemented with 10% fetal bovine serum, 2 mmol/L L-glutamine, 1 mmol/L sodium pyruvate, 100 U/ml penicillin, and 100 mg/ml streptomycin (GIBCO, Grand Island, NY). The radiolabeling of cell surface proteins was performed as described previously.19 Cells were lysed for 60 minutes at 4°C in 1% Triton-X 100, 10 mmol/L Tris/HC1, pH 8.0, 150 mmol/L NaCl, 1 mmol/L CaCl2, 1 mmol/L MgCl2, 1 mmol/L MnCl2, and 2% bovine serum albumin (BSA; Sigma). After lysis, insoluble debris was removed by centrifugation at 15,000g for 30 minutes, and immunoprecipitation of radio labeled protein was performed as described.24 One-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed under nondenaturing conditions on vertical 7.5% gels according to the method of Laemmli.25 Autoradiography was performed using intensifying screens (Cronex Lightening Plus; Du Pont, Boston, MA) at −80°C with Kodak XR film (Eastman Kodak, Rochester, NY).

Protein adherence assays. Ninety-six-well microtiter plates (Nunc Maxi Sorb Immuno Plate; PGC Sciences Corp, Gaithersburg, MD) were coated overnight (18 to 20 hours) at room temperature with 100-μL aliquots of human laminin (obtained from pepsin digestion of placenta and ≥95% purity by SDS-PAGE [Telios Pharmaceuticals], type 1 collagen [Collaborative Research Inc, Bedford, MA], or type 4 collagen [Telios, San Diego, CA]) diluted in PBS-CM at varying concentrations (from 0.1 to 60 μg/ml). In some experiments, wells were also coated overnight (4°C) with a soluble form of VCAM-1 (a generous gift of Dr Walter Newman, Otsuka America Pharmaceuticals, Rockville, MD) as described.19 The wells were then blocked by incubation with PBS containing 2% BSA for 4 to 6 hours at 37°C to prevent nonspecific adherence to plastic. Control adherence was measured in wells coated overnight with PBS-CM alone and blocked with PBS containing 2% BSA. Wells were washed once, and 100-μL aliquots of the 51Cr-labeled eosinophil suspension (containing 2.5 × 105 cells) were added to wells in triplicate. Cells were allowed to adhere for 30 minutes at 37°C, after which the nonadherent cells were removed by gentle aspiration and rinsing. Adherent cells were then lysed with 1 mol/L NH4OH for 20 minutes at room temperature, and radioactivity of adherent cell lysates was determined using a gamma counter. In some experiments, PBS without calcium or magnesium was used in the adherence assay and in all wash steps. Percent adherence was calculated by comparing the radioactivity of adherent cell lysates with the radioactivity of separate 100-μL aliquots of cells. Percent of control adherence was calculated according to the formula: percentage of control adherence = ([observed adherence]/[control adherence to PBS-CM–coated wells]) × 100.

In blocking experiments, eosinophils were preincubated (4°C for 30 minutes) with saturating concentrations of the indicated intact MoAbs before the adhesion assay.

Statistical analysis. Data are presented as mean ± standard error. Paired Student’s t-test was used to determine significance.

RESULTS

Eosinophil surface expression of integrins. In initial experiments, integrin expression on eosinophils purified from allergic donors was determined using indirect immunofluorescence and flow cytometry. Figure 1A shows representative histograms from a single donor. Consistent with previous observations, eosinophils were found to express the α4 and β1 subunits; however, expression of the α6 subunit can be seen also (Fig 1A). This pattern was consistently observed on eosinophils purified from numerous additional donors (Fig 1B). No significant expression of the α1, α2, α3, or α5 subunits was detected (Fig 1B). The average mean fluorescence intensity (MFI) for α4 and α6 was 8.5 ± 1.1 and 7.2 ± 1.2, respectively (Fig 1B). β1-subunit fluorescence (MFI, 8.3 ± 1.5) was less than that of the β2 subunit (MFI, 67.5 ± 9.8; n = 13). No expression of the β4 subunit was observed after labeling with MoAb 439-9B (n = 4, not shown).

Platelets are known to express large amounts of β1 integrins including α2β1 and α6β1 and can complicate the study of these surface molecules by adhering to eosinophils in vitro.25 However we did not observe platelets adhering to the purified eosinophils used in these studies using light microscopy. To further exclude the possibility that adherent platelets might contribute to our detection of α6β1 expression by flow cytometry, eosinophils were labeled with a...
panel of platelet-specific MoAbs. No significant expression of any of these platelet antigens was detected using MoAbs recognizing the β3 (CD61) or αv (CD51) subunits, or platelet glycoproteins gpIb (CD42b) or gpIIb (CD41b) (n = 4, data not shown).

