

Original article

Selective upregulation of a functional β_7 integrin on differentiating eosinophils

Background: The sequence of adhesion-molecule expression during eosinophil differentiation remains unclear.

Methods: We analyzed the surface expression of α_4 , β_1 , and β_7 integrins and compared it to established myeloid developmental markers, using the eosinophilic cell line HL-60 clone 15, as well as cord and peripheral blood differentiation assays.

Results: Cells induced to eosinophil differentiation by treatment with butyric acid, IL-5, and GM-CSF showed a significant upregulation of β_7 integrin expression coincident with a marked upregulation of CD35 and attenuation of CD33 and β_1 integrin expression. In addition, adhesion of induced HL-60 clone 15 cells to fibronectin was attenuated by a β_7 integrin antibody.

Conclusions: Our data show that protein synthesis-dependent upregulation of the functional β_7 integrin occurs under conditions when α_4 and β_1 integrins are fully expressed, indicating a sequential appearance of specific adhesion molecules on differentiating eosinophil progenitors.

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To understand the role of eosinophils in allergic inflammatory diseases such as asthma, we must explore and understand the mechanisms that stimulate the specific recruitment of these cells. The ingress of mature eosinophils from circulatory pools through specific upregulation of adhesion molecules and directional migration along a positive chemotactic gradient is a key part of the process of recruitment of these proinflammatory cells (1–3). In addition, we have proposed that a selective hemopoietic process, by which upregulation and release of bone-marrow-derived, eosinophil-lineage committed progenitors into the peripheral circulation occurs, increases the accessible pool of differentiating effector cells to the affected tissues (4, 5).

Besides the action of soluble and membrane-bound cytokines, adequate hemopoiesis is dependent upon adhesive interactions between progenitors and the bone-marrow microenvironment, which provides a broad array of ligands, such as fibronectin and vascular cell adhesion molecule (VCAM)-1 (6–10). In addition, several adhesion molecules contribute to bone-marrow-related adhesive interactions; namely, members of the β_1 and β_2 integrin families and the selectins. It has recently been demonstrated that expression of the β_1 integrin, the very late activation antigen (VLA)-4, and the β_2 integrin, lymphocyte

function-associated antigen (LFA)-1, is lower on blood-derived progenitors than bone-marrow-derived progenitors (11, 12). In addition, *in vivo* administration of anti-VLA-4 has been shown to mobilize progenitors into the peripheral circulation (13), and antibodies directed against its ligand, VCAM-1, disrupt the interaction between hemopoietic progenitor cells and bone-marrow stromal cells (14). From these observations, it is reasonable to propose that mobilization of progenitors and mature cells, from the bone marrow to the circulation, is at least in part, the result of changes in expression and/or function of adhesion molecules and the corresponding ligands, allowing an ordered, microenvironment-specific, progression of hemopoiesis.

In general, the pattern of expression of adhesion receptors on mature eosinophils is similar to that of other leukocytes, although eosinophils, unlike human neutrophils, express functional forms of VLA-4, VLA-6, and $\alpha_4\beta_7$ (15). Several studies have proposed that VLA-4/VCAM-1 and β_2 integrin/ICAM-1 adhesion pathways may be involved in the selective recruitment of eosinophils to sites of allergic inflammation (15, 16). MAdCAM-1, the ligand for $\alpha_4\beta_7$, and almost exclusively expressed on the gut epithelium, may be important in directing eosinophils to the intestinal walls, where they normally reside (17). The

β_1 integrin, VLA-4, is expressed at high levels on primitive progenitor cells and is lost as cells differentiate toward the neutrophil-lineage (18, 19). The role and sequence of expression of other adhesion molecules in relation to eosinophil differentiation have not yet been investigated.

