

Up-Regulation and Activation of Eosinophil Integrins in Blood and Airway after Segmental Lung Antigen Challenge¹

Mats W. Johansson,^{2*} Elizabeth A. B. Kelly,[†] William W. Busse,[†] Nizar N. Jarjour,[†] and Deane F. Mosher^{*†}

We hypothesized that there are clinically relevant differences in eosinophil integrin expression and activation in patients with asthma. To evaluate this, surface densities and activation states of integrins on eosinophils in blood and bronchoalveolar lavage (BAL) of 19 asthmatic subjects were studied before and 48 h after segmental Ag challenge. At 48 h, there was increased expression of α_D and the N29 epitope of activated β_1 integrins on blood eosinophils and of α_M , β_2 , and the mAb24 epitope of activated β_2 integrins on airway eosinophils. Changes correlated with the late-phase fall in forced expiratory volume in 1 s (FEV₁) after whole-lung inhalation of the Ag that was subsequently used in segmental challenge and were greater in subjects defined as dual responders. Increased surface densities of α_M and β_2 and activation of β_2 on airway eosinophils correlated with the concentration of IL-5 in BAL fluid. Activation of β_1 and β_2 on airway eosinophils correlated with eosinophil percentage in BAL. Thus, eosinophils respond to an allergic stimulus by activation of integrins in a sequence that likely promotes eosinophilic inflammation of the airway. Before challenge, β_1 and β_2 integrins of circulating eosinophils are in low-activation conformations and $\alpha_D\beta_2$ surface expression is low. After Ag challenge, circulating eosinophils adopt a phenotype with activated β_1 integrins and up-regulated $\alpha_D\beta_2$, changes that are predicted to facilitate eosinophil arrest on VCAM-1 in bronchial vessels. Finally, eosinophils present in IL-5-rich airway fluid have a hyperadhesive phenotype associated with increased surface expression of $\alpha_M\beta_2$ and activation of β_2 integrins. *The Journal of Immunology*, 2008, 180: 7622–7635.

The contribution of eosinophils to certain aspects of asthma, such as airway hyperresponsiveness, remains controversial; nevertheless, there is evidence that eosinophil recruitment to the airway contributes to asthma exacerbations and the chronic character of asthma by regulating airway inflammation and remodeling (1–15). Thus, the study of how eosinophils traffic from blood to airway is of considerable importance. Integrins, which are versatile cellular adhesion receptors (16, 17), are likely determinants of how eosinophils roll and arrest on lung endothelium, extravasate and migrate through the endothelium, underlying basement membrane, and tissue to the airway wall, and traverse the bronchial epithelium to the airway lumen (18–20).

Human eosinophils express seven integrin heterodimers: $\alpha_4\beta_1$ (CD49d/CD29), $\alpha_6\beta_1$ (CD49f/CD29), $\alpha_L\beta_2$ (CD11a/CD18), $\alpha_M\beta_2$ (CD11b/CD18), $\alpha_X\beta_2$ (CD11c/CD18), $\alpha_D\beta_2$ (CD11d/CD18), and $\alpha_4\beta_7$ (CD49d/ β_7) (13, 21, 22). Each integrin heterodimer interacts with its own set of ligands, which are counter-receptors on other cells or extracellular matrix components (17, 23). The functions of an integrin on a given cell is regulated by expression level and activation state (24–26).

We hypothesized that there are clinically relevant differences in eosinophil integrin expression levels and activation states in pa-

tients with asthma. The purpose of the present study was to define changes in the expression levels and activation states of blood and airway eosinophil integrins 48 h following segmental allergen challenge in asthmatic subjects, thus reflecting the development of allergic inflammation. Segmental Ag challenge induces a strong local recruitment of eosinophils (27). We found characteristic integrin changes on blood and airway eosinophils, differences between single and dual responder asthmatics, and associations among integrin changes, magnitude of eosinophil recruitment to airway, and IL-5 concentration in bronchoalveolar lavage (BAL)³ fluid. We propose a scenario in which the following occur: 1) Ag challenge leads to activation of β_1 integrins and increased surface expression of $\alpha_D\beta_2$ on blood eosinophils; 2) such activated eosinophils are more prone to arrest on VCAM-1 (CD106)-bearing endothelium in challenged segments; and 3) IL-5 and other cytokines trigger activation of β_2 integrins, contributing to the hyperadhesive phenotype of airway eosinophils.

Materials and Methods

Subjects and screening

Nineteen subjects with mild asthma as diagnosed by an allergist were studied (Table I). These subjects had a history of asthma exacerbation to aeroallergen, a provocative concentration of methacholine producing a 20% fall in forced expiratory volume in 1 s (FEV₁) of <8 mg/ml and/or reversibility to β -agonist of >12%. Subjects were screened as described previously with allergen skin prick tests, determination of airway hyperresponsiveness, and spirometry (28). All subjects had a positive skin prick test to one or more aeroallergens, were nonsmokers, did not have a respiratory infection within 30 days of study, and had not received antihistamines within seven days or corticosteroids within 30 days of study enrollment. The studies were reviewed and approved by the University of Wisconsin Health

*Department of Biomolecular Chemistry and [†]Department of Medicine, University of Wisconsin, Madison, WI 53706

Received for publication November 20, 2007. Accepted for publication March 21, 2008.

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

¹ This work was supported by Specialized Center of Research Grant HL56396, Program Project Grant HL88594, and General Clinical Research Center Grant M01 RR03186 from the National Institutes of Health.

² Address correspondence to Dr. Mats W. Johansson, Department of Biomolecular Chemistry, University of Wisconsin, 4285A Medical Sciences Center, 1300 University Avenue, Madison, WI 53706. E-mail address: mwjohansson@wisc.edu

³ Abbreviations used in this paper: BAL, bronchoalveolar lavage; AgPD₂₀, provocative dose of Ag producing a 20% fall in FEV₁; FEV₁, forced expiratory volume in 1 s; gMCF, geometric mean channel fluorescence.

Copyright © 2008 by The American Association of Immunologists, Inc. 0022-1767/08/\$2.00

Table I. Subject characteristics

Subject No	Age (years)	Sex ^a	Ag ^b	Screening		Whole-Lung Challenge			Segmental Challenge	
				PC ₂₀ (mg/ml) ^c	FEV ₁ (liters/%) ^d	AgPD ₂₀ (CBU) ^e	FEV ₁ Fall Early (%)	FEV ₁ Fall Late (%)	Eosinophils in BAL 48 h after Challenge (%)	Total Eosinophils in BAL 48 h after Challenge (×10 ⁶)
Single responders ^f										
1	22	M	RW	9.0	3.8 (90)	81	37	0	26	9
2	21	F	HDM	1.2	3.2 (85)	6	52	4	37	150
3	26	F	HDM	1.8	4.0 (99)	1	26	7	21	7
4	19	M	CD	0.7	4.1 (85)	85	31	4	ND	ND
5	19	F	HDM	3.5	3.7 (105)	118	22	0	47	150
6	21	M	RW	0.2	4.4 (93)	115	23	0	26	24
7	20	M	CD	5.5	4.3 (98)	68	44	4	60	83
8	27	M	HDM	20	3.7 (89)	52	35	8	38	24
9	23	F	CD	0.4	3.0 (91)	2	32	11	24	10
10	26	F	CD	2.9	4.0 (109)	174	40	7	62	88
Median	22			2.4	3.9 (92)		34	4	37	24
Dual responders ^g										
11	30	F	HDM	4.8	2.9 (95)	11	23	25	72	220
12	19	M	CD	3.8	4.9 (96)	37	22	17	63	230
13	20	F	RW	0.3	2.9 (87)	1	48	15	34	29
14	20	F	HDM	1.3	3.7 (99)	8	31	15	59	410
15	18	M	HDM	20	5.1 (104)	21	21	15	65	120
16	22	M	HDM	2.5	3.9 (87)	31	23	32	74	760
17	22	M	RW	20	4.7 (97)	48	35	18	66	420
18	20	F	HDM	0.4	3.4 (96)	27	31	21	67	280
19	23	M	RW	20	4.4 (106)	52	48	24	55	380
Median	20			3.8	3.9 (96)		31	18***	65**	280**

^a F, Female; M, male.^b CD, Cat dander; HDM, house dust mite; RW, ragweed.^c PC₂₀, Provocative concentration of methacholine producing a 20% fall in FEV₁.^d FEV₁ values from the screening visit are expressed in liters (number on left) and as a percentage of the predicted value (number in parentheses).^e CBU, Cumulative breath units.^f Single responders are subjects with FEV₁ fall 3–8 h after whole-lung Ag challenge (late-phase fall) < 15% (see Ref. 28).^g Dual responders are subjects with FEV₁ fall 3–8 h after whole-lung Ag challenge ≥ 15% (see Ref. 28).**, *p* ≤ 0.01; ***, *p* ≤ 0.001 versus single responders.

Sciences Human Subjects Committee (Madison, WI). Informed written consent was obtained from each subject before participation.

Determination of provocative allergen dose and early- and late-phase response to whole-lung allergen challenge

At least 4 wk before bronchoscopy, a graded whole-lung inhaled Ag challenge was performed as described (28) to determine the provocative dose of Ag producing a 20% fall in FEV₁ (AgPD₂₀) and the magnitude of early- and late-phase responses. Briefly, baseline spirometry was performed and repeated after five breaths of saline diluent. If FEV₁ remained within 10% of baseline, five breaths of allergen were inhaled and spirometry was repeated 10 min later. Consecutively greater concentrations of allergen were given until FEV₁ fell by ≥20% from baseline. The maximum immediate- or early-phase (within 15–30 min) fall in FEV₁ was determined and subjects were then monitored every 15 min until FEV₁ returned to within 10% of baseline. Thereafter, the subjects were monitored at 1-h intervals for 8 h to determine whether a late-phase response was present (28, 29). Subjects having a FEV₁ fall of ≥15% 3–8 h after the whole-lung Ag challenge were considered to have a dual response phenotype; the other subjects were considered single responders (28).

Segmental bronchoprovocation with allergen and BAL

Bronchoscopy, segmental bronchoprovocation with allergen, and subsequent BAL were performed in two different bronchopulmonary segments as described (27, 28, 30). Baseline BAL at 0 h immediately before segmental bronchoprovocation was performed in two segments. Then, a total dose of 30% of the subject's AgPD₂₀ was administered incrementally to enhance subject safety as follows: 10% of the AgPD₂₀ in the first segment and, when this dose was well tolerated, 20% in the second segment. Forty-eight hours later, a second bronchoscopy was performed by instilling 160 ml of sterile 0.9% NaCl warmed to 37°C in each segment. BAL fluid recovered from the two segments was pooled for analysis, and the volume of recovered fluid was measured.

Abs for flow cytometry and ELISA and recombinant protein standards

Anti-α_D integrin mAb 240I (31) was obtained as a gift from ICOS. Activation-sensitive β₂ integrin mAb24 (32, 33) was a gift from N. Hogg (Cancer Research U.K. London Research Institute, London, U.K.). Anti-β₁ mAb MAR4, anti-β₂ L130, anti-β₇ Fib504, anti-α₄ 9F10, anti-α₆ Goh3, anti-α_L A1111, anti-α_X Bly6, PE-conjugated goat anti-mouse and anti-rat IgG, FITC-conjugated anti-CD14 and anti-CD16, the isotype controls mouse IgG1 κ (clone A112-2) and rat IgG2a κ (A110-2), and the unlabeled and biotinylated anti-IL-3, anti-IL-5, and anti-IFN-γ mAbs and corresponding recombinant protein standards for ELISA were from BD Biosciences. Anti-α_M LM11 and activation-sensitive anti-β₁ N29 (34) were from Chemicon. Unlabeled and biotinylated anti-GM-CSF mAb and recombinant protein standard for ELISA were from R&D Systems.