We next undertook SDS-PAGE analysis of the structure of integrins immunoprecipitated by anti-β1, anti-β4, anti-α4, and anti-α6 MoAb from cell-surface-labeled eosinophils and cells of the T-cell tumor HPB-ALL (Fig 2). Because MoAb 18D3 reacts with the β1 subunit, immunoprecipitates should contain all of the α-β1 heterodimers expressed by a particular cell type. The MoAb 18D3 immunoprecipitate contained bands of 180 kD, 140 kD, 115 kD, 85 kD, and 75 kD from HPB-ALL cells (Fig 2, lane 2). Flow cytfluorometry indicated that HPB-ALL cells expressed the α4 and α6 but not the α2, α3, α5, or αV subunits (data not shown), indicating that the anti-β1 immunoprecipitate contained the α4β1 and α6β1 heterodimers. Little if any of the 200-kD α1 subunit was detected in the anti-β1 immunoprecipitate (Fig 2, lane 2). The SDS-PAGE–band pattern of the α4-specific MoAb L25 immunoprecipitate was nearly identical to that of MoAb 18D3 (Fig 2, lane 4). From previous analyses of αβ1 structure, the two bands of 85 kD and 75 kD found in both the anti-β1 and anti-α4 immunoprecipitates were most likely fragments of the 140-kD α4 subunit. These α4 fragments remain associated with the 115-kD β1 subunit and can be immunoprecipitated by the addition of anti-β1 MoAb. The identity of the 180-kD band found in both anti-β1 and anti-α4 immunoprecipitates is unknown. However, it might be related to the high molecular weight form of α4 described by Hemler et al.

Immunoprecipitation with the anti-α6 MoAb GoH3 immunoprecipitated the 130-kD α6 polypeptide and a 115-kD β polypeptide from the HPB-ALL lysate (Fig 2, lane 3). Because HPB-ALL cells lacked the β4 subunit (Fig 2, lane 5) and the only other β subunit known to associate with α6 is β1, it is, therefore, likely that the 115-kD β subunit seen in the anti-α6 immunoprecipitate is β1.

As expected from the flow cytometry results, the anti-β4 MoAb 439-9B did not immunoprecipitate detectable radio-
Fig 2. The surface proteins of HPB-ALL cells (lanes 1 through 5) and eosinophils (lanes 6 through 10) were surface-labeled with $^{125}$I, and the cells lysed in Triton X-100 with divalent cations as described in Materials and Methods. Immunoprecipitations were performed using P3 ascites (lanes 1 and 6), MoAb 18D3 (anti-$\alpha$1, lanes 2 and 7), MoAb GoH3 (anti-$\alpha$6, lanes 3 and 8), MoAb L25 (anti-$\alpha$4, lanes 4 and 9). and MoAb 439-9B (anti-$\beta$4, lanes 5 and 10). The precipitates were analyzed by SDS-PAGE on a 7.5% polyacrylamide gel under nonreducing conditions.

labeled protein from an eosinophil lysate (Fig 2, lane 10). However, MoAb GoH3 immunoprecipitated two polypeptides of 130 kD and 115 kD (Fig 2, lane 8), suggesting that, similar to HPB-ALL cells, eosinophils express the $\alpha 6\beta 1$ but not the $\alpha 6\beta 4$ heterodimer. The MoAb L25 immunoprecipitate from eosinophils resolved as three diffuse bands of 115 to 150 kD, 85 to 95 kD, and 70 to 75 kD (Fig 2, lane 9). A similar result was observed when the anti-$\beta 1$ immunoprecipitate was analyzed (Fig 2, lane 7). However, a fairly discrete 130-kD band similar to $\alpha 6$ could be detected in the anti-$\beta 1$ immunoprecipitate (Fig 2, lane 7). The diffuse nature of the $\alpha 4$ and $\beta 1$ bands seen after SDS-PAGE analysis was consistently observed in immunoprecipitates from eosinophils. Although the reason for this is unknown, it may reflect heterogeneity in the structure of these polypeptides at the level of posttranslational processing events.