Given that the timing of the appearance of integrins on differentiating cells is critical to determining the quantity and type of effector cells which can accumulate in tissues, and the consequent contribution of hemopoietic mechanisms to cell recruitment, the regulation of integrin expression must be subject to stringent control. As such, the aim of the present study was to examine the appearance of α_4 (CD49d), β_1 (CD29), and β_7 integrins, as compared to established differentiation markers such as CD9 (a mature eosinophil/basophil marker), CD15 (a mature neutrophil marker), CD33 (an early myeloid-lineage marker), and CD35 (a marker of eosinophil-lineage commitment), on differentiating eosinophils. These cells were derived from induced HL-60 clone 15 cells (20, 21), which represent a subclone of HL-60 cells that can be induced by butyric acid treatment to differentiate into cells with many features of eosinophils (22–26) and (hemopoietically related) basophils, including expression of specific granule proteins and functional IL-5 receptors (27–29).

Material and methods

Materials

HL-60 clone 15 cells were obtained from the American Type Culture Collection (Rockville, MD, USA). RPMI 1640 medium, fetal calf serum (FCS), and McCoy's 5A were obtained from Gibco Lab. (NY, USA); HEPES buffer from Boehringer Mannheim (Quebec, Canada); and Nunclon[™] culture wells from NUNC (Denmark). Fibronectin was purchased from Boehringer Mannheim (Bioch., Germany), and sodium butyrate and *n*-octyl-beta-D-glucopyranoside from Sigma, St Louis, MO, USA. Human recombinant GM-CSF, IL-3, and IL-5 were obtained from R&D Systems, Inc. (Minneapolis, MN, USA), and Lymphoprep from NycoMed Pharma AS (Oslo, Norway). MACS microbeads were bought from Miltenyi (Auburn, CA, USA). The adhesion molecule-blocking antibodies anti-CD29 (β_1 integrin, clone 3S3), anti-CD49d (α_4 integrin, clone HP2/1), fluorescein isothiocyanate (FITC)-linked anti-CD35 (clone E11), anti- α_4 integrin/CD49d (clone 44H6), anti- α_5 integrin/CD49e (clone SAM-1), antivimentin (clone J144), and phycoerythrin (PE)-conjugated anti- β_1 integrin/CD29 (clone 1195M) were all purchased from Serotec Ltd, (Oxford, UK). FITC-conjugated anti-CD15 (clone MMA), anti-CD62L (Leu-8, clone

SK11), and PE-conjugated anti-CD11b (clone D12) were purchased from Becton Dickinson Immunocytometry Systems, Mountain View, CA, USA. PE-conjugated anti- β_7 integrin (clone FIB504) and FITC-conjugated anti-CD33 (clone HIM3-4) were purchased from PharMingen (PharMingen, Mississauga, ON, Canada).

Culture of HL-60 clone 15 cells

The promyelocytic cell line HL-60 clone 15 cells were maintained in RPMI 1640 with 10% heat-inactivated FCS and 25 mM HEPES buffer, pH 7.8, at 37°C in an atmosphere containing 5% CO₂. Cells were induced to differentiate to eosinophil-like cells with 0.3 mM sodium butyrate, IL-5 (1 ng/ml), and GM-CSF (10 ng/ml) for 5 days, as previously described (30, 31). Clone 15 cells incubated at pH 7.8 alone were run in parallel and are referred to as uninduced cells. Cytospin preparations were made and stained with May-Grünwald Giemsa to assess the eosinophilic content of each cell-culture population. Viability was measured by trypan blue exclusion.

Histamine assay

Cell-associated histamine was assayed by isotopic radioenzyme conversion (32, 33). Cell aliquots were boiled for 5 min to inactivate histaminase and stored at -70°C until assayed. Histamine values obtained were divided by viable cell numbers as judged by trypan blue exclusion. Differences in histamine content were calculated by subtracting histamine content in uninduced clone 15 cells from corresponding values in induced cells, and were expressed as Δ -pg/10⁵ viable cells.