Flow cytometry of blood and BAL cell samples

Because purification of blood eosinophils has been found to cause the activation of β₁ (35), flow cytometry was done on unfractionated blood and BAL cells. Surface expressions of α_M, α_L, α_X, α_D, α₄, α₆, β₁, β₂, β₇, activated β₁, and activated β₂ were determined. Blood was drawn routinely into lavender top standard tubes (giving a final EDTA concentration of 1.8 mg/ml) (BD Vacutainer Systems). For determination of mAb24 reactivity, blood was drawn into green top tubes (giving a final heparin concentration of 14 U.S. Pharmacopeia (USP) units/ml). Control experiments revealed that the anticoagulant had no effect on the results except, as reported, for mAb24, whose epitope is not exposed in the presence of EDTA (32). Not all samples were subjected to complete analysis due to changes in the mAb panel that were made based on ongoing analysis of results from this study and other studies. Originally, we focused on differences between blood and BAL fluid 48 h after segmental challenge. Blood from before segmental challenge was included as a routine later, when we had indications of interesting differences between blood after vs before challenge. Similarly, we originally focused on subunits of the integrins known to be able to bind

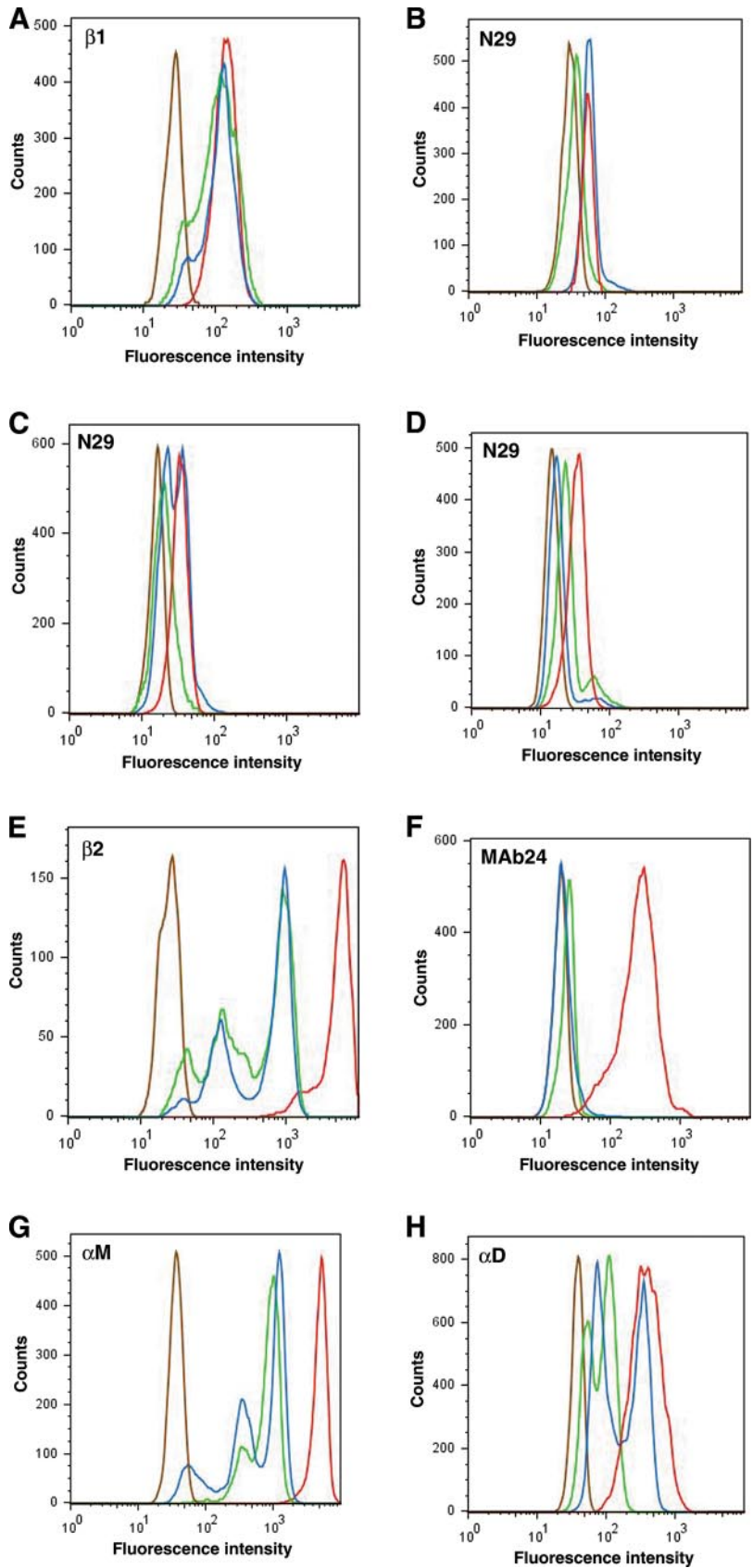


FIGURE 1. Integrin expression on blood and BAL eosinophils before and after segmental Ag challenge. Representative flow cytometry histograms of expression of total β_1 integrin (A), activation-sensitive β_1 integrin mAb N29 epitope (B–D), total β_2 integrin (E), activation-sensitive β_2 integrin mAb24 epitope (F), α_M integrin (G), or α_D integrin (H) on blood eosinophils before segmental Ag challenge (green), blood eosinophils 48 h after segmental Ag challenge (blue), or BAL eosinophils 48 h after segmental Ag challenge (red), or isotype control (brown). For N29 epitope expression, three examples are shown: one homogeneous, symmetric peak on blood eosinophils before and after challenge and higher mean expression level after challenge than before (B); one peak on blood eosinophils before challenge and two peaks after challenge, and higher mean expression level after challenge (C), and an asymmetric peak with a “shoulder” to the right on blood eosinophils before and after challenge, and decrease in the size of the “shoulder” and lower mean expression level after challenge (D). The numbers of individuals with the various patterns are summarized in Table II.

VCAM-1, $\alpha_4\beta_1$, $\alpha_4\beta_7$, and $\alpha_D\beta_2$ (13, 21) and added α_M later when we had evidence that $\alpha_M\beta_2$ is involved in the adhesion of purified airway eosinophils to VCAM-1 and other ligands (22, 36). BAL fluid cells were recovered, cytospun, and stained for differential counts as described (29, 30).

EDTA-treated blood (100 μ l) was incubated with 0.5 μ g of primary Ab or isotype control in 100 μ l of FACS buffer (PBS with 2% BSA and 0.2% NaN_3) for 30 min. For mAb24 (and its isotype control), heparin-treated blood was used because the mAb24 epitope is not exposed in EDTA (32)

Table II. Integrin expression on blood and BAL eosinophils as distribution patterns and percentage of positive cells before and 48 h after segmental Ag challenge

Integrin (mAb)	Blood Eosinophils before Segmental Ag Challenge		Blood Eosinophils 48 h after Segmental Ag Challenge		BAL Eosinophils 48 h after Segmental Ag Challenge	
	Homogenous/Total ^a	Percentage Positive ^b	Homogenous/Total ^a	Percentage Positive ^b	Homogenous/Total ^a	Percentage Positive ^b
All tested subjects						
Total β_1 (MAR4)	2/9	82 $\pm$ 4	6/16	80 $\pm$ 5	17/17	85 $\pm$ 6
Activated β_1 (N29)	8/9	54 $\pm$ 7	11/16	57 $\pm$ 7	15/15	55 $\pm$ 8
Total β_2 (L130)	1/10	91 $\pm$ 2	1/17	93 $\pm$ 2	12/14	97 $\pm$ 1*†
Activated β_2 (mAb24)	3/3	23 $\pm$ 17	3/3	18 $\pm$ 11	10/13	65 $\pm$ 11
α_M	1/4	92 $\pm$ 5	2/12	95 $\pm$ 3	8/9	96 $\pm$ 3
α_D	2/9	79 $\pm$ 2	4/15	87 $\pm$ 6**	13/16	81 $\pm$ 8
Single responders ^c						
Total β_1 (MAR4)	2/5	85 $\pm$ 7	5/9	79 $\pm$ 8	9/9	80 $\pm$ 9
Activated β_1 (N29)	4/5	53 $\pm$ 11	5/9	48 $\pm$ 10	8/8	49 $\pm$ 10
Total β_2 (L130)	1/5	94 $\pm$ 2	0/9	92 $\pm$ 4	6/7	95 $\pm$ 3
Activated β_2 (mAb24) ^d	2/2	30 $\pm$ 27	2/2	26 $\pm$ 14	6/6	42 $\pm$ 19
α_M	1/2	100 $\pm$ 0	1/7	93 $\pm$ 4	4/5	95 $\pm$ 5
α_D	2/5	82 $\pm$ 4	3/8	80 $\pm$ 12	7/8	77 $\pm$ 11
Dual responders ^e						
Total β_1 (MAR4)	0/4	78 $\pm$ 3	1/7	83 $\pm$ 5	8/8	90 $\pm$ 7*
Activated β_1 (N29)	4/4	56 $\pm$ 8	6/7	70 $\pm$ 7	7/7	61 $\pm$ 14
Total β_2 (L130)	0/5	88 $\pm$ 4	1/8	94 $\pm$ 2	6/7	99 $\pm$ 0*
Activated β_2 (mAb24)	1/1	8	1/1	3	4/7	85 $\pm$ 4
α_M	0/2	84 $\pm$ 4	1/5	97 $\pm$ 1	4/4	98 $\pm$ 2
α_D	0/4	76 $\pm$ 1‡	1/7	95 $\pm$ 2**	6/8	85 $\pm$ 11*

^a Number of subjects with homogeneous expression distribution out of the total number of subjects.^b Expression as percentage of positive cells (mean $\pm$ SEM).^c Single responders are subjects with FEV₁ fall 3–8 h after whole-lung Ag challenge < 15% (see Ref. 28).^d Data with $n < 3$ are within the ranges of our overall data on blood eosinophils from unchallenged donors.^e Dual responders are subjects with FEV₁ fall 3–8 h after whole-lung Ag challenge $\geq$ 15% (see Ref. 28).*, $p \leq 0.05$ and **, $p \leq 0.01$ versus blood eosinophils before segmental challenge; †, $p \leq 0.05$ versus blood eosinophils after segmental challenge; ‡, $p \leq 0.05$ versus single responders.

and was incubated with primary Ab in RPMI 1640 with 10% FBS at 37°C following the protocol especially designed for mAb24 (37). After primary Ab incubation, samples were washed with 1 ml PBS, washed with 250 μ l of FACS buffer, and resuspended in 250 μ l of FACS buffer containing PE-conjugated goat anti-mouse or anti-rat IgG (2 μ g/ml). After incubation for 30 min, samples were washed again with PBS, resuspended in 100 μ l FACS buffer with a mixture of FITC-conjugated anti-CD14 (0.125 μ g) and anti-CD16 (0.625 μ g) and incubated for 30 min. RBC were lysed by incubation with 2 ml of FACS lysing solution (BD Biosciences) for 10 min, followed by centrifugation. Incubations were at room temperature until after RBC lysis and then at 4°C. Samples were washed with 500 μ l of FACS buffer, resuspended in 250 μ l of FACS fixative (1% paraformaldehyde, 67.5 mM sodium cacodylate, and 113 mM NaCl (pH 7.2)), stored at 4°C in the dark, and washed with 1 ml of PBS and resuspended in 250 μ l of FACS buffer just before data collection. Fixation did not decrease signals (data not shown). Data were collected from 30,000–170,000 events, using a FACSCalibur (BD Biosciences; available through the Flow Cytometry Facility, Comprehensive Cancer Center, University of Wisconsin, Madison, WI) and CellQuest software (BD Biosciences). Rainbow calibration fluorescent beads (Spherotech) were run each day to calibrate and check the performance of the instrument and channels. Data were analyzed using CellQuest and Flow Jo (Tree Star). Eosinophils were gated based both on scattering (high side scatter) and lack of staining with anti-CD14 and anti-CD16 (30, 35). Thus, the cells that were analyzed for the PE signal fit two criteria for eosinophils by being gated inside both characteristic regions in a plot of side scatter vs FITC staining and a plot of side vs forward scatter. When cells gated on side vs forward scatter were collected by cell sorting followed by cytospin, $\geq 96\%$ stained for the eosinophil marker eosinophil major basic protein. To analyze circulating neutrophils and monocytes in the same data sets, these leukocyte types were gated inside characteristic regions in the two plots, neutrophils having intermediate side scatter and FITC positivity and monocytes having relatively low side scatter and FITC positivity. Data are expressed as specific geometric mean channel fluorescence (gMCF; specific gMCF = gMCF with a specific integrin mAb – gMCF with isotype control) and as percentage of positive cells (isotype control set with a marker to 2% positive cells) as before (35). For unfractionated BAL cells, flow cytometry was performed

with 2×10^5 cells (in 100 μ l) and the protocol was as described for blood samples, except that all incubations were at 4°C and the erythrocyte lysis step was omitted and replaced by a wash with 1 ml of PBS.

ELISA for cytokines in BAL fluid

To measure cytokine concentrations, BAL fluid was concentrated 10-fold at 4°C using a low protein-binding Centriprep centrifugal filter unit (Millipore) with a molecular mass cutoff limit of 3 kDa. A sensitive two-step sandwich ELISA was used as described (38). The assay sensitivities were below 3 pg/ml for IL-5 and GM-CSF, 12 pg/ml for IL-3, and 25 pg/ml for IFN- γ . Values are presented as the concentration in recovered BAL fluid before the concentration step.

Statistics

The Mann-Whitney U test was used to compare data between groups. The Spearman rank correlation test was used to analyze correlations. A level of $p \leq 0.05$ was considered significant. Analyses were performed using Prism 3.0 (GraphPad).

