

Increased eosinophil-lineage committed progenitors in the lung of allergen-challenged mice

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Background: There is increasing evidence that hemopoietic progenitor cells may traffic from bone marrow to sites of allergen exposure in asthma and undergo *in situ* differentiation, contributing to ongoing airway inflammation. However, the isolation and detailed phenotyping of true CD34⁺ progenitors from lung tissue during an allergen-induced airway eosinophilia has not been performed.

Objective: We attempted to isolate and investigate the *in vivo* kinetics of hemopoietic progenitor cells and production of eosinophilopoietic mediators in the lung.

Methods: In a mouse model of allergic airway inflammation, cells were extracted from lung tissue by enzymatic digestion. Total (CD34⁺45⁺) and eosinophil lineage committed (CD34⁺45⁺IL-5Rα⁺) progenitors were enumerated by flow cytometry. Outcome measurements were made 2, 6, 12, 24, 48, and 72 hours and 7 and 14 days after allergen challenge.

Results: Compared with saline control, CD34⁺45⁺ progenitors were elevated between 6 and 48 hours ($P < .05$), attenuated by 72 hours and subsequently increased by 14 days ($P > .05$).

CD34⁺45⁺IL-5Rα⁺ progenitors were transiently elevated at 6 hours ($P < .05$) before a return to preallergen levels by 12 hours and a subsequent increase at 14 days ($P < .05$). Bronchoalveolar lavage eosinophils were increased at 2 hours, peaking at 72 hours ($P < .00625$) and declining by 14 days. Both IL-5 and eotaxin levels were increased by 2 hours, peaking at 12 hours ($P < .05$) and 24 hours ($P < .05$), respectively.

Conclusion: We propose that the increase in CD34⁺45⁺IL-5Rα⁺ cells and the eosinophilopoietic mediators IL-5 and eotaxin in the lung after allergen exposure may promote *in situ* differentiation of eosinophils that contribute to ongoing allergic airway inflammation. (*J Allergy Clin Immunol* 2005;115:95-102.)

Key words: Asthma, eosinophils, progenitors, CD34⁺, IL-5, eotaxin, lungs, bone marrow, *in situ*, hematopoiesis

The bone marrow (BM) is well established as a site of inflammatory cell production. However, there is increasing evidence that in atopic subjects after exposure to

Abbreviations used

BAL: Bronchoalveolar lavage
BM: Bone marrow
Eo-CFU: Eosinophil colony-forming units
FITC: Fluorescein isothiocyanate
NAMNC: Nonadherent mononuclear cell
SDF-1: Stromal cell-derived factor 1

allergen, distinct phenotypic changes occur within the BM progenitor cell population that, in addition to triggering the activation of eosinopoiesis, may also induce the migration of progenitor cells from the BM to the site of allergic inflammation.^{1,2} These progenitor cells may undergo *in situ* differentiation in the presence of locally elaborated cytokines and provide an ongoing source of inflammatory effector cells (Fig 1).

Eosinophils, like other leukocytes, develop from CD34⁺ pluripotent progenitor cells.^{1,3,4} The CD34 antigen is a monomeric *O*-sialylated glycoprotein whose expression within the hemopoietic system is restricted to primitive progenitor cells of all lineages.^{1,5} By flow cytometry, we have shown increased numbers of BM and peripheral blood CD34⁺ progenitor cells in atopic compared with nonatopic control subjects.^{1,6} Through further phenotypic analyses, we have shown that CD34⁺ progenitor cells can coexpress the low-affinity binding sites for an eosinophil-specific cytokine, IL-5 (IL-5 receptor α-subunit; IL-5Rα) and suggest that CD34⁺IL-5Rα⁺ cells may be the phenotype of the earliest eosinophil lineage committed progenitor cells.^{1,7} Chemokines including stromal cell-derived factor 1 (SDF-1)^{1,8,9} and eotaxin^{1,10,11} are reported to cause the mobilization of CD34⁺ progenitor cells; however, the ability of these mediators *in situ* to induce recruitment to the site of allergic inflammation is not established. The traffic of CD34⁺ progenitor cells from the BM to allergic tissue during inflammatory conditions is inferred by findings that CD34⁺ cells can be extracted from human nasal polyp tissue and have the capacity to undergo cytokine-stimulated proliferation and differentiation into eosinophil-basophil colony-forming units.¹² Similarly, increased numbers of both CD34-immuno⁺ cells and CD34-immuno⁺IL-5RαmRNA⁺ cells have been detected in bronchial biopsies from atopic patients with asthma compared with control subjects without asthma.⁴ Experiments using nasal explant tissue from allergic subjects with rhinitis have