Immunostaining and flow cytometry

Cell samples were incubated at 4°C for 20 min in PBS plus 5% mouse and rat sera to block nonspecific binding sites. Saturating concentrations of the antibody were added to the cells and incubated for 30 min, washed twice in PBS and resuspended in 1% paraformaldehyde (PFA), and analyzed with a FACScan flow cytometer equipped with an argon ion laser (Becton Dickinson Instrument Systems). The instrument was calibrated with Standard-Brite (Flow Cytometry Standards Corp., San Juan, Puerto Rico). Off-line analysis was performed with WinMDI version 2.0.6 software.

In some experiments, results are expressed as the ratio between SMFI units obtained without and with permeabilization, which were taken as proportional to surface and total pool, respectively. Isotype-matched control antibodies were run in parallel to determine nonspecific binding; this was either subtracted from

specific binding to give the specific mean fluorescence intensity (SMFI) or used to define the cutoff for positively labeled cells within a 98% confidence limit.

To measure the total detectable pool of antigen, cells were permeabilized by the FOG method (paraformaldehyde fixation with subsequent OG treatment) (34). Briefly, cells were fixed in PFA at 20°C for 5 min, washed and incubated with 0.74% of *n*-octyl-beta-D-glucopyranoside 0.74% at 20°C for 6 min, washed, and immunostained. To confirm that accurate permeabilization had been obtained, we measured intracellular binding of antivimentin antibodies. Over 90% of permeabilized cells stained positive for intracellular vimentin.

Adhesion assay

The leukocyte adhesion assay was performed as previously described, with a slight modification (35). Briefly, culture wells with a growth area of 1.77 cm were precoated with fibronectin (plasma origin, 40 µg/ml) diluted in PBS, incubated for 2 h at 37°C, 5% CO₂-95% air atmosphere, and washed once in PBS. Nonspecific binding sites were blocked with 1% BSA for 1 h at 37°C. Control wells were coated with BSA only to determine the basal adhesion level. The wells were then rinsed with PBS and air-dried, and the cells (0.1×10^6 in 200 µl per well) in RPMI buffer were added and incubated at 37°C, 5% CO₂-95% air atmosphere. After varying incubation periods, non-adhering cells were removed by gentle rinsing with RPMI 1640 medium at 37°C. Adherent cells were fixed in 1% PFA and stained with trypan blue, and the percentage of adherent cells was determined by counting five random fields at $\times 40$ magnification, representing 18% of the total area of each well, under

inverted light microscopy. In preliminary experiments, optimal binding to fibronectin was obtained in 60 min. Viability, determined by trypan blue exclusion, did not differ between the adherent and nonadherent populations, excluding the possibility that adherence is mainly related to viability.

Statistics

Results are presented as medians (*ranges*), unless otherwise stated. Data were analyzed by a nonparametric method (Wilcoxon paired test), and significance was accepted at $P < 0.05$.

Results

HL-60 clone 15 cell characteristics

After incubation of HL-60 clone 15 cells for 5 days at alkaline pH with sodium butyrate, IL-5, and GM-CSF, a mixed eosinophil/basophil (Eo/Baso) population was observed by May-Grünwald Giemsa staining, and a significant increase in histamine content was detected ($11.1 [5.0-17.8] \Delta\text{-pg}/10^5$ viable cells, $n=5$). In association with this, we observed a significant induction-related increase in both surface expression and intracellular pool of the complement receptor type 1 (CR1, CD35), as judged by percent positive cells and specific mean fluorescence intensity (SMFI) units (Table 1 and Fig. 1). These findings agree with a previous report from our group (21).