**Eosinophil $\alpha 6\beta 1$ is a laminin receptor.** Because $\alpha 6\beta 1$ has been reported to be a laminin receptor on other cell types, we investigated the ability of eosinophils to adhere to plastic wells coated with human laminin. As shown in Fig 3, eosinophils purified from allergic donors adhered to laminin over a range of concentrations from 10 to 60 $\mu$g/mL. The adhesion at 30 $\mu$g/mL and 60 $\mu$g/mL was 26% ± 3% and 35% ± 3% of control, respectively (Fig 3, n = 4 to 12, P < .05). Visual inspection using an inverted microscope showed that adhesion occurred evenly throughout the wells (not shown). Eosinophils did not adhere to either type 1 or type 4 collagen over a similar concentration range (n = 4, data not shown). The adherence to laminin required divalent cations because adhesion did not occur in PBS lacking calcium and magnesium (percentage of control adherence to 60 $\mu$g/mL laminin = 102% ± 37%, n = 3). To determine the surface molecules involved in adherence to laminin, eosinophils were preincubated with various anti-integrin MoAbs before the adherence assay. As shown in Fig 4, adherence to laminin was completely inhibited by preincubation with the anti-$\alpha 6$ MoAb GoH3 and by the anti-$\beta 1$ MoAb 33B6. Adhesion was not significantly affected by isotype-matched control antibodies, nor by anti-$\alpha 4$ (19H8) or anti-$\beta 2$ (H52) antibodies (Fig 4). Interestingly, the anti-$\beta 1$ MoAb 18D3 did not block eosinophil adhesion to laminin despite complete inhibition by 33B6 (Fig 4). MoAbs against the $\alpha 1$, $\alpha 2$, or $\alpha 3$ subunits were also ineffective in this assay (n = 1 to 3, data not shown).

Similar studies were performed using eosinophils purified from four healthy, nonallergic donors without peripheral eosinophilia. Adhesion to laminin was less than that of eosinophils obtained from allergic subjects (14% ± 4% v
express a6/1, including monocytes, "memory" T cells, thy-
ins, and that eosinophils bind laminin in a divalent cat-
tation that human eosinophils also express cell surface
molecules," but recent investigators were able to detect func-
tional a6/1 on human neutrophils.\textsuperscript{1} In this report, we have
shown by both immunofluorescence and immunoprecipi-
tation that human eosinophils also express cell surface
a6/1, and that eosinophils bind laminin in a divergent cat-
ion-dependent manner using this molecule. Unlike neutro-
phils that adhere to laminin primarily via $\beta_2$ integrins,\textsuperscript{30,33}
we did not detect any $\beta_2$-mediated adhesion to laminin by
eosinophils. Furthermore, we did not detect expression of
other $\beta_1$-integrin laminin receptors on eosinophils by flow
cytometry (Fig 1), and the anti-$\alpha_1$, $\alpha_2$, or $\alpha_3$ MoAbs did not
inhibit eosinophil adhesion to laminin in our assay. The
observation that eosinophils did not adhere to laminin in
the absence of calcium and magnesium is compatible with
previous observations in other cell types that divalent cat-
ions are required for optimal function of $\alpha_6\beta_1$.\textsuperscript{29,33}

Importantly, the anti-$\alpha_6$ MoAb GoH3 completely inhib-
et eosinophil adhesion to laminin without affecting cell
binding to VCAM-1, another known eosinophil ligand.
This observation supports the idea that $\alpha_6\beta_1$ is a specific
laminin receptor on human eosinophils. Several noninte-
grin laminin receptors have also been described,\textsuperscript{34} but the
fact that GoH3 and 33B6 completely inhibit eosinophil ad-
hesion to laminin argues that other laminin receptors, if
present,\textsuperscript{35} have little or no role in this adhesive event.
The domain of murine laminin recognized by $\alpha_6\beta_1$ has been
mapped primarily to the elastase fragment E8.\textsuperscript{36} Individual
domains of human laminin have been less well character-
ized, but our inability to inhibit adhesion by pretreating
eosinophils with an arginine-glycine-aspartic acid (RGD)-
containing peptide (data not shown) is compatible with ad-
hesion to an E8-like domain that lacks RGD. Further stud-
ies will be needed to clarify this issue.