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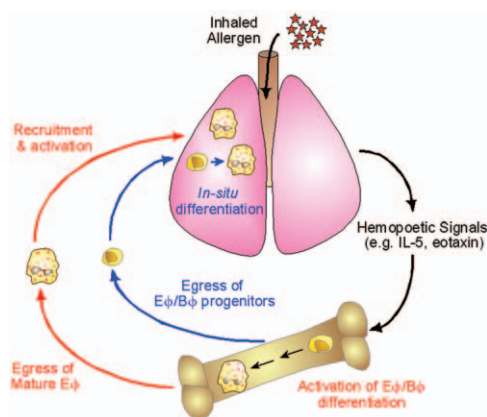


FIG 1. Hypothesized role of bone marrow progenitor cells in the development of ongoing airway eosinophilia.

shown increases in major basic protein-positive eosinophils in conjunction with a reduction in CD34-immuno⁺IL-5R α mRNA⁺ cells after *in vitro* antigen challenge, suggesting the potential for *in situ* differentiation of eosinophil progenitors at the site of allergic inflammation.¹³ These findings raise the possibility that control of CD34⁺ cell egress from the BM and control of local differentiation and expansion of CD34⁺IL-5R α ⁺ cells within allergic tissue may both be prerequisites for the complete attenuation of eosinophilic inflammation in the allergic airway.⁴ Although these studies support the concept of progenitor cell involvement in the asthmatic etiology, the isolation and detailed phenotyping of true CD34⁺ progenitor cells from lung tissue at the time of allergen-induced airway eosinophilia has not been performed. In addition, detailed kinetics of the *in vivo* changes in the number and phenotype of these CD34⁺ progenitor cells compared with the development of airway eosinophilia have not been determined. Furthermore, the production of eosinophilopoietic cytokines and chemokines during the development of airway eosinophilia has not been studied.

We hypothesize that after airway allergen challenge, there is an elevated level of CD34⁺ progenitor cells in lung tissue that precedes an increase in bronchoalveolar lavage (BAL) eosinophils but succeeds an increase in airways eosinopoietic cytokines and eosinophilic chemokines. We further hypothesize that these cells undergo *in situ* differentiation and contribute to the development of airway eosinophilia. In an attempt to address this hypothesis, this study used a novel approach of using a well-established mouse model of allergen-induced airway inflammation and airway hyperreactivity to explore 3 specific objectives. First, we wanted to isolate, for the first time, total (CD34⁺45⁺) and eosinophil lineage-committed (CD34⁺45⁺IL-5R α ⁺) progenitor cells from the lung tissue of allergen-sensitized and allergen-challenged mice. Second, we wanted to compare the kinetics of CD34⁺45⁺ and CD34⁺45⁺IL-5R α ⁺ progenitor cells with the kinetics of airway eosinophilia. Finally, we wanted to begin to investigate the mechanisms of *in situ* progenitor

cell accumulation by comparing the kinetics of CD34⁺45⁺IL-5R α ⁺ progenitor cell numbers in the lung with the production of the cytokine IL-5 and chemokines eotaxin and SDF-1.

METHODS

Animals

Female BALB/c mice age 10 to 12 weeks were purchased from Harlan Sprague Dawley (Indianapolis, Ind) and housed in environmentally controlled specific pathogen-free conditions for 1 week before the study, and for the duration of the experiments. All procedures were reviewed and approved by the Animal Research Ethics Board at McMaster University and followed National Institutes of Health guidelines for animal care.

Sensitization and challenge

The ovalbumin sensitization and challenge protocol was performed as previously described.¹⁴ Briefly, mice were sensitized with intraperitoneal ovalbumin conjugated to aluminum potassium sulphate injected on days 1 and 11, and intranasal ovalbumin on day 11. Sensitized mice were exposed to intranasal ovalbumin (100- μ g in 25- μ L saline) challenges on days 19 and 20. Control mice received intraperitoneal and intranasal saline. Mice were studied at 2, 6, 12, 24, 48, and 72 hours and 7 and 14 days after the final exposure to allergen and 24 hours after the final exposure to saline.

Outcomes

Separate groups of 10 mice were studied at each time point, with the following outcome measurements made: (1) total and differential cell counts in BAL fluid; (2) IL-5, SDF-1 α , and eotaxin levels in BAL supernatant; (3) CD34⁺CD45⁺ and CD34⁺CD45⁺IL-5R α ⁺ cell counts in lung digest by using flow cytometry; and (4) colony assays.

BAL

Bronchoalveolar lavage was performed as described previously.¹⁴ Briefly, 2 aliquots of 250- μ L PBS were injected and withdrawn through the tracheal cannula. After centrifugation, the supernatant was stored at -20°C for subsequent measurements, and cytocentrifuge slides were prepared (Cytospin 3; Shandon Scientific, Sewickley, Pa) and stained with Diff-Quik (Dade Behring, Newark, Del). Differential cell counts were performed on 400 cells by 1 investigator blind to the experimental conditions. Cells were classified as macrophages, neutrophils, mononuclear cells, or eosinophils. ELISA analysis for IL-5, SDF-1 α , and eotaxin (OptEIA; PharMingen, Mississauga, Ontario, Canada; Quantikine; R&D Systems, Minneapolis, Minn) levels were performed on BAL supernatant. Measurements below 7.8 pg/mL, 18 pg/mL, and 3 pg/mL, respectively, are considered by the manufacturers to be below the level of detection.