In addition, differentiating eosinophils also expressed lower levels of CD33 and CD15 than uninduced cells (Table 1). Only traces of CD9 were found on the surface of both uninduced and induced

Table 1. Expression of CD35, CD15, and CD33 in uninduced and induced clone 15 cells

	Uninduced		Induced	
CD35 (surface)	12.8% (9.7–15.9)	1.7 SMFI (0.5–2.3)	38.5% (29.9–40.0)	11.3 SMFI (6.4–13.5)
			$P=0.008$	$P=0.008$
CD35 (unpermeabilized/ permeabilized)	0.96 (0.76–1.03)		0.75 (0.49–0.85)	
			$P=0.028$	
CD15	96.8% (93.8–97.0)	138.5 SMFI (112.0–171.2)	95.3% (94.2–96.2)	125.3 SMFI (92.9–152.0)
				$P=0.038$
CD33	93.0% (87.5–95.2)	13.4 SMFI (9.0–15.8)	78.4% (60.4–85.1)	13.0 SMFI (9.9–13.9)
			$P=0.028$	

Results are given as percent positive labeled cells (%), as well as specific mean fluorescence intensity (SMFI), and are expressed as median (IQR). Appearance of intracellular pool of CD35 is expressed as ratio of CD35 expression (SMFI) in unpermeabilized to that in permeabilized cells, which were taken as proportional to surface and total pool, respectively. P values represent statistical differences between induced and uninduced cell populations ($n=6-9$).

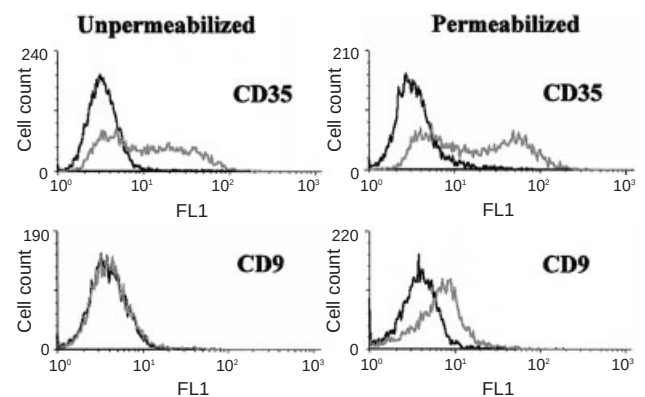


Figure 1. Flow cytometric analysis of CD35 and CD9 expression in induced HL-60 clone 15 cells. Histograms show staining with either specific or control antibody (gray and black lines, respectively). Values were taken to represent surface and total pool of adhesion receptor expression, in unpermeabilized and permeabilized cells, respectively. For CD35, specific mean fluorescence intensity (SMFI) values were 14.3 and 26.0 units, respectively, yielding surface/total pool ratio of 0.55. In contrast, significant expression of CD9 was detected only in permeabilized cells.