Results

Response to whole-lung allergen challenge

All subjects responded to whole-lung inhaled Ag challenge with a drop in FEV₁ within 15–30 min to a median value of 31% early FEV₁ fall (Table I). Nine of the 19 subjects had a late-phase response (39) as defined by a FEV₁ fall $\geq 15\%$ from baseline 3–8 h after challenge (Table I). Using this criterion (28), the nine subjects were classified as having a dual-response phenotype. The other 10 subjects were considered single responders (Table I). There were no significant differences between the single and dual responders in regards to age, sex, airway responsiveness to methacholine, FEV₁ at the screening visit, or early-phase fall in response to whole-lung Ag challenge (Table I). FEV₁ 48 h after segmental

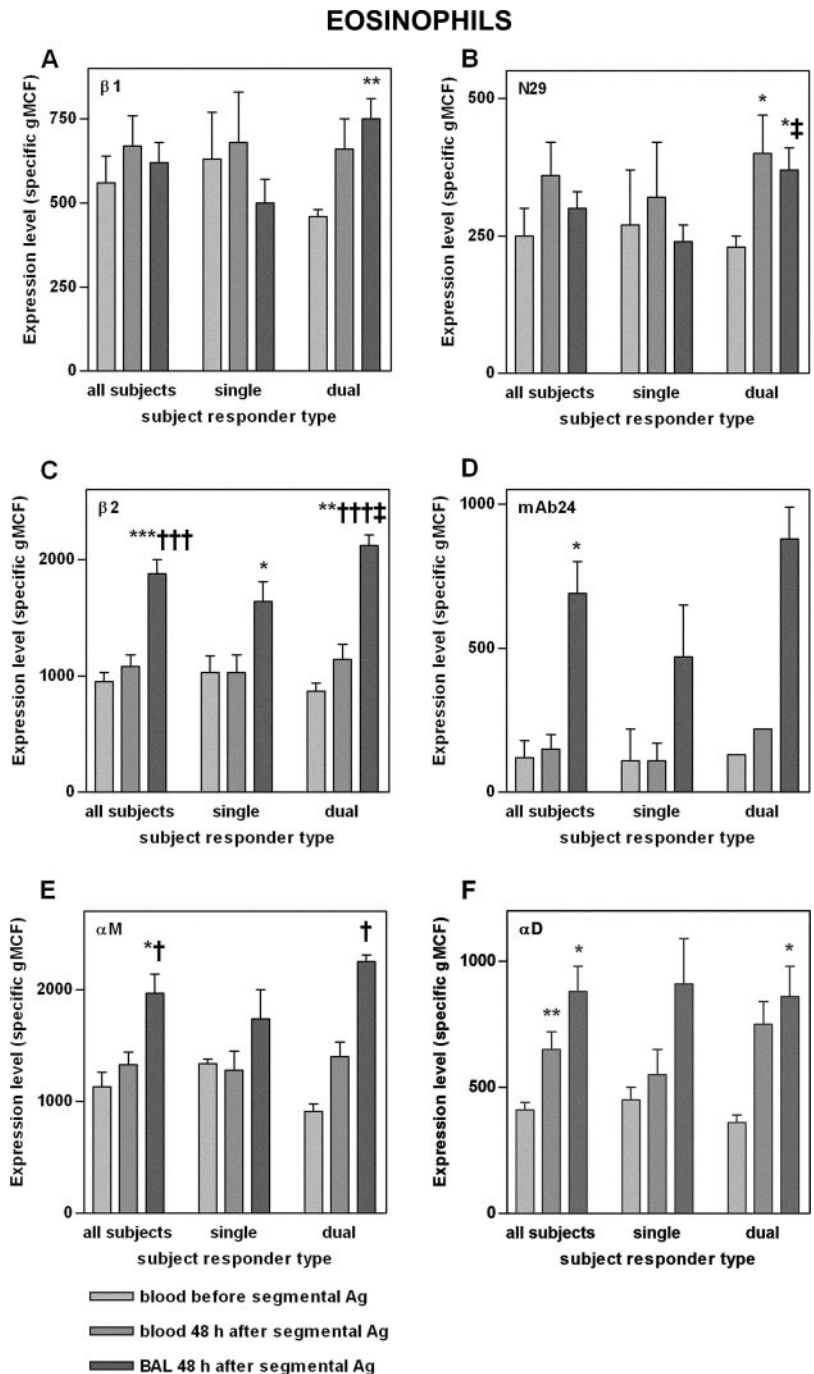


FIGURE 2. Integrin expression on blood and BAL eosinophils before and 48 h after segmental Ag challenge. Expression of total β_1 integrin (A), activation-sensitive β_1 integrin mAb N29 epitope (B), total β_2 integrin (C), activation-sensitive β_2 integrin mAb24 epitope (D), α_M integrin (E), and α_D integrin (F) on blood eosinophils before segmental Ag challenge (light gray), blood eosinophils 48 h after segmental Ag challenge (medium gray), and BAL eosinophils 48 h after segmental Ag challenge (dark gray) in all tested subjects, single responders, and dual responders. Values shown are specific gMCF (mean $\pm$ SEM). For n values and expression given as percentage of positive cells, see Table II. *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$ vs blood eosinophils before segmental challenge; †, $p \leq 0.05$; †††, $p \leq 0.001$ vs blood eosinophils after segmental challenge; ‡, $p \leq 0.05$ vs single responders.

Ag challenge was decreased minimally compared with before segmental Ag challenge and was not significantly different between single and dual responders (medians 98 and 96% of the value before segmental challenge in single and dual responders, respectively).

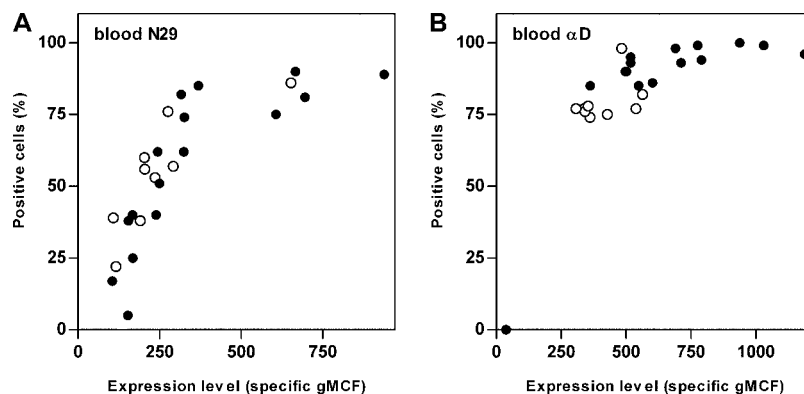
Median number of BAL eosinophils after segmental challenge was >10 -fold higher in the dual responders than in the single responders (Table I). Median numbers of BAL neutrophils and macrophages, in contrast, were ~ 2 -fold higher and not significantly different in dual vs single responders (medians in single and dual responders were 3.1×10^6 and 7.5×10^6 neutrophils and 43×10^6 and 93×10^6 macrophages; respectively). The neutrophil percentage was not significantly different and the macrophage percentage was significantly lower in dual responders due to the increased proportion of eosinophils (medians in single and dual

responders were 2.3 and 3.0% neutrophils and 47 and 22% macrophages, respectively).

Integrin expression on blood and BAL eosinophils after segmental allergen challenge

Sample flow cytometry histograms are shown in Fig. 1 for eosinophil expression of total β_1 (A), the activation-sensitive β_1 epitope for mAb N29 (B–D), total β_2 (E), the activation-sensitive β_2 epitope for mAb24 (F), α_M (G), and α_D (H). Expression distributions were homogeneous in some samples (Fig. 1, B and F) and heterogeneous and asymmetric with one or more “shoulders” in others (Fig. 1, A, C, D, E, G, and H). β_1 distributions were mostly heterogeneous on blood eosinophils and uniformly homogeneous on BAL eosinophils (Fig. 1A and Table II). N29 reactivity of blood eosinophils was variable (Fig. 1, B–D, and Table II). Some samples

FIGURE 3. Integrin expression on blood eosinophils as the percentage of positive cells and the expression level. Blood eosinophil expression of activation-sensitive β_1 integrin mAb N29 epitope (A) or α_D integrin (B) before (open symbols) and 48 h after (filled symbols) segmental Ag challenge, expressed as the percentage of positive cells (y axes) or as expression level (specific gMCF) (x axes).



had two peaks (Fig. 1C) or a “shoulder” of reactivity (Fig. 1D). N29 reactivity of BAL eosinophils was uniformly homogeneous (Fig. 1, B–D, and Table II). β_2 was heterogeneous on blood eosinophils, often with two or several distinct populations, whereas the peak of BAL eosinophils was mostly homogeneous and shifted to the right with higher fluorescence intensity compared with the most positive blood eosinophil population (Fig. 1E and Table II). mAb24 reactivity was homogeneous and low on blood eosinophils and mostly homogeneous and shifted to the right on BAL eosinophils (Fig. 1F and Table II). α_M was mostly heterogeneous on blood eosinophils and homogeneous and shifted to the right on BAL eosinophils (Fig. 1G and Table II). α_D was mostly heterogeneous on blood eosinophils and mostly homogeneous on BAL eosinophils (Fig. 1H and Table II). Thus, distributions on BAL eosinophils were typically homogeneous whereas distributions of β_1 , β_2 , α_M , and α_D on blood eosinophils were mostly heterogeneous.

Expression data were scored either as percentage of positive cells or as expression level (specific gMCF) (Table II and Fig. 2). Fig. 3 shows a comparison between the results with these two scoring methods on blood eosinophils for N29 (Fig. 3A) and α_D (Fig. 3B). At lower fluorescence intensity the percentage of positive cells and the expression level correlate, whereas at higher intensity the percentage of positive cells plateaus (Fig. 3, A and B). Because of its greater dynamic range, specific gMCF was chosen as the more informative measure of integrin expression and activation (Fig. 2). It should be emphasized that for the samples displaying heterogeneous bimodal or multimodal distributions of the β_2 integrin subunits, neither gMCF nor percentage of positive cells fully captures the complexity of the distributions.

On circulating eosinophils of dual responders the expression level of the epitope for activation-sensitive anti- β_1 mAb N29 (34, 40, 41) was higher 48 h after Ag segmental challenge as compared with the level before challenge (Fig. 2B). Circulating eosinophils also had significantly higher expression of the α_D integrin subunit compared with before segmental challenge (Fig. 2F). α_D and N29 epitope expression on blood eosinophils 48 h after segmental challenge correlated with each other (r_s (Spearman rank correlation coefficient) = 0.73; p = 0.002). Thus, the results demonstrate that segmental Ag challenge causes significantly increased surface expression of the α_D subunit of β_2 integrins and, in dual responders, activation of β_1 integrins at 48 h on blood eosinophils.

Eosinophils in BAL 48 h after segmental Ag challenge had significantly higher β_2 and α_M subunit expression than eosinophils in blood obtained before or 48 h after segmental challenge (Fig. 2, C and E). The expression of the epitope for the activation-sensitive

anti- β_2 mAb24 (32, 33) was significantly increased on BAL eosinophils; the binding of mAb24 to blood eosinophils was low in blood both before and after segmental challenge (Fig. 2D). α_D expression was significantly increased on BAL eosinophils as compared with blood eosinophils before challenge but not as compared with blood also sampled at 48 h (Fig. 2F). In dual responders, BAL eosinophils had significantly more total and activated β_1 (as ascertained by N29) than blood eosinophils before challenge (Fig. 2, A and B). α_6 expression was lower on BAL eosinophils than on blood eosinophils after challenge (specific gMCF = 560 ± 140 (mean $\pm$ SEM) vs 920 ± 110 , $p \leq 0.05$) (data not shown). No differences were found in the expression of α_L , α_X , α_4 , or β_7 (data not shown). Overall, the results indicate that $\alpha_M\beta_2$ is up-regulated on BAL eosinophils and that BAL eosinophil β_2 integrins are in a conformationally altered and activated state compared with β_2 integrins on blood eosinophils.

To examine the specificity of the changes on blood eosinophils, circulating neutrophils and monocytes were also analyzed. Before segmental challenge, neutrophils had higher N29 reactivity than eosinophils and monocytes had even higher N29 reactivity (Figs. 4B and 5B; compare with Fig. 2B). At 48 h after segmental Ag challenge, circulating neutrophils and monocytes, like eosinophils, had significantly higher α_D expression compared with before challenge (Figs. 4F and 5F). Monocytes from dual responders had higher α_D after segmental challenge than those from single responders (Fig. 5F). N29 reactivity of neutrophils (as with eosinophils) increased significantly in dual responders upon segmental challenge, although to a lesser degree (1.4-fold for neutrophils, 1.7-fold for eosinophils) (Fig. 4B; compare with Fig. 2B), whereas N29 reactivity of monocytes did not increase significantly (Fig. 5B). Furthermore, α_M on blood neutrophils and monocytes and β_2 on blood monocytes increased significantly upon segmental challenge (Figs. 4E and 5, C and E) in contrast to blood eosinophil α_M and β_2 , which did not (Fig. 2C,E).

Thus, we determined the following: 1) α_D is up-regulated on all three leukocyte populations, not just eosinophils; 2) β_1 at baseline is more activated on neutrophils and monocytes compared with eosinophils; 3) in dual responders we found an increased activation state of β_1 on neutrophils despite the high baseline activation state; and 4) surface expression of α_M is up-regulated on monocytes and neutrophils, but not on blood eosinophils.