Platelets, by virtue of their ability to bind to leukocytes,
can interfere with the study of $\beta_1$ integrins in vitro. Indeed,
a preliminary report has described platelets adhering to puri-
fied eosinophils.\textsuperscript{37} We were unable to detect platelets adher-
ing to the eosinophils used in our studies either by light
microscopy or by immunolabeling eosinophils with specific
antiplatelet MoAbs (against either the $\beta_3$ or $\alpha_\text{IIa}$ subunits or
platelet glycoproteins gpIb or gpIIb). In addition, we did not
detect expression of other $\beta_1$ integrins known to be ex-
pressed by platelets (ie, $\alpha_2\beta_1$ and $\alpha_5\beta_1$). Reasons for this
discrepancy include (1) the methods for purification in the
present studies involved an initial density gradient centrifug-
ation step in which the platelet-rich fraction was carefully
removed, and (2) the eosinophils were extensively washed
before analysis.

The ability of certain cell types to adhere to laminin-
coated substrates in vitro often requires cell stimulation.
For example, neutrophils (either congenitally lacking CD18
or blocked with anti-CD18 MoAbs) were recently shown to
adhere to laminin via $\alpha_6\beta_1$ only after stimulation with
phorbol myristate acetate (PMA).\textsuperscript{30} Macrophage adhesion
to laminin also requires PMA activation.\textsuperscript{37} Because receptor
numbers are not increased after PMA treatment,\textsuperscript{30} this
agent apparently acts by enhancing $\alpha_6\beta_1$ ligand affinity,
possibly through phosphorylation of the $\alpha_6$ or $\beta_1$ subunit.
Stimulated mast cells can also adhere to laminin but in an
integrin-independent manner.\textsuperscript{38} In contrast, the adhesion of
eosinophils to laminin described in this report does not re-
quire additional in vitro stimulation. In preliminary experi-
ments, stimulation of eosinophils with additional agonists

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig4.png}
\caption{The ability of various MoAbs to inhibit eosinophil adhe-
sion to plate-bound laminin (10 $\mu$g/mL) was determined ($n = 3$ to
10). Adhesion in the absence of MoAbs was $31\% \pm 5\%$, $^{*}P < .05$
for adherence with MoAb versus adherence without MoAb pre-
treatment.}
\end{figure}

32\% $\pm$ 4\% adhesion to laminin at 10 $\mu$g/mL). However, the
observed adhesion was still significantly inhibited by pre-
treatment with anti-$\alpha_6$ MoAb (24\% $\pm$ 9\% of control adhe-
sion, $P < .003$, $n = 4$).

To verify the specificity of inhibition of adhesion to lami-
in by the anti-$\alpha_6$ antibody GoH3, we tested the ability of
this MoAb to inhibit eosinophil adherence to plate-
bound VCAM-1. Consistent with previous observations,\textsuperscript{14}
eosinophil adhesion to VCAM-1 was mediated by cell-sur-
f ace $\alpha_4$. Importantly, preincubation with GoH3 did not af-
dict eosinophil adhesion to VCAM-1. In the absence of
MoAb, adhesion to VCAM-1 was 200\% $\pm$ 28\% of control
(control adherence, 15\% $\pm$ 2\%); in the presence of anti-$\alpha_4$
or anti-$\alpha_6$ MoAbs, adhesion was 86\% $\pm$ 14\% and 183\% $\pm$ 29\%
of control, respectively ($n = 4$).

\section*{DISCUSSION}

The integrin $\alpha_6\beta_1$ was first described on human platelets,
where it was shown to be the major component of platelet
glycoproteins Ic/IId.\textsuperscript{26,29} Initial attempts at characteriz-
ing this molecule were hampered by its comigration with other
integrin subunits on gel electrophoresis.\textsuperscript{18} It was subse-
quently shown that $\alpha_6\beta_1$ was a specific receptor for the
basement membrane protein laminin.\textsuperscript{28} Unlike other $\beta_1$
integrins that can bind laminin in addition to other ligands
(eg, $\alpha_1$, $\alpha_2$, and $\alpha_3$), $\alpha_6\beta_1$ appears to be monospecific.\textsuperscript{18}
Many cell types in addition to platelets are now known to
express $\alpha_6\beta_1$, including monocytes, "memory" T cells, thy-
mocytes, epithelial cells, EC, and certain tumor cells.\textsuperscript{18,31,32}
Granulocytes were initially not thought to express this mole-
cule,\textsuperscript{18} but recent investigators were able to detect func-
tional $\alpha_6\beta_1$ on human neutrophils.\textsuperscript{30} In this report, we have
shown by both immunofluorescence and immunoprecipi-
tation that human eosinophils also express cell surface
$\alpha_6\beta_1$, and that eosinophils bind laminin in a divergent cat-