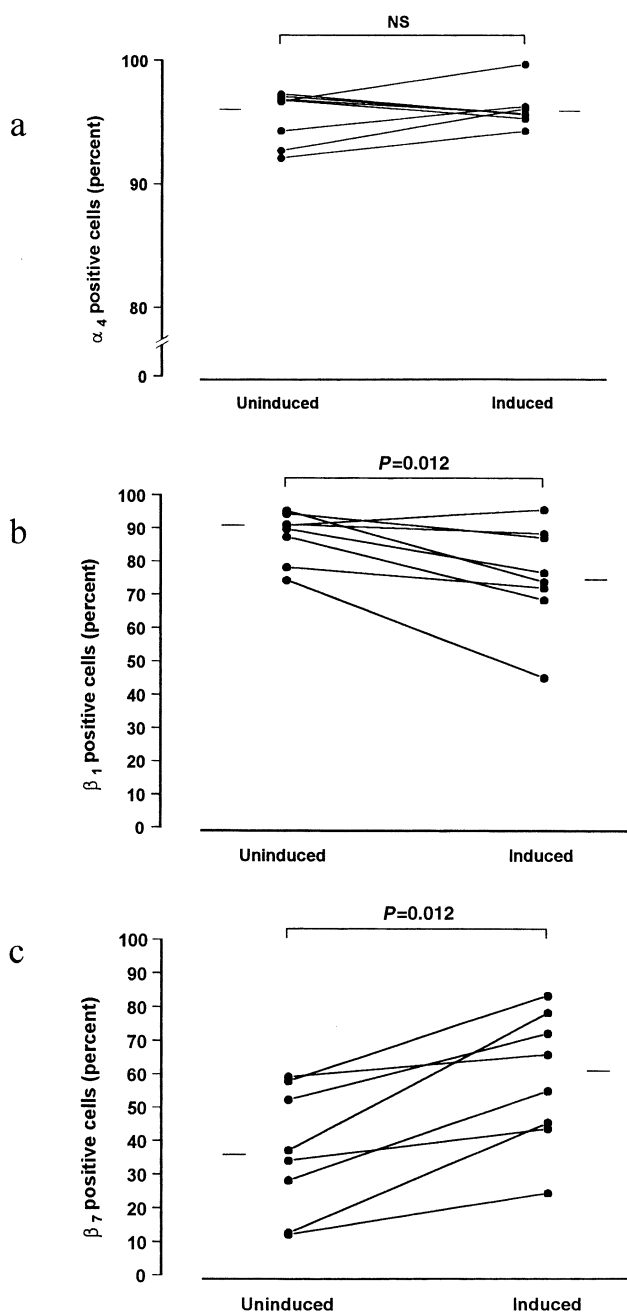


Figure 2. Flow cytometric analysis of α_4 , β_1 , and β_7 (a–c, respectively) expression in uninduced and induced HL-60 clone 15 cells. Data are expressed as percent positive labeled cells from separate experiments ($n=8$).

cells, but, after permeabilization, a significant intracellular pool was detected, and it remained stable during the induction process (Fig. 1).

Expression of adhesion molecules on HL-60 clone 15 cells

The effect of induction of HL-60 clone 15 cells to eosinophil differentiation on expression of integrins α_4 , β_1 , and β_7 is shown in Fig. 2, and representative histograms are given in Fig. 3. We observed a

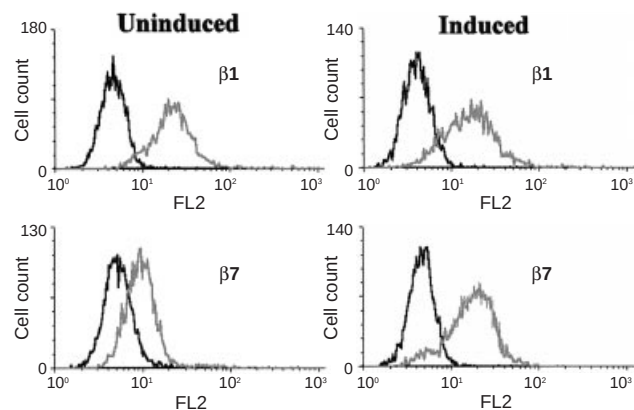


Figure 3. Flow cytometric analysis of β_1 and β_7 expression in uninduced (left panel) and induced (right panel) HL-60 clone 15 cells. Each histogram shows staining with specific antibody (grey line) and respective control antibody (black line).

significant induction-related increase in β_7 integrin, judged by both percent positive labeled cells (35.7 [20.5–55.3]% vs 60.7 [44.9–75.4]%, $P=0.012$, $n=8$) and intensity of expression as shown by SMFI units (10.3 [9.8–11.7] units vs 15.9 [14.7–18.0] units, $P=0.012$, $n=8$) (Fig. 2c). In double-staining experiments, we found that β_7 integrin was coexpressed with α_4 integrin in both uninduced and induced cells ($n=4$).