Correlations between integrin expression and eosinophil numbers in BAL after segmental allergen challenge

As with the variability in eosinophil numbers in BAL after segmental Ag challenge in the 19 subjects (Table I), there was considerable variability in integrin activation or expression on BAL eosinophils (Fig. 2). BAL eosinophils from dual responders had

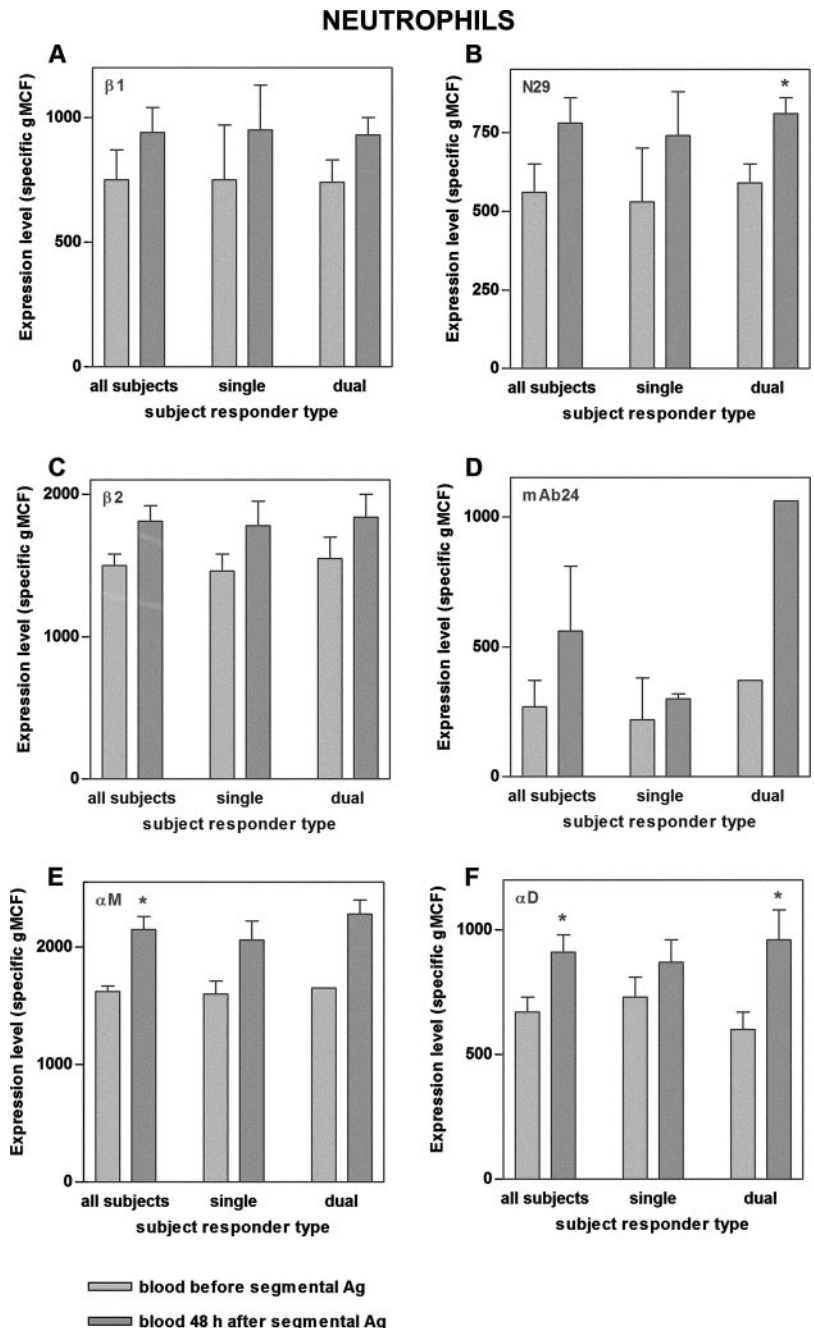


FIGURE 4. Integrin expression on blood neutrophils before and 48 h after segmental Ag challenge. Expression of total β_1 integrin (A), activation-sensitive β_1 integrin mAb N29 epitope (B), total β_2 integrin (C), activation-sensitive β_2 integrin mAb24 epitope (D), α_M integrin (E), and α_D integrin (F) on blood neutrophils before segmental Ag challenge (light gray) and 48 h after segmental Ag challenge (medium gray) in all tested subjects, single responders, and dual responders. Values shown are specific gMCF (mean $\pm$ SEM). *, $p \leq 0.05$ vs before segmental challenge.

significantly higher N29 epitope and total β_2 expression than BAL eosinophils from single responders (Figs. 2 and 6). Grouping all subjects together, the reactivity of BAL eosinophils with the activation-sensitive mAbs, N29 against β_1 and mAb24 against β_2 , correlated significantly with the percentage of eosinophils in BAL (Fig. 7). Surface expression of other integrin subunits on BAL eosinophils did not correlate with BAL eosinophil percentage (data not shown). These results indicate that activation of both β_1 and β_2 integrins are associated with eosinophil recruitment to the airway.

Correlations between integrin expression after segmental allergen challenge and the magnitude of the late-phase fall in FEV₁ after whole-lung allergen challenge

We also analyzed for possible correlations between eosinophil integrins after segmental challenge and the magnitude of the maxi-

mum fall in FEV₁ 3–8 h after whole-lung allergen challenge (late-phase fall). α_D expression and reactivity with N29 of blood eosinophils 48 h after segmental challenge each correlated with the magnitude of the late-phase fall in FEV₁ after whole-lung challenge (Fig. 8, A and B). β_2 expression and reactivity with mAb24 of BAL eosinophils 48 h after segmental challenge also correlated with the magnitude of the late-phase FEV₁ fall (Fig. 8, C and D), as did α_M expression of BAL eosinophils ($r_s = 0.83$, $p = 0.008$) (data not shown). In addition, the late-phase FEV₁ fall correlated with the percentage of eosinophils in BAL 48 h after segmental challenge ($r_s = 0.70$, $p = 0.001$) (data not shown). Thus, the results indicate that greater α_D up-regulation and β_1 activation on blood eosinophils and greater $\alpha_M\beta_2$ up-regulation and β_2 activation on BAL eosinophils after segmental challenge in a subject are associated with a greater late-phase FEV₁ fall in response to whole-lung challenge.

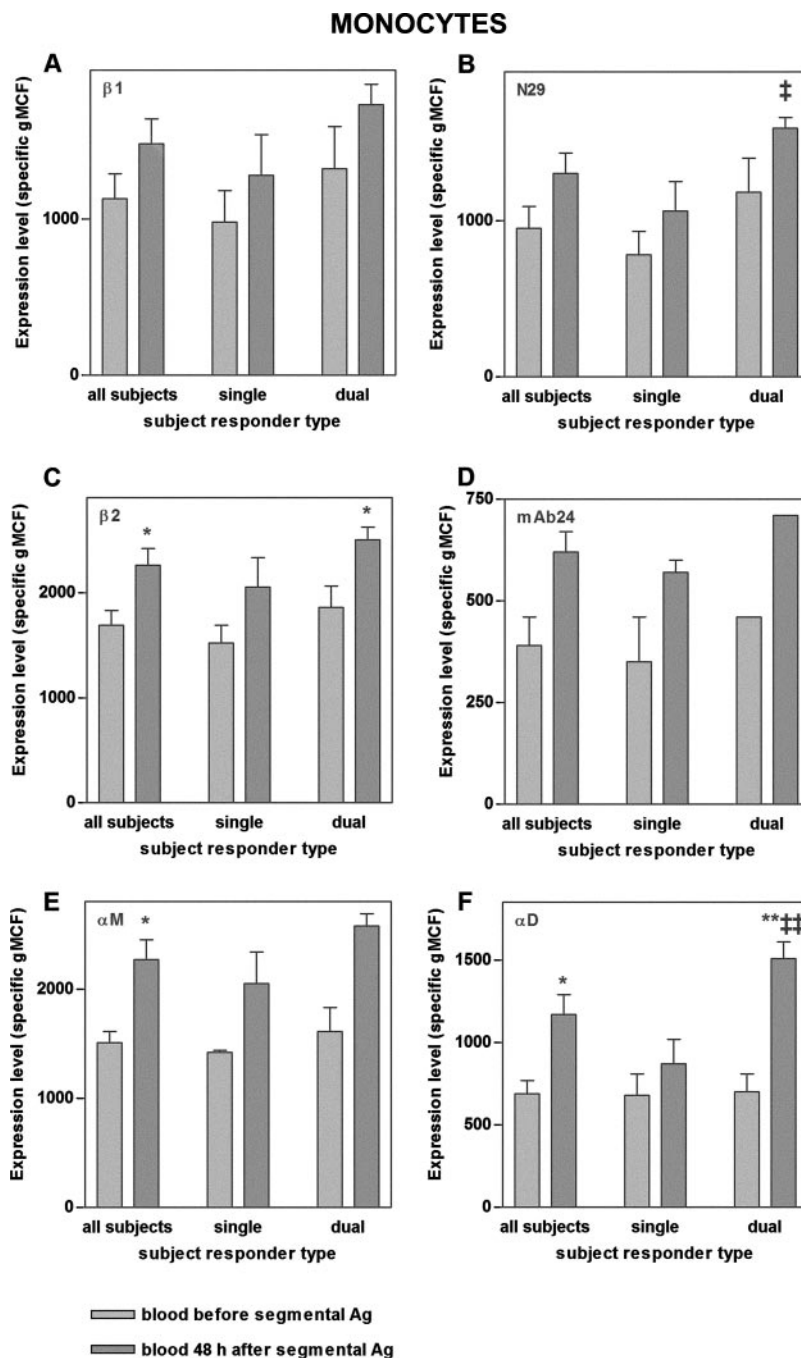


FIGURE 5. Integrin expression on blood monocytes before and 48 h after segmental Ag challenge. Expression of total β_1 integrin (A), activation-sensitive β_1 integrin mAb N29 epitope (B), total β_2 integrin (C), activation-sensitive β_2 integrin mAb24 epitope (D), α_M integrin (E), and α_D integrin (F) on blood monocytes before segmental Ag challenge (light gray) and 48 h after segmental Ag challenge (medium gray) in all tested subjects, single responders, and dual responders. Values shown are specific gMCF (mean $\pm$ SEM). *, $p \leq 0.05$; **, $p \leq 0.01$ vs before segmental challenge; †, $p \leq 0.05$; ‡, $p \leq 0.01$ vs single responders.

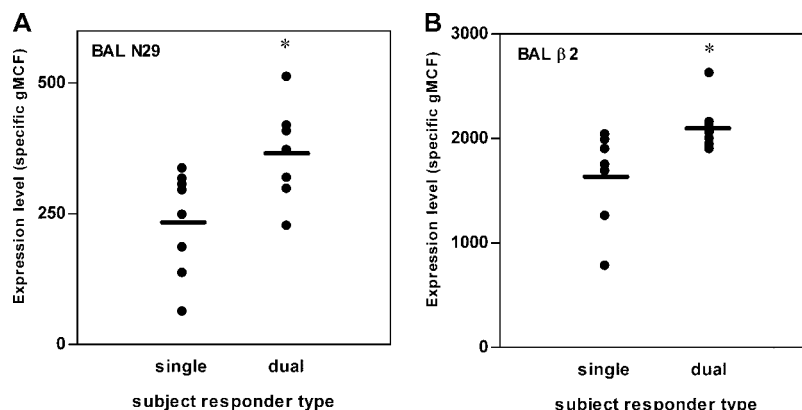
Correlations between integrin expression and cytokine concentrations in BAL fluid after segmental allergen challenge

To identify factor(s) possibly responsible for the changes in integrins observed following segmental Ag challenge, the concentrations of IL-5, GM-CSF, IL-3, and IFN- γ in BAL fluid were measured (Fig. 9). The concentration of all four cytokines was significantly higher 48 h after segmental challenge than in samples obtained immediately before segmental challenge (Fig. 9). There was a highly significant difference in BAL fluid IL-5 after segmental challenge between single and dual responders; median IL-5 concentration in BAL fluid from dual responders was $\sim$ 40-fold greater than in BAL fluid from single responders (Fig. 9). Also IL-3 and IFN- γ were significantly different between single and dual responders; medians from dual responders were $\sim$ 10- and $\sim$ 3-fold those from single responders for IL-3 and IFN- γ , respec-

tively (Fig. 9). GM-CSF was not different between single and dual responders (Fig. 9).

The concentration of BAL fluid IL-5, but not that of the other cytokines, correlated significantly and strongly with the percentage of eosinophils in BAL 48 h after segmental Ag challenge ($r_s = 0.69$, $p = 0.001$ for IL-5; $r_s = 0.23$, $p = 0.36$ for GM-CSF; $r_s = 0.32$, $p = 0.19$ for IL-3; and $r_s = 0.39$, $p = 0.11$ for IFN- γ). It is possible that one or both of the other IL-5 family cytokines would correlate with the BAL eosinophil percentage if a higher number of subjects were studied. IL-5 also correlated inversely with the percentage of macrophages (data not shown), reflecting the decreased macrophage percentage as eosinophil percentage increases. Further, BAL fluid IL-5 after segmental challenge correlated strongly with the magnitude of the late-phase fall in FEV₁ after whole-lung challenge ($r_s = 0.79$, $p < 0.0001$). IL-3 also

FIGURE 6. Differences in integrin activation and expression on BAL eosinophils between single and dual responders. BAL eosinophil expression 48 h after segmental Ag challenge of activation-sensitive β_1 integrin mAb N29 epitope (A) or total β_2 integrin (B) in single and dual responders. Bar, mean; *. $p \leq 0.05$ vs single responders.



correlated with late-phase FEV₁ fall, but less well ($r_s = 0.51$, $p = 0.03$). GM-CSF ($r_s = 0.37$, $p = 0.12$) and IFN- γ ($r_s = 0.36$, $p = 0.13$) did not correlate with late-phase FEV₁ fall.