(eg, platelet-activating factor, C5a) or incubation with cytokines (eg, IL-5) did not significantly augment eosinophil adhesion to laminin but did enhance their adhesion to plastic (unpublished observations). We cannot exclude the possibility that some degree of cell activation occurred in vitro during the eosinophil purification process. It is interesting to note that in four experiments, eosinophils purified from normal donors showed less adhesion to laminin than those from allergic donors, although the adhesion was still α6-dependent. This observation may reflect eosinophil priming in vivo in allergic subjects. The present data are consistent with the recent observation that eosinophil purified from normal subjects adhered to fibronecin via α4β1 without additional cell activation. Specific factors that regulate the function of eosinophil α6β1 in vitro or in vivo are currently under investigation.

An unexpected observation was that the anti-β1 MoAb 18D3 did not inhibit eosinophil adhesion to laminin, despite complete inhibition by the anti-α6 MoAb GoH3 and the anti-β1 MoAb 33B6. 18D3 has been previously shown to inhibit eosinophil (and basophil) adherence to IL-4-treated human EC36 and gives a positive signal by flow cytometry (Fig 1) and immunoprecipitation (Fig 2). Furthermore, unlike other recently reported anti-β1 MoAbs that can enhance various integrin-mediated functions (including binding to ECM proteins), 43, 45 18D3 did not enhance binding to laminin in our experiments. A probable explanation for the different effectiveness of the two anti-β1 MoAbs is that 18D3 recognizes an epitope on β1 not involved in eosinophil adherence to laminin. This is supported by recent studies in other cell types showing that 18D3 and 33B6 recognize functionally distinct epitopes of the β1 molecule. 20

In addition to associating with β1, the α6 subunit is now known to associate with the β4 molecule, forming a heterodimer that can also mediate adhesion to laminin. 46 The distribution of α6β4 appears to be limited to epithelial cells, some carcinoma cell lines, and peripheral nerves; little or no β4 has been detected to date in association with α6 on leukocytes or platelets. 18, 22, 44, 46 We did not find any evidence that eosinophils express cell-surface α6β4, because (1) we did not observe expression of β4 by flow cytometry, (2) we did not detect the characteristically high molecular weight bands (approximately 200 kD) associated with this subunit by immunoprecipitation with anti-α6 MoAb, and (3) MoAb to α6 and β1 completely inhibited binding of eosinophils to laminin.

The role of α6β1-laminin interactions in vivo have not been directly tested but are probably cell- and/or tissue-specific. On epithelial cells and EC, for example, laminin-binding integrins including α6β1 presumably help anchor cells to the underlying basement membrane. 31, 32 The function of leukocyte α6β1 is less obvious. Several reports have shown that binding of cells to laminin can directly influence various aspects of cell function, including phagocytosis and bactericidal activity, although the receptors involved in these studies have generally not been well characterized. 37-39

The fact that laminin is a major constituent of basement membranes suggests that α6β1 may be involved in leukocyte diapedesis and also raises the possibility that circulating cells may bind directly to exposed laminin at sites of increased vascular permeability (such as in inflammation). Although studies are currently underway to examine the role of α6β1 in eosinophil transendothelial migration in vitro, the exact role of laminin and other ECM proteins in influencing eosinophil function and/or tissue localization in vivo remains to be elucidated.

ACKNOWLEDGMENT

We thank Dr James Hildreth for providing the H52 MoAb; Dr Steven Kennel for providing the 439-9B MoAb; Dr Walter Newman for providing recombinant soluble VCAM-1; Dr Edward Knol for thoughtful discussion; and Carol Bickel and Dr Toshi Yamada for expert assistance with eosinophil purification.

REFERENCES

12. Dobrina A, Menegazzi R, Carlos TM, Nardon E, Cramer R,
Zacchi T, Harlan JM, Patriarca P: Mechanisms of eosinophil adherence to cultured vascular endothelial cells: Eosinophils bind to the cytokine-induced endothelial ligand vascular cell adhesion molecule-1 via the very late activation antigen-4 integrin receptor. J Clin Invest 88:20, 1991


From www.bloodjournal.org by guest on November 16, 2017. For personal use only.


Expression of a functional laminin receptor (alpha 6 beta 1, very late activation antigen-6) on human eosinophils

SN Georas, BW McIntyre, M Ebisawa, JL Bednarczyk, SA Sterbinsky, RP Schleimer and BS Bochner