In contrast to these findings, α_4 integrin was expressed on over 90% of both induced and uninduced HL-60 clone 15 cells, and induction to eosinophilic differentiation was accompanied by a slight attenuation in α_4 density, as judged by SMFI units (30.1 [26.2–30.9] units vs 25.0 [23.0–26.2] units), but not percent positive cells (Fig. 2a). By comparison,

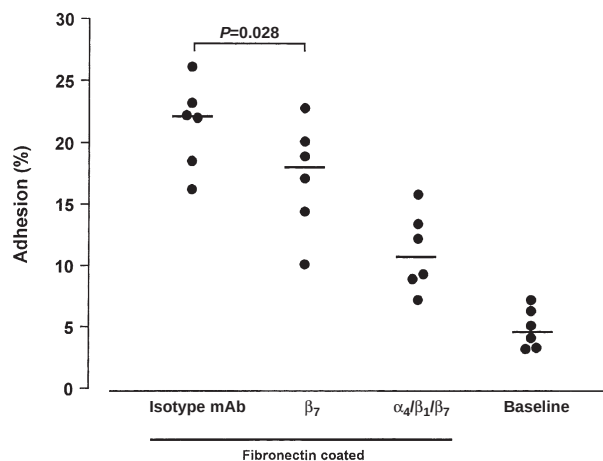


Figure 4. Adhesion of clone 15 cells to fibronectin-coated wells. Induced cells were allowed to adhere to fibronectin-coated wells for 1 h. Before adhesion to fibronectin, induced HL-60 clone 15 cells were incubated with either anti- β_7 alone or combination of anti- β_7 with anti- α_4 and anti- β_1 . At baseline, induced clone 15 cells were allowed to adhere to BSA-coated wells for 1 h under culture conditions without preincubation. Individual values and medians are presented for $n=6$ experiments.

induced cells expressed significantly lower levels of β_1 integrin (90.4 [82.9–92.9]% *vs* 75.3 [70.3–87.9]%, $P=0.012$, $n=8$) (Fig. 2b). The percentage of cells expressing the α_5 integrin was 90% in uninduced and 66% in induced cells.

That these findings are relevant to eosinophil differentiation *in vivo* is supported by our findings with cord-blood-derived progenitor cells. Eosinophil differentiation was induced by coculture for 28 days with a cocktail of cytokines including IL-3, IL-5, and GM-CSF. In the eosinophil population derived from cord-blood CD34⁺ progenitors, over 80% of the cells were judged to comprise eosinophil/basophil granules by May-Grünwald Giemsa staining at day 28; viability was >95%. At day 0, over 90% of the cells stained positive for CD34 and less than 10% expressed β_7 integrin. However, at day 28, 54 (33–72)% of the cells expressed β_7 integrin ($n=5$). Over 90% of cells, both at day 0 and at day 28, expressed the α_4 integrin, and a concurrent decrease in β_1 integrin expression was noted (>90% positive day 0 *vs* 67 [58–79]% day 28, $n=5$).

To determine whether upregulation of β_7 integrin was the result of translocation of a preformed intracellular pool, we analyzed the total detectable pool in both uninduced and induced HL-60 clone 15 cells and the effect of a protein synthesis inhibitor, cycloheximide (10 μ g/ml). We did not find any permeabilization-dependent pool of β_7 integrin in either uninduced or induced clone 15 cells ($n=3$). Addition of cycloheximide during the early phase of induction toward eosinophil differentiation (within the first 24–48 h) abolished the β_7 integrin upregulation, without affecting the constitutive expression of α_4 and β_1 integrins ($n=2$). Coculturing with cycloheximide did not affect the viability of these cells.

We also analyzed the expression of CD11b and CD62L (or L-selectin), both of which are involved in the transmigration process. Induction of differentiation increased the percentage of cells expressing the CD11b molecule significantly (5.3 [4.7–11.1]% *vs* 15.5 [10.7–24.0]%, $P=0.018$, $n=7$), but no significant levels of CD62L were detectable on either uninduced or induced cells ($n=3$).

Adhesion to fibronectin

β_7 integrin has been suggested to play a role in adhesion of mature eosinophils to fibronectin (36, 37). In the present study, the contribution of β_7 integrin to the adherence of differentiating eosinophils to fibronectin was investigated. Induced [HL-60] clone 15 cells were preincubated with blocking antibodies against β_7 integrin alone or in combination with α_4 and β_1 integrins, and were allowed to adhere to fibronectin-coated wells. There was a significant blocking effect of anti- β_7 antibody on adhesion to fibronectin (22.1 [16.2–22.7]% *vs* 18.0 [10.1–19.5]%, $P=0.028$, $n=6$)

(Fig. 4). Preincubation with a combination of antibodies against α_4 , β_1 , and β_7 integrins further abrogated adhesion to fibronectin, but not down to baseline levels (adhesion to BSA-coated wells) (Fig. 4).