Levels of β_2 and reactivity with mAb24 of BAL eosinophils correlated significantly with the concentration of IL-5 in BAL fluid 48 h after segmental Ag challenge (Fig. 10), as did level of α_M ($r_s = 0.70$, $p = 0.04$) (data not shown). These integrins did not correlate with concentrations of the other cytokines (data not shown). There was no correlation with BAL fluid IL-5 before segmental challenge (data not shown). Integrins of blood eosinophils did not correlate with cytokine concentrations in BAL fluid 48 h after segmental Ag challenge (data not shown). Thus, a greater IL-5 concentration in BAL fluid in a subject is associated with greater $\alpha_M\beta_2$ up-regulation and β_2 activation on BAL eosinophils after segmental challenge.

Discussion

We observed changes in integrins on eosinophils in the blood and airways of 19 subjects with mild allergic asthma after segmental bronchial allergen challenge that were related to the reactivities of subjects in the whole Ag challenge protocol. Eosinophils in blood 48 h after challenge had increased expression of the α_D integrin subunit and, in asthmatics with a dual response phenotype, increased reactivity with the activation-sensitive anti- β_1 integrin mAb N29 compared with before challenge. Eosinophils in BAL obtained 48 h after challenge had increased expression of β_2 and α_M integrin subunits and increased reactivity of activation-sensitive anti- β_2 integrin mAb24 compared with eosinophils in blood. β_2 , α_M , and mAb24 epitope expression on BAL eosinophils correlated with the concentration of IL-5 in BAL fluid obtained 48 h after segmental Ag challenge. In addition, α_D expression and, in dual responders, N29 reactivity were higher on BAL eosinophils

than on blood eosinophils before challenge. Integrin expression patterns on BAL eosinophils were more homogeneous and symmetric than on blood eosinophils. The observations of N29 epitope and α_D expression are compatible with a scenario whereby extravasated eosinophils appearing in the airway are derived from a subpopulation of the total, more heterogeneous population of circulating eosinophils. However, the observations of $\alpha_M\beta_2$ expression, β_2 activation state, and IL-5 levels in BAL fluid indicate that, after leaving the circulation, eosinophils undergo IL-5-triggered activation of β_2 and up-regulation of $\alpha_M\beta_2$. Analysis of neutrophils and monocytes in the same blood samples revealed that α_D expression increased on all three leukocyte types upon segmental challenge; thus, α_D up-regulation may occur through a common mechanism. At baseline, N29 reactivity was higher on neutrophils than on eosinophils and even higher on monocytes. N29 reactivity increased significantly on neutrophils in dual responders upon challenge but to a relatively lesser degree than on eosinophils, whereas it did not change significantly on monocytes. The mechanisms responsible for maintaining the baseline β_1 activation levels on the different leukocytes and those responsible for the elevation of activation state upon challenge are unknown. Finally, neutrophil and monocyte α_M and monocyte β_2 were up-regulated upon challenge, in contrast to eosinophil α_M and β_2 , which were up-regulated in BAL but not in blood. Thus, circulating eosinophils are less sensitive than neutrophils or monocytes to increased surface expression of $\alpha_M\beta_2$.

β_1 activation on blood eosinophils, assessed by reactivity with N29, has been shown to correlate inversely with FEV₁ in an inhaled corticosteroid withdrawal study (35). The present results complement the steroid withdrawal study by demonstrating persistent β_1 activation in dual responders with more eosinophilic inflammation in the airway. Blood eosinophils from dual

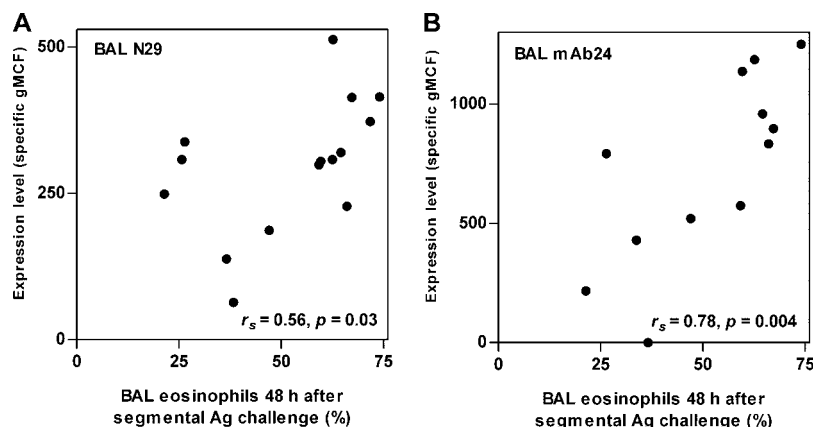
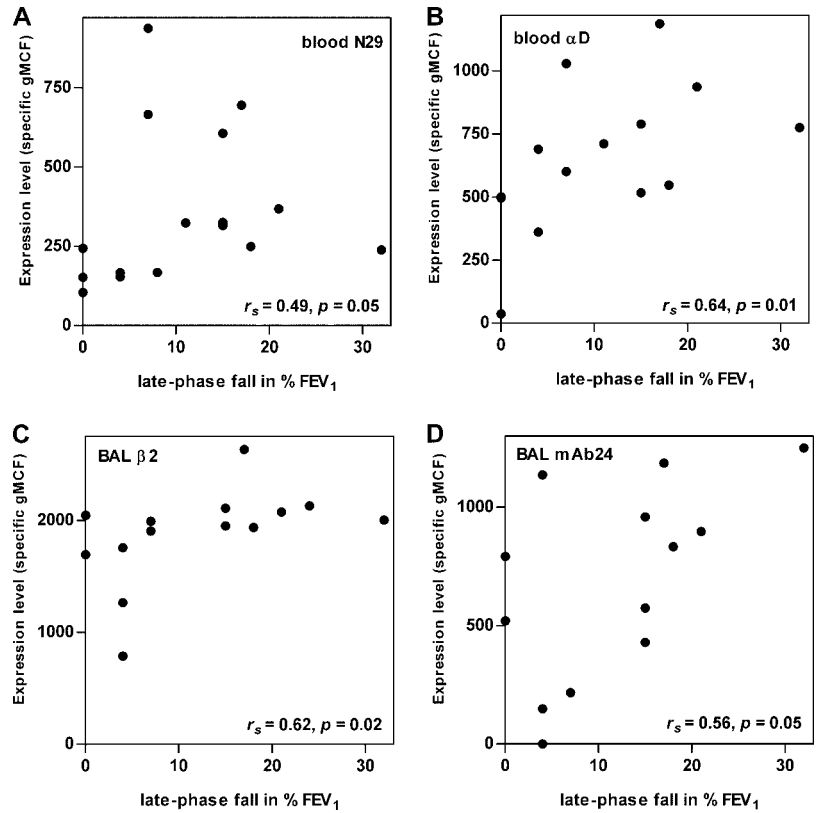


FIGURE 7. Correlations between integrin activation on BAL eosinophils and BAL eosinophil percentage. Correlations between BAL eosinophil expression of activation-sensitive β_1 integrin mAb N29 epitope (A) or activation-sensitive β_2 integrin mAb24 epitope (B) and the percentage of eosinophils in BAL 48 h after segmental Ag challenge. r_s , Spearman rank correlation coefficient.

FIGURE 8. Correlations between integrin activation and expression on blood and BAL eosinophils and the magnitude of the fall in FEV₁ 3–8 h after whole-lung Ag challenge. Correlations between blood eosinophil expression of activation-sensitive β_1 integrin mAb N29 epitope (A) or α_D integrin (B) or BAL eosinophil expression of total β_2 integrin (C) or activation-sensitive β_2 integrin mAb24 epitope (D) 48 h after segmental Ag challenge and the magnitude of the late-phase fall in FEV₁ 3–8 h after whole-lung Ag challenge. r_s , Spearman rank correlation coefficient.



responders, but not from single responders, have also been shown to have enhanced activation of Fc γ RII (CD32) as assessed with the phage mAb A17 6 h after challenge (42). Whether activation of β_1

integrin and CD32 on circulating eosinophils are triggered by the same or different stimuli and signaling pathways is an interesting question that remains to be investigated.

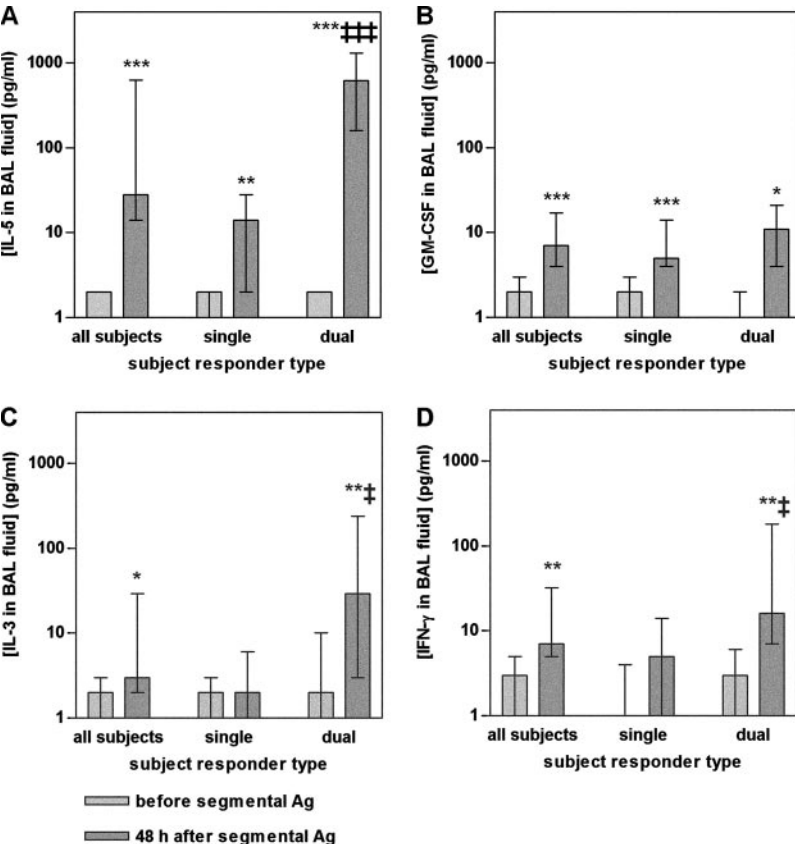
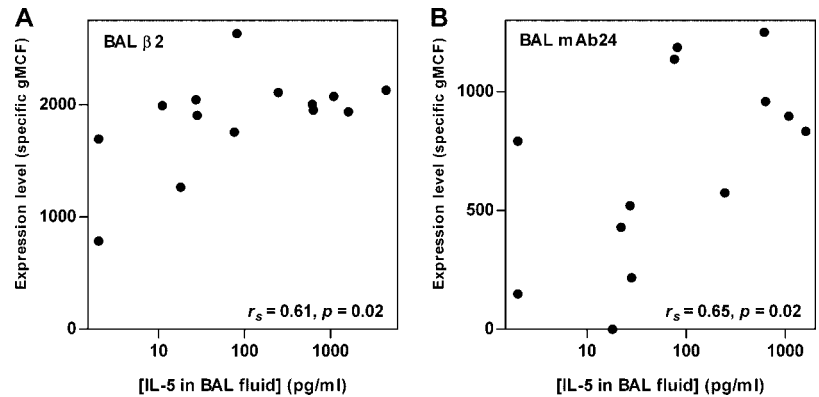


FIGURE 9. Cytokine concentrations in BAL fluid before and 48 h after segmental Ag challenge. Concentrations of IL-5 (A), GM-CSF (B), IL-3 (C), and IFN- γ (D) in BAL fluid before (light gray bars) and 48 h after (medium gray bars) segmental Ag challenge from all tested subjects ($n = 19$), single responders ($n = 10$), or dual responders ($n = 9$). Values shown are medians with 25th and 75th percentiles of concentrations in recovered, unconcentrated BAL fluid. *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$ vs before segmental challenge; †, $p \leq 0.05$; †††, $p \leq 0.001$ vs single responders.

FIGURE 10. Correlations between integrin activation and expression on BAL eosinophils and IL-5 in BAL fluid. Correlations between blood eosinophil expression of total β_2 integrin (A) or activation-sensitive β_2 integrin mAb24 epitope (B) and the concentration of IL-5 in BAL fluid 48 h after segmental Ag challenge. r_s , Spearman rank correlation coefficient.