Discussion

The novel finding of this study is that eosinophil differentiation is associated with a protein synthesis-dependent upregulation of a functional β_7 integrin, under conditions in which α_4 and β_1 integrins are fully expressed. These observations indicate a sequential appearance of disparate members of the integrin family on progenitors as they differentiate to become mature eosinophils. This may reflect the roles of specific adhesion pathways at distinct stages of eosinophil maturation.

We have previously reported that the CD35 antigen is upregulated in HL-60 cells during eosinophil differentiation; this subpopulation stains metachromatically and is enriched in histamine content (21). Herein we confirm this finding in an eosinophilic subline of HL-60 and also include data indicating that eosinophil differentiation involves storage of an additional intracellular pool of CD35. HL-60 cells have previously been shown to contain substantial levels of CD35 mRNA, a feature which correlates to the ability to generate the soluble form of the receptor (designated sCR1) (38), a potent inhibitor of complement that reduces inflammation-induced tissue damage in animals (39).

The CD9 antigen is a glycoprotein expressed on mature eosinophils, basophils, and platelets, and it has been suggested that CD9 mediates signaling involved in the survival of eosinophils (20). In our study, we identified a permeabilization-dependent pool of CD9 that was not present in KG1 cells and that remained stable during induction of differentiation. The relevance of this finding is not clear, but it may indicate that lineage-specific surface antigens can be produced and stored intracellularly before they appear on the cell surface.

In man, various adhesion pathways, including β_1 integrins (40, 41), β_2 integrins (42), and selectins (43), have been proposed to be involved in the interaction of hemopoietic progenitors within the bone marrow, as well as in the recruitment process of mature leukocytes into tissues (44). In the present study, we demonstrate a significant upregulation of β_7 integrin in differentiating eosinophils derived from an HL-60 leukemic cell line. That these data relate to the eosinophil differentiation from primitive progenitor cells *in vivo* is supported by similar findings with differentiating eosinophils from cord-blood-derived CD34⁺ cells. Our findings demonstrate that β_7 integrin expression accompanies Eo/Baso lineage commitment, appearing before full maturation of eosinophils/basophils.

The β_7 integrin subfamily comprises two members,

$\alpha_4\beta_7$ and $\alpha_E\beta_7$, each of whose notable characteristic is the ability to recognize ligands expressed on endothelial and epithelial cells at mucosal sites. Studies in man have revealed that β_7 integrin distribution is mainly restricted to leukocytes, and that the $\alpha_4\beta_7$ heterodimer mediates adhesion to VCAM-1 and fibronectin (45), as well as to MAdCAM-1 in Peyer's patches (46). In contrast, the $\alpha_4\beta_1$ heterodimer mediates adhesion to VCAM-1 and fibronectin, but not to MAdCAM-1 (47). The β_7 integrin is expressed on lymphocytes, eosinophils, basophils, and NK cells, but not on peripheral monocytes or neutrophils. A reciprocal association between $\alpha_4\beta_7$ and β_1 on different lymphocyte subsets has also been described (48, 49). This reciprocal relationship was noted in our study, where an upregulation of β_7 integrin accompanied a decrease in β_1 integrin expression.

The sensitivity of β_7 upregulation during early differentiation to cycloheximide, a known protein synthesis inhibitor that blocks translation of proteins, suggests that continuous protein synthesis is required for β_7 expression, and not simply translocation from an intracellular pool. This concept is also supported by our and others' inability to identify any additional permeabilization-dependent pool of β_7 integrin in both uninduced and induced clone 15 cells (50).