The changes observed here in BAL eosinophils reproduce observations in the literature, made mostly on isolated eosinophils, that BAL eosinophils have higher α_M and β_2 than blood eosinophils (43–46), activated $\alpha_M\beta$ as monitored by anti-active α_M mAb CBRM1/5 (22, 36), and increased $\alpha_M\beta_2$ -inhibitable adhesion to diverse ligands (22, 36). Our finding that dual responders have higher total numbers of cells and eosinophils and a higher proportion of eosinophils in BAL after segmental Ag challenge than single responders also is in accord with prior reports (47–50). We also found that the β_1 activation state and β_2 surface expression were significantly higher on BAL eosinophils from dual responders than on those from single responders. Activation of β_1 and β_2 integrins, as assessed with the activation-sensitive mAbs N29 and mAb24, respectively, on BAL eosinophils correlated with eosinophil percentage in BAL, indicating that activation of both of these subfamilies of integrins is important for eosinophil recruitment. This idea is consistent with *in vivo* studies supporting the involvement of $\alpha_4\beta_1$ in eosinophil appearance in the airway and with *in vitro* studies showing the involvement of both β_1 and β_2 integrins in eosinophil transendothelial migration (22, 51–56). The evidence for involvement of both $\alpha_4\beta_1$ and β_2 integrins in eosinophil migration *in vivo* is further strengthened by a recent report that eosinophil recruitment to airway after Ag challenge was severely attenuated in both a conditional α_4 integrin knockout mouse and a β_2 -deficient mouse (57). Finally, α_D expression and β_1 activation on blood eosinophils as well as β_2 and α_M expression and β_2 activation on BAL eosinophils after segmental Ag challenge all correlated with the magnitude of the late-phase fall in FEV₁ in response to whole-lung Ag challenge.

A number of mediators are increased in the airway after challenge (28, 29, 49, 58, 59). Of these, the most likely candidates to account for the up-regulation and activation of $\alpha_M\beta_2$ are the IL-5 family cytokines (36, 60, 61). Concentrations of IL-5, GM-CSF, IL-3, and IFN- γ in BAL fluid recovered 48 h after segmental Ag challenge were all increased significantly compared with those in BAL fluid recovered before challenge. IL-5 correlated with BAL eosinophil α_M and β_2 expression and β_2 activation 48 h after segmental challenge. The median concentration of IL-5 in BAL fluid from dual responders after segmental challenge was ~ 600 pg/ml, which was >20 -fold the median concentration of the other cytokines. Further, the IL-5 concentration in the five BAL samples containing eosinophils with the most highly activated β_2 was 100 to >1000 pg/ml. During the recovery of BAL, the volume of the fluid lining the airway epithelium *in vivo* is estimated to become diluted 100-fold (62). Thus, the levels of IL-5 in the epithelial lining fluid of these subjects (all of whom were dual responders) are estimated to be ~ 10 – 100 ng/ml. Treatment of blood eosinophils *in vitro* with IL-5 at concentrations in this range is known to

saturate the IL-5 receptor (63–68) and lead to $\alpha_M\beta_2$ up-regulation and activation and induction of adhesion to ICAM-1 and other substrates (36, 60, 61), priming and enhanced response to chemoattractants (69), enhanced viability (70), degranulation and granule protein release (30), IL-5 receptor α down-regulation (71), and enhanced expression of certain genes (72). In contrast, median concentrations of the other IL-5 family cytokines before dilution are estimated to be ~ 1 – 3 ng/ml. Thus, the concentration of IL-5 in the lining fluid *in vivo*, assuming that a significant portion of the IL-5 measured represents active IL-5, is estimated to be sufficient to cause $\alpha_M\beta_2$ up-regulation and activation of β_2 integrins. These results are in accord with the earlier observation that the IL-5 receptor is down-regulated specifically compared with the GM-CSF receptor on eosinophils recovered by BAL 48 h after segmental Ag challenge (30). We also found that BAL fluid IL-5 correlated with the percentage of eosinophils in BAL and with the magnitude of the late-phase fall in FEV₁. A correlation between BAL fluid IL-5 and eosinophil recruitment to the airway after segmental Ag challenge has been reported previously (38, 73–76) and is consistent with the observation that anti-IL-5 therapy causes a significant decrease in sputum eosinophils (1).

Our study has several limitations. The data sets were incomplete in that all integrins were not assayed from all samples. Sampling of blood and BAL was performed only at one time point (48 h) after segmental Ag challenge. Future experiments with blood sampling at various time points after segmental and/or whole-lung Ag challenge are required to record the time course of α_D and N29 expression on blood eosinophils in dual and single responders and to learn when the values diverge. Further, the importance of the integrin expression heterogeneity observed on blood eosinophils, particularly regarding β_2 and α_M , is not known. Such heterogeneity, to our knowledge, has not been described or discussed before. Of note, we subjected whole blood to primary Ab incubation without previous cell isolation or centrifugation. Earlier studies on blood eosinophil integrin expression have been performed on a buffy coat preparation or purified eosinophils (43–45). Dextran sedimentation during buffy coat preparation has been shown to cause up-regulation of α_M on blood eosinophils (and neutrophils) compared to that in whole, unfractionated blood (77). Eosinophils are likely similar to neutrophils, which are known to store a large proportion of α_M in granules, wherefrom it is translocated to the plasma membrane after cell stimulation (78). Remarkably, eosinophils in BAL had strong homogeneous labeling for β_2 and α_M . One possibility is that higher reacting subpopulations of circulating leukocytes represent cells that have undergone “retrograde” migration back to the blood from tissues, as has been demonstrated for zebrafish neutrophils *in vivo* (79) and human neutrophils in

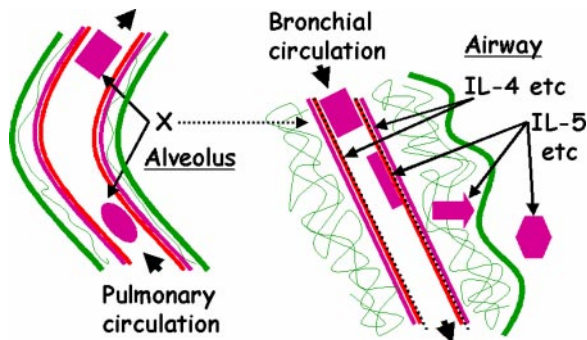


FIGURE 11. Sequential up-regulation and activation of eosinophil integrins during recruitment to airway after exposure to allergen. Schematic relating activation of β_1 and β_2 integrins to eosinophil trafficking during and after segmental Ag challenge. *Left*, An eosinophil (oval) with unactivated β_1 integrins and low surface expression of α_D is shown entering the pulmonary circulation of a segment subjected to Ag challenge. A yet to be identified stimulus or stimuli (X) cause(s) activation of β_1 integrins and increased surface expression of α_D during transit of the eosinophil (rectangle) through the vessel. *Right*, Concurrently, IL-4 and other mediators are released and specifically induce surface expression of VCAM-1 (discontinuous black line) on the endothelial cells of a bronchial blood vessel in the challenged segment. An eosinophil with activated β_1 and up-regulated α_D (rectangle) is shown entering the bronchial circulation. The eosinophil arrests and adheres (elongated rectangle) to VCAM-1. After exposure to IL-5 the eosinophil becomes responsive to chemotactic factors, migrates (arrow), and assumes a hyperadhesive airway phenotype (hexagon) in the lumen. The airway eosinophil displays activated β_1 , up-regulated α_D , activated β_2 , and up-regulated α_M and β_2 .

vitro (80). Blood sampling at various time points after Ag challenge, including times beyond 48 h, may shed further light on circulating eosinophil subpopulations.

Our results and the literature are compatible with the schematic of eosinophil recruitment after segmental Ag challenge that is depicted in Fig. 11. At baseline, β_1 and β_2 integrins on a circulating eosinophil are in a low activation state, the level of eosinophil surface α_D is low, and VCAM-1 is absent from endothelial surfaces of the bronchial circulation. Yet to be identified stimuli resulting from Ag challenge cause enhanced activation of β_1 integrins and increased surface expression of α_D on circulating eosinophils. Because increased expression of the β_1 activation epitope occurs on most eosinophils, the activation likely takes place in the pulmonary circulation through which the eosinophils constantly pass or in response to the release of an activating substance into the circulation (Fig. 11). Such activation contrasts with the model for the recruitment of leukocytes (16), including eosinophils (20), in which integrin activation is assumed to occur locally and concurrently with rolling and tethering on endothelium. Interestingly, bone marrow-derived progenitor cells or circulating eosinophils of asthmatics have been shown to be activated upon Ag challenge also as measured by the up-regulation of IL-5 receptor α and CCR3, the activation of CD32, and greater responsiveness to chemoattractants (42, 81–83). Further, eosinophils from allergic asthmatics have been shown to have a greater capacity to adhere to and transmigrate through endothelium than eosinophils from normal donors (51, 84).

The question then arises as to how activated circulating eosinophils localize to the parts of the lung subjected to Ag challenge in preference to neutrophils and monocytes. As a parallel response to Ag challenge, mediators, including IL-4 and IL-13, are elaborated and activate the bronchial endothelium to specifically synthesize and express VCAM-1. VCAM-1 has been shown to be preferentially expressed in the asthmatic lung or after Ag challenge (85,

86). Further, VCAM-1 can support the adhesion of eosinophils but not neutrophils (9, 18, 22, 87–91). Circulating eosinophils with activated β_1 and up-regulated α_D are presumed to have a higher probability of arresting on VCAM-1 on the activated endothelium of the bronchial circulation (Fig. 11), because adhesion of purified blood eosinophils to VCAM-1 is mediated by $\alpha_4\beta_1$ (22, 92), with a possible contribution by $\alpha_D\beta_2$ (22, 31).

α_M and β_2 are shown as being up-regulated and β_2 integrins as being activated by IL-5, also elaborated in response to allergen. Eosinophils appearing in the airway lumen have a hyperadhesive phenotype that is marked by activated β_1 integrins, up-regulated $\alpha_D\beta_2$, activated β_2 integrins, and up-regulated $\alpha_M\beta_2$ (Fig. 11). Eosinophils, particularly after priming by IL-5 (69), responds to chemoattractants, such as eotaxin (9, 13) (Fig. 11). $\alpha_M\beta_2$ is believed to be important for eosinophil migration (22, 51, 53, 93–95). Thus, the scenario has migrating eosinophils using activated $\alpha_M\beta_2$ and possibly other integrins to interact with multiple substrates (22, 36, 92), including VCAM-1, ICAM-1, and extracellular matrix proteins, on endothelial cells, in connective tissue, and on epithelium of the bronchial wall.

Acknowledgments

We thank Mary Jo Jackson and Erin Billmeyer for patient recruitment, screening, and assistance with bronchoscopy; Lin-Ying Liu, Sarah Panzer, Rebecca Lawniczak, and Rose DeGrauw for processing BAL samples and providing blood samples; Melissa Heim and Lisa Skoggs for assistance with ELISA; Nancy Hogg for providing mAb24; ICOS for providing anti- α_D mAb 240I; Kathleen Schell, Joan Batchelder, and Joel Puchalski for help with flow cytometry data collection and analysis; Michael Evans for advice on statistics; and Julie Sedgwick and Lin-Ying Liu for advice.

Disclosures

M.W.J., W.W.B., and D.F.M. have, with the help of the Wisconsin Alumni Research Foundation, a patent application pending on “Beta1 integrin activation as a marker for asthma,” based on the associations between N29 reactivity and FEV₁ reported by Johansson et al. 2006 (Ref. 35). W.W.B. has or has had research support from Novartis, Dynavax, Wyeth, Centocor, GlaxoSmithKline, Medicinova, Pfizer, and Biowa/MedImmune. E.A.B.K. and N.N.J. have no financial conflict of interest.