We found a slight but significant decrease in α_4 expression in induced clone 15 cells, in line with the observation that circulating CD34⁺ progenitors express lower levels of VLA-4 (α_4 integrin) than CD34⁺ progenitors localized in the bone marrow (12). A downregulation of α_4 mRNA has also been proposed in phorbol ester-treated HL-60 cells concomitant with β_7 mRNA upregulation, suggesting that β_7 associates with an α chain distinct from α_4 (51, 52). Shaw et al. have reported that, besides pairing with the α_4 chain, the β_7 chain can form a heterodimer with the α_E chain, expressed mainly on T cells, which mediates adhesion to epithelial cells (53). Our double-staining experiments revealed that β_7 was coexpressed with α_4 , favoring the view that α_4 downregulation does not limit β_7 expression, and that β_7 forms a functional heterodimer with α_4 (data not shown).

We have previously examined the role of the bone marrow in the delivery of inflammatory effector cells to local tissues, hypothesizing that myelopoiesis is skewed toward eosinophil differentiation in atopic individuals, and that generation of eosinophil progeny occurs in discrete steps (4, 5). Our observation in the present paper that β_7 integrin is upregulated on a relatively immature Eo/Baso cell has several implications for the understanding of selective Eo/Baso accumulation in atopic states. Since upregulation of β_7 integrin appears to occur before terminal differentiation of the Eo/Baso lineage, concomitant with a downregulation of α_4 and β_1 integrins, this may provide nascent Eo/Baso cells with a selective adhesion pathway to exit the bone marrow;

e.g., by binding to VCAM-1 on bone-marrow endothelial cells. In addition, expression of a functional β_7 integrin on subsets of circulating Eo/Baso "intermediate" progenitors may facilitate their selective homing to local tissues, both lymphoid and non-lymphoid.

The respective contribution of α_4 , β_1 , and β_7 integrins to adhesion of leukocytes to fibronectin, VCAM-1, and MAdCAM-1 is not fully understood. Our data show that β_7 integrin plays a partial role, together with α_4 and β_1 integrins, in adhesion to fibronectin of immature human eosinophils. This concept is in line with data from studies in mature human eosinophils which suggest that β_7 integrin significantly contributes to the overall adhesion to fibronectin, VCAM-1, and MAdCAM-1 (36, 37). The extent to which cell activation or differentiation is required for β_7 integrin-dependent adhesion to fibronectin to occur is not clear. Using two human α_4/β_7 -expressing lymphoma cell lines, HUT 78 and RPMI 8866, Erle et al. demonstrated that HUT 78 required stimulation to use α_4/β_7 to adhere to fibronectin, whereas RPMI 8866 adhered efficiently via α_4/β_7 in the absence of stimulation (48). Differences may therefore exist in the functional state of α_4/β_7 integrin in different human cell lines. In our model, unlike mature eosinophils, stimulation of HL-60 clone 15 cells was not required to demonstrate a β_7 -mediated adhesion to fibronectin (36, 37). Reported discrepancies in the contribution of β_7 integrin-mediated adhesion may be related to the use of different antibodies. The Act-1 antibody is proposed to recognize an epitope that includes residues from both α_4 and β_7 subunits, whereas the FIB504 antibody, which was used in our study, recognizes an epitope exclusively within the β_7 subunit (54, 55). Additional adhesion pathways, including the α_5 integrin and the CD11/CD18 complex, could explain the observed residual adhesion of induced clone 15 cells to fibronectin. Indeed, we noted that the majority of induced cells expressed the α_5 integrin (56–58) concomitant with an upregulation of complement receptor type 3 (CR3 or CD11b/CD18).

In conclusion, using a human eosinophilic cell line, we have shown that upregulation of a functional β_7 integrin occurs on lineage-committed eosinophil progenitors under conditions where α_4 and β_1 integrins are fully expressed, demonstrating a sequential appearance of adhesion molecules during eosinophil differentiation.

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