References

- Leckie, M. J., A. ten Brinke, J. Khan, Z. Diamant, B. J. O'Connor, C. M. Walls, A. K. Mathur, H. C. Cowley, K. F. Chung, R. Djukanovic, et al. 2000. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 356: 2144–2148.
- Busse, W. W., and R. F. Lemanske, Jr. 2001. Asthma. *N. Engl. J. Med.* 344: 350–362.
- Green, R. H., C. E. Brightling, S. McKenna, B. Hargadon, D. Parker, P. Bradding, A. J. Wardlaw, and I. D. Pavord. 2002. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet* 360: 1715–1721.
- Mattes, J., M. Yang, S. Mahalingam, J. Kuehr, D. C. Webb, L. Simson, S. P. Hogan, A. Koskinen, A. N. McKenzie, L. A. Dent, et al. 2002. Intrinsic defect in T cell production of interleukin (IL)-13 in the absence of both IL-5 and eotaxin precludes the development of eosinophilia and airways hyperreactivity in experimental asthma. *J. Exp. Med.* 195: 1433–1444.
- Flood-Page, P. T., A. N. Menzies-Gow, A. B. Kay, and D. S. Robinson. 2003. Eosinophil's role remains uncertain as anti-interleukin-5 only partially depletes numbers in asthmatic airway. *Am. J. Respir. Crit. Care Med.* 167: 199–204.
- Flood-Page, P., A. Menzies-Gow, S. Phipps, S. Ying, A. Wangoo, M. S. Ludwig, N. Barnes, D. Robinson, and A. B. Kay. 2003. Anti-IL-5 treatment reduces deposition of ECM proteins in the bronchial subepithelial basement membrane of mild atopic asthmatics. *J. Clin. Invest.* 112: 1029–1036.
- Justice, J. P., M. T. Borchers, J. R. Crosby, E. M. Hines, H. H. Shen, S. I. Ochkur, M. P. McGarry, N. A. Lee, and J. J. Lee. 2003. Ablation of eosinophils leads to a reduction of allergen-induced pulmonary pathology. *Am. J. Physiol.* 284: L169–L178.
- Shen, H. H., S. I. Ochkur, M. P. McGarry, J. R. Crosby, E. M. Hines, M. T. Borchers, H. Wang, T. L. Biechelle, K. R. O'Neill, T. L. Ansay, et al. 2003. A causative relationship exists between eosinophils and the development of allergic pulmonary pathologies in the mouse. *J. Immunol.* 170: 3296–3305.
- Wills-Karp, M., and C. L. Karp. 2004. Eosinophils in asthma: remodeling a tangled tale. *Science* 305: 1726–1729.
- Bochner, B. S., and W. W. Busse. 2005. Allergy and asthma. *J. Allergy Clin. Immunol.* 115: 953–959.

11. Kay, A. B. 2005. The role of eosinophils in the pathogenesis of asthma. *Trends Mol. Med.* 11: 148–152.
12. Liu, L. Y., S. K. Mathur, J. B. Sedgwick, N. N. Jarjour, W. W. Busse, and E. A. Kelly. 2006. Human airway and peripheral blood eosinophils enhance Th1 and Th2 cytokine secretion. *Allergy* 61: 589–597.
13. Rothenberg, M. E., and S. P. Hogan. 2006. The eosinophil. *Annu. Rev. Immunol.* 24: 147–174.
14. Kariyawasam, H. H., and D. S. Robinson. 2007. The role of eosinophils in airway tissue remodelling in asthma. *Curr. Opin. Immunol.* 19: 681–686.
15. Liu, L. Y., M. E. Bates, N. N. Jarjour, W. W. Busse, P. J. Bertics, and E. A. Kelly. 2007. Generation of Th1 and Th2 chemokines by human eosinophils: evidence for a critical role of TNF- α . *J. Immunol.* 179: 4840–4848.
16. Springer, T. A. 1994. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* 76: 301–314.
17. Hynes, R. O. 2002. Integrins: bidirectional, allosteric signaling machines. *Cell* 110: 673–687.
18. Broide, D., and P. Sriramarao. 2001. Eosinophil trafficking to sites of allergic inflammation. *Immunol. Rev.* 179: 163–172.
19. Wardlaw, A. J. 2001. Eosinophil trafficking in asthma. *Clin. Med.* 1: 214–218.
20. Rosenberg, H. F., S. Phipps, and P. S. Foster. 2007. Eosinophil trafficking in allergy and asthma. *J. Allergy Clin. Immunol.* 119: 1303–1310.
21. Tachimoto, H., and B. S. Bochner. 2000. The surface phenotype of human eosinophils. *Chem. Immunol.* 76: 45–62.
22. Barthel, S. R., M. W. Johansson, D. M. McNamee, and D. F. Mosher. 2008. Roles of integrin activation in eosinophil function and the eosinophilic inflammation of asthma. *J. Leukocyte Biol.* 83: 1–12.
23. Humphries, J. D., A. Byron, and M. J. Humphries. 2006. Integrin ligands at a glance. *J. Cell Sci.* 119: 3901–3903.
24. Palecek, S. P., J. C. Loftus, M. H. Ginsberg, D. A. Lauffenburger, and A. F. Horwitz. 1997. Integrin-ligand binding properties govern cell migration speed through cell-substratum adhesiveness. *Nature* 385: 537–540.
25. Humphries, M. J. 2004. Monoclonal antibodies as probes of integrin priming and activation. *Biochem. Soc. Trans.* 32: 407–411.
26. Xiao, T., J. Takagi, B. S. Coller, J. H. Wang, and T. A. Springer. 2004. Structural basis for allostery in integrins and binding to fibrinogen-mimetic therapeutics. *Nature* 432: 59–67.
27. Calhoun, W. J., N. N. Jarjour, G. J. Gleich, C. A. Stevens, and W. W. Busse. 1993. Increased airway inflammation with segmental versus aerosol antigen challenge. *Am. Rev. Respir. Dis.* 147: 1465–1471.
28. Liu, L., N. N. Jarjour, W. W. Busse, and E. A. Kelly. 2004. Enhanced generation of helper T type 1 and 2 chemokines in allergen-induced asthma. *Am. J. Respir. Crit. Care Med.* 169: 1118–1124.
29. Kelly, E. A., W. W. Busse, and N. N. Jarjour. 2000. Inhaled budesonide decreases airway inflammatory response to allergen. *Am. J. Respir. Crit. Care Med.* 162: 883–890.
30. Liu, L. Y., J. B. Sedgwick, M. E. Bates, R. F. Vrtis, J. E. Gern, H. Kita, N. N. Jarjour, W. W. Busse, and E. A. Kelly. 2002. Decreased expression of membrane IL-5 receptor α on human eosinophils: I. Loss of membrane IL-5 receptor α on airway eosinophils and increased soluble IL-5 receptor α in the airway after allergen challenge. *J. Immunol.* 169: 6452–6458.
31. Grayson, M. H., M. Van der Vieren, S. A. Sterbinsky, M. W. Gallatin, P. A. Hoffman, D. E. Staunton, and B. S. Bochner. 1998. α β 2 integrin is expressed on human eosinophils and functions as an alternative ligand for vascular cell adhesion molecule 1 (VCAM-1). *J. Exp. Med.* 188: 2187–2191.
32. Dransfield, I., and N. Hogg. 1989. Regulated expression of Mg²⁺ binding epitope on leukocyte integrin α subunits. *EMBO J.* 8: 3759–3765.
33. Lu, C., M. Shimaoka, Q. Zang, J. Takagi, and T. A. Springer. 2001. Locking in alternate conformations of the integrin α β 2 I domain with disulfide bonds reveals functional relationships among integrin domains. *Proc. Natl. Acad. Sci. USA* 98: 2393–2398.
34. Wilkins, J. A., A. Li, H. Ni, D. G. Stupack, and C. Shen. 1996. Control of β 1 integrin function: localization of stimulatory epitopes. *J. Biol. Chem.* 271: 3046–3051.
35. Johansson, M. W., S. R. Barthel, C. A. Swenson, M. D. Evans, N. N. Jarjour, D. F. Mosher, and W. W. Busse. 2006. Eosinophil β 1 integrin activation state correlates with asthma activity in a blind study of inhaled corticosteroid withdrawal. *J. Allergy Clin. Immunol.* 117: 1502–1504.
36. Barthel, S. R., N. N. Jarjour, D. F. Mosher, and M. W. Johansson. 2006. Dissection of the hyperadhesive phenotype of airway eosinophils in asthma. *Am. J. Respir. Cell Mol. Biol.* 35: 378–386.
37. Leitinger, B., and N. Hogg. 2000. Effects of I domain deletion on the function of the β 2 integrin lymphocyte function-associated antigen-1. *Mol. Biol. Cell* 11: 677–690.
38. Kelly, E. A., R. R. Rodriguez, W. W. Busse, and N. N. Jarjour. 1997. The effect of segmental bronchoprovocation with allergen on airway lymphocyte function. *Am. J. Respir. Crit. Care Med.* 156: 1421–1428.
39. Lemanske, R. F. Jr. 1991. The late phase response: clinical implications. *Adv. Intern. Med.* 36: 171–193.
40. Ni, H., A. Li, N. Simonsen, and J. A. Wilkins. 1998. Integrin activation by dithiothreitol or Mn²⁺ induces a ligand-occupied conformation and exposure of a novel NH2-terminal regulatory site on the β 1 integrin chain. *J. Biol. Chem.* 273: 7981–7987.
41. Mould, A. P., M. A. Travis, S. J. Barton, J. A. Hamilton, J. A. Askari, S. E. Craig, P. R. Macdonald, R. A. Kammerer, P. A. Buckley, and M. J. Humphries. 2005. Evidence that monoclonal antibodies directed against the integrin β subunit plexin/semaphorin/integrin domain stimulate function by inducing receptor extension. *J. Biol. Chem.* 280: 4238–4246.
42. Kanters, D., W. ten Hove, B. Lujik, C. van Aalst, R. C. Schweizer, J. W. Lammers, H. G. Leufkens, J. A. Raaijmakers, M. Bracke, and L. Koenderman. 2007. Expression of activated Fc γ RII discriminates between multiple granulocyte-priming phenotypes in peripheral blood of allergic asthmatic subjects. *J. Allergy Clin. Immunol.* 120: 1073–1081.
43. Georas, S. N., M. C. Liu, W. Newman, L. D. Beall, B. A. Stealey, and B. S. Bochner. 1992. Altered adhesion molecule expression and endothelial cell activation accompany the recruitment of human granulocytes to the lung after segmental antigen challenge. *Am. J. Respir. Cell Mol. Biol.* 7: 261–269.
44. Sedgwick, J. B., W. J. Calhoun, R. F. Vrtis, M. E. Bates, P. K. McAllister, and W. W. Busse. 1992. Comparison of airway and blood eosinophil function after in vivo antigen challenge. *J. Immunol.* 149: 3710–3718.
45. Kroegel, C., M. C. Liu, W. C. Hubbard, L. M. Lichtenstein, and B. S. Bochner. 1994. Blood and bronchoalveolar eosinophils in allergic subjects after segmental antigen challenge: surface phenotype, density heterogeneity, and prostanoic production. *J. Allergy Clin. Immunol.* 93: 725–734.
46. Mengelers, H. J., T. Maikoe, L. Brinkman, B. Hooibrink, J. W. Lammers, and L. Koenderman. 1994. Immunophenotyping of eosinophils recovered from blood and BAL of allergic asthmatics. *Am. J. Respir. Crit. Care Med.* 149: 345–351.
47. Aalbers, R., H. F. Kauffman, B. Vrugt, M. Smith, G. H. Koeter, W. Timens, and J. G. de Monchy. 1993. Bronchial lavage and bronchoalveolar lavage in allergen-induced single early and dual asthmatic responders. *Am. Rev. Respir. Dis.* 147: 76–81.
48. Shaver, J. R., J. J. O'Connor, M. Pollice, S. K. Cho, G. C. Kane, J. E. Fish, and S. P. Peters. 1995. Pulmonary inflammation after segmental ragweed challenge in allergic asthmatic and nonasthmatic subjects. *Am. J. Respir. Crit. Care Med.* 152: 1189–1191.
49. Gauvreau, G. M., R. M. Watson, and P. M. O'Byrne. 1999. Kinetics of allergen-induced airway eosinophilic cytokine production and airway inflammation. *Am. J. Respir. Crit. Care Med.* 160: 640–647.
50. Wood, L. J., R. Schmi, S. Dorman, Q. Hamid, M. K. Tulic, R. M. Watson, R. Foley, P. Wasi, J. A. Denburg, G. Gauvreau, and P. M. O'Byrne. 2002. Allergen-induced increases in bone marrow T lymphocytes and interleukin-5 expression in subjects with asthma. *Am. J. Respir. Crit. Care Med.* 166: 883–889.
51. Moser, R., J. Fehr, and P. L. B. Bruijnzeel. 1992. IL-4 controls the selective endothelium-driven transmigration of eosinophils from allergic individuals. *J. Immunol.* 149: 1432–1438.
52. Ebisawa, M., T. Yamada, C. Bickel, D. Klunk, and R. P. Schleimer. 1994. Eosinophil transendothelial migration induced by cytokines: III. Effect of the chemokine RANTES. *J. Immunol.* 153: 2153–2160.
53. Jia, G. Q., J. A. Gonzalo, A. Hidalgo, D. Wagner, M. Cybulsky, and J. C. Gutierrez-Ramos. 1999. Selective eosinophil transendothelial migration triggered by eotaxin via modulation of Mac-1/ICAM-1 and VLA-4/VCAM-1 interactions. *Int. Immunol.* 11: 1–10.
54. Nagata, M., H. Yamamoto, K. Tabe, and Y. Sakamoto. 2001. Eosinophil transmigration across VCAM-1-expressing endothelial cells is upregulated by antigen-stimulated mononuclear cells. *Int. Arch. Allergy Immunol.* 125 (Suppl. 1): 7–11.
55. Norris, V., L. Choong, D. Tran, Z. Corden, M. Boyce, H. Arshad, S. Holgate, B. O'Connor, S. Millet, B. Miller, et al. 2005. Effect of IVL745, a VLA-4 antagonist, on allergen-induced bronchoconstriction in patients with asthma. *J. Allergy Clin. Immunol.* 116: 761–767.
56. Yamamoto, H., M. Nagata, and Y. Sakamoto. 2005. CC chemokines and transmigration of eosinophils in the presence of vascular cell adhesion molecule 1. *Ann. Allergy Asthma Immunol.* 94: 292–300.
57. Banerjee, E. R., Y. Jiang, W. R. Henderson, Jr., L. M. Scott, and T. Papayannopoulou. 2007. α 4 and β 2 integrins have nonredundant roles for asthma development, but for optimal allergen sensitization only α 4 is critical. *Exp. Hematol.* 35: 605–617.
58. Liu, L. Y., C. A. Swenson, E. A. Kelly, H. Kita, and W. W. Busse. 2000. The relationship of sputum eosinophilia and sputum cell generation of IL-5. *J. Allergy Clin. Immunol.* 106: 1063–1069.
59. Kelly, E. A., W. W. Busse, and N. N. Jarjour. 2003. A comparison of the airway response to segmental antigen bronchoprovocation in atopic asthma and allergic rhinitis. *J. Allergy Clin. Immunol.* 111: 79–86.
60. Zhu, X., N. M. Munoz, K. P. Kim, H. Sano, W. Cho, and A. R. Leff. 1999. Cytosolic phospholipase A2 activation is essential for β 1 and β 2 integrin-dependent adhesion of human eosinophils. *J. Immunol.* 163: 3423–3429.
61. Wong, C. K., W. K. Ip, and C. W. Lam. 2003. Interleukin-3, -5, and granulocyte macrophage colony-stimulating factor-induced adhesion molecule expression on eosinophils by p38 mitogen-activated protein kinase and nuclear factor- κ B. *Am. J. Respir. Cell Mol. Biol.* 29: 133–147.
62. Rennard, S. I., G. Basset, D. Lecossier, K. M. O'Donnell, P. Pinkston, P. G. Martin, and R. G. Crystal. 1986. Estimation of volume of epithelial lining fluid recovered by lavage using urea as marker of dilution. *J. Appl. Physiol.* 60: 532–538.
63. Chihara, J., J. Plumas, V. Gruart, J. Tavernier, L. Prin, A. Capron, and M. Capron. 1990. Characterization of a receptor for interleukin 5 on human eosinophils: variable expression and induction by granulocyte/macrophage colony-stimulating factor. *J. Exp. Med.* 172: 1347–1351.
64. Plaetinck, G., J. Van der Heyden, J. Tavernier, I. Fache, T. Tuypens, S. Fischkoff, W. Fiers, and R. Devos. 1990. Characterization of interleukin 5 receptors on eosinophilic sublines from human promyelocytic leukemia (HL-60) cells. *J. Exp. Med.* 172: 683–691.
65. Ingley, E., and I. G. Young. 1991. Characterization of a receptor for interleukin-5 on human eosinophils and the myeloid leukemia line HL-60. *Blood* 78: 339–344.

66. Migita, M., N. Yamaguchi, S. Mita, S. Higuchi, Y. Hitoshi, Y. Yoshida, M. Tomonaga, I. Matsuda, A. Tominaga, and K. Takatsu. 1991. Characterization of the human IL-5 receptors on eosinophils. *Cell. Immunol.* 133: 484–497.
67. Tavernier, J., R. Devos, S. Cornelis, T. Tuypens, J. Van der Heyden, W. Fiers, and G. Plaetinck. 1991. A human high affinity interleukin-5 receptor (IL5R) is composed of an IL5-specific α chain and a β chain shared with the receptor for GM-CSF. *Cell* 66: 1175–1184.
68. Zhang, J., P. Wu, R. Kuvelkar, J. L. Schwartz, R. W. Egan, M. M. Billah, and P. Wang. 1999. A scintillation proximity assay for human interleukin-5 (hIL-5) high-affinity binding in insect cells coexpressing hIL-5 receptor α and β subunits. *Anal. Biochem.* 268: 134–142.
69. Bates, M. E., V. L. Green, and P. J. Bertics. 2000. ERK1 and ERK2 activation by chemotactic factors in human eosinophils is interleukin 5-dependent and contributes to leukotriene C₄ biosynthesis. *J. Biol. Chem.* 275: 10968–10975.
70. Hall, D. J., J. Cui, M. E. Bates, B. A. Stout, L. Koenderman, P. J. Coffey, and P. J. Bertics. 2001. Transduction of a dominant-negative H-Ras into human eosinophils attenuates extracellular signal-regulated kinase activation and interleukin-5-mediated cell viability. *Blood* 98: 2014–2021.
71. Liu, L. Y., J. B. Sedgwick, M. E. Bates, R. F. Vrtis, J. E. Gern, H. Kita, N. N. Jarjour, W. W. Busse, and E. A. Kelly. 2002. Decreased expression of membrane IL-5 receptor α on human eosinophils: IL-5 down-modulates its receptor via a proteinase-mediated process. *J. Immunol.* 169: 6459–6466.
72. Bates, M. E., L. Y. Liu, S. Esnault, B. A. Stout, E. Fonkem, V. Kung, J. B. Sedgwick, E. A. Kelly, D. M. Bates, J. S. Malter, et al. 2004. Expression of interleukin-5- and granulocyte macrophage-colony-stimulating factor-responsive genes in blood and airway eosinophils. *Am. J. Respir. Cell Mol. Biol.* 30: 736–743.
73. Zangrilli, J. G., J. R. Shaver, R. A. Cirelli, S. K. Cho, C. G. Garlisi, A. Falcone, F. M. Cuss, J. E. Fish, and S. P. Peters. 1995. sVCAM-1 levels after segmental antigen challenge correlate with eosinophil influx, IL-4 and IL-5 production, and the late phase response. *Am. J. Respir. Crit. Care Med.* 151: 1346–1353.
74. Sur, S., H. Kita, G. J. Gleich, T. C. Chenier, and L. W. Hunt. 1996. Eosinophil recruitment is associated with IL-5, but not with RANTES, twenty-four hours after allergen challenge. *J. Allergy Clin. Immunol.* 97: 1272–1278.
75. Jarjour, N. N., W. J. Calhoun, E. A. Kelly, G. J. Gleich, L. B. Schwartz, and W. W. Busse. 1997. The immediate and late allergic response to segmental bronchopulmonary provocation in asthma. *Am. J. Respir. Crit. Care Med.* 155: 1515–1521.
76. Sedgwick, J. B., W. J. Calhoun, G. J. Gleich, H. Kita, J. S. Abrams, L. B. Schwartz, B. Volovitz, M. Ben-Yaakov, and W. W. Busse. 1991. Immediate and late airway response of allergic rhinitis patients to segmental antigen challenge. Characterization of eosinophil and mast cell mediators. *Am. Rev. Respir. Dis.* 144: 1274–1281.
77. Berends, C., B. Dijkhuizen, J. G. de Monchy, J. Gerritsen, and H. F. Kauffman. 1994. Induction of low density and up-regulation of CD11b expression of neutrophils and eosinophils by dextran sedimentation and centrifugation. *J. Immunol. Methods* 167: 183–193.
78. Todd, R. F., III, M. A. Arnaout, R. E. Rosin, C. A. Crowley, W. A. Peters, and B. M. Babior. 1984. Subcellular localization of the large subunit of Mo1 (Mo1 α ; formerly gp 110), a surface glycoprotein associated with neutrophil adhesion. *J. Clin. Invest.* 74: 1280–1290.
79. Mathias, J. R., B. J. Perrin, T. X. Liu, J. Kanki, A. T. Look, and A. Huttenlocher. 2006. Resolution of inflammation by retrograde chemotaxis of neutrophils in transgenic zebrafish. *J. Leukocyte Biol.* 80: 1281–1288.
80. Buckley, C. D., E. A. Ross, H. M. McGettrick, C. E. Osborne, O. Haworth, C. Schmutz, P. C. Stone, M. Salmon, N. M. Matharu, R. K. Vohra, et al. 2006. Identification of a phenotypically and functionally distinct population of long-lived neutrophils in a model of reverse endothelial migration. *J. Leukocyte Biol.* 79: 303–311.
81. Warringa, R. A., H. J. Mengelers, J. A. Raaijmakers, P. L. Bruijnzeel, and L. Koenderman. 1993. Upregulation of formyl-peptide and interleukin-8-induced eosinophil chemotaxis in patients with allergic asthma. *J. Allergy Clin. Immunol.* 91: 1198–1205.
82. Sehmi, R., L. J. Wood, R. Watson, R. Foley, Q. Hamid, P. M. O'Byrne, and J. A. Denburg. 1997. Allergen-induced increases in IL-5 receptor α -subunit expression on bone marrow-derived CD34⁺ cells from asthmatic subjects: a novel marker of progenitor cell commitment towards eosinophilic differentiation. *J. Clin. Invest.* 100: 2466–2475.
83. Sehmi, R., S. Dorman, A. Baatjes, R. Watson, R. Foley, S. Ying, D. S. Robinson, A. B. Kay, P. M. O'Byrne, and J. A. Denburg. 2003. Allergen-induced fluctuation in CC chemokine receptor 3 expression on bone marrow CD34⁺ cells from asthmatic subjects: significance for mobilization of haemopoietic progenitor cells in allergic inflammation. *Immunology* 109: 536–546.
84. Moser, R., J. Fehr, L. Olgiati, and P. L. Bruijnzeel. 1992. Migration of primed human eosinophils across cytokine-activated endothelial cell monolayers. *Blood* 79: 2937–2945.
85. Nakajima, H., H. Sano, T. Nishimura, S. Yoshida, and I. Iwamoto. 1994. Role of vascular cell adhesion molecule 1/very late activation antigen 4 and intercellular adhesion molecule 1/lymphocyte function-associated antigen 1 interactions in antigen-induced eosinophil and T cell recruitment into the tissue. *J. Exp. Med.* 179: 1145–1154.
86. ten Hacken, N. H., D. S. Postma, F. Bosma, G. Drok, B. Rutgers, J. Kraan, and W. Timens. 1998. Vascular adhesion molecules in nocturnal asthma: a possible role for VCAM-1 in ongoing airway wall inflammation. *Clin. Exp. Allergy* 28: 1518–1525.
87. Masinovsky, B., D. Urdal, and W. M. Gallatin. 1990. IL-4 acts synergistically with IL-1 β to promote lymphocyte adhesion to microvascular endothelium by induction of vascular cell adhesion molecule-1. *J. Immunol.* 145: 2886–2895.
88. Thornhill, M. H., and D. O. Haskard. 1990. IL-4 regulates endothelial cell activation by IL-1, tumor necrosis factor, or IFN- γ . *J. Immunol.* 145: 865–872.
89. Thornhill, M. H., S. M. Wellicome, D. L. Mahiouz, J. S. Lanchbury, U. Kyan-Aung, and D. O. Haskard. 1991. Tumor necrosis factor combines with IL-4 or IFN- γ to selectively enhance endothelial cell adhesiveness for T cells: the contribution of vascular cell adhesion molecule-1-dependent and -independent binding mechanisms. *J. Immunol.* 146: 592–598.
90. Weller, P. F., T. H. Rand, S. E. Goelz, G. Chi-Rosso, and R. R. Lobb. 1991. Human eosinophil adherence to vascular endothelium mediated by binding to vascular cell adhesion molecule 1 and endothelial leukocyte adhesion molecule 1. *Proc. Natl. Acad. Sci. USA* 88: 7430–7433.
91. Schleimer, R. P., S. A. Sterbinsky, J. Kaiser, C. A. Bickel, D. A. Klunk, K. Tomioka, W. Newman, F. W. Lusinskas, M. A. Gimbrone, Jr., B. W. McIntyre, and B. S. Bochner. 1992. IL-4 induces adherence of human eosinophils and basophils but not neutrophils to endothelium: association with expression of VCAM-1. *J. Immunol.* 148: 1086–1092.
92. Barthel, S. R., D. S. Annis, D. F. Mosher, and M. W. Johansson. 2006. Differential engagement of modules 1 and 4 of vascular cell adhesion molecule-1 (CD106) by integrins $\alpha_4\beta_1$ (CD49d/29) and $\alpha_M\beta_2$ (CD11b/18) of eosinophils. *J. Biol. Chem.* 281: 32175–32187.
93. Ebisawa, M., B. S. Bochner, S. N. Georas, and R. P. Schleimer. 1992. Eosinophil transendothelial migration induced by cytokines: I. Role of endothelial and eosinophil adhesion molecules in IL-1 β -induced transendothelial migration. *J. Immunol.* 149: 4021–4028.
94. Liu, L., L. Hakansson, P. Ridefelt, R. C. Garcia, and P. Venge. 2003. Priming of eosinophil migration across lung epithelial cell monolayers and upregulation of CD11b/CD18 are elicited by extracellular Ca²⁺. *Am. J. Respir. Cell Mol. Biol.* 28: 713–721.
95. Ulfman, L. H., J. Alblas, C. W. van Aalst, J. J. Zwaginga, and L. Koenderman. 2005. Differences in potency of CXC chemokine ligand 8-, CC chemokine ligand 11-, and C5a-induced modulation of integrin function on human eosinophils. *J. Immunol.* 175: 6092–6099.