Lung cell isolation

Mice were anesthetized by an intraperitoneal injection of 2,2,2-tribromoethanol (Avertin, 240 mg/kg; Aldrich Chemical Co, Milwaukee, Wis). When anesthesia was established, the chest cavity was opened, and the trachea was exposed and cannulated by using a blunted 18-gauge needle. Mice were then killed by terminal exsanguination via cardiac puncture. The heart and lungs were dissected and removed from the thoracic cavity of each mouse. The lungs were then minced and incubated for 60 minutes at 37°C with Dulbecco PBS containing 1 mg/mL hyaluronidase, 0.4 mg/mL collagenase (type 1A), and 5 μ L/mL DNase (all reagents: Sigma, Oakville, Ontario, Canada). Lung digest was filtered through a

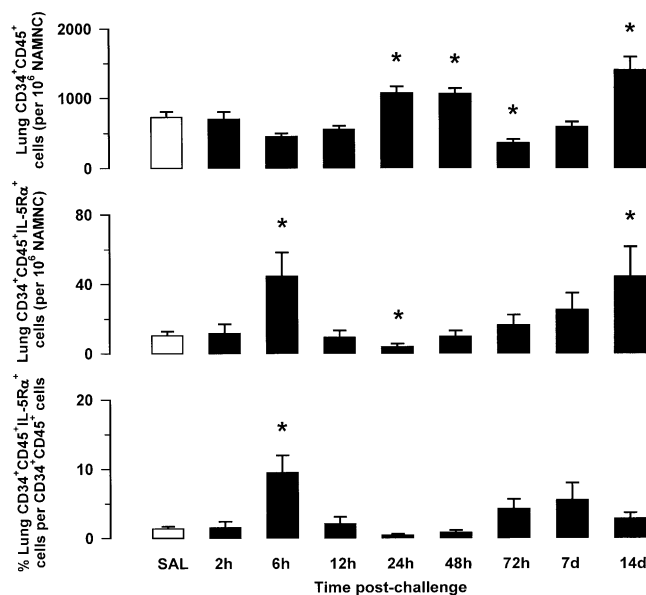


FIG 2. Flow-cytometric enumeration of the time course of the absolute number of lung extracted total (CD34⁺CD45⁺) progenitor cells, eosinophil-lineage committed (CD34⁺CD45⁺IL-5Rα⁺) progenitor cells, and proportion of lung extracted CD34⁺CD45⁺IL-5Rα⁺ per CD34⁺CD45⁺ after airway ovalbumin challenge (mean $\pm$ SEM). n = 8-10/group. **P* < .05 compared with saline (SAL) control at 24-hour time point.

52- μ m filter and centrifuged at 425g for 10 minutes. Erythrocytes were lysed with ice-cold NH₄Cl-Tris buffer, and cells were washed with 10% FBS-PBS (Sigma). Cell suspension was centrifuged at 425 g for 10 minutes and resuspended in 1 mL 10% FBS-PBS. In preliminary studies, pulmonary circulation was perfused with intravascular saline. No difference in the number of CD34⁺45⁺ or CD34⁺45⁺IL-5Rα⁺ progenitor cells was determined between perfused and nonperfused lungs.

Lung colony assay

Colony assays were performed as previously described, with modifications.¹⁴ Briefly, lung cells were isolated as previously outlined from saline perfused lungs. After lung cell filtration, mononuclear cells were washed once with McCoy's 3⁺ (Invitrogen Corp, Grand Island, NY), then incubated overnight in plastic flasks at 37°C and 5% CO₂ to remove adherent cells. The nonadherent cells were cultured in microassay 24-well plates (Becton Dickinson, Lincoln Park, NJ), 1.4 $\times$ 10⁵ cells per well. The culture medium was made up of 0.9% methylcellulose (Baledon Lab, Georgetown, Ontario, Canada), 30% FBS, and an optimal concentration (10 ng/mL) of recombinant mouse IL-5 (R&D Systems). After 10 days, colonies greater than 40 cells were counted by using inverse microscopy and identified by using morphologic and histologic criteria. To confirm the identification of colonies as eosinophil colony-forming units (Eo-CFU), selected samples were removed from the culture wells for viewing under light microscopy after staining with Diff-Quik.

Monoclonal antibodies

Fluorescein isothiocyanate (FITC)-conjugated rat antimouse IgG_{2a}, CD34 mAb (RAM34), r-phycoerythrin (PE)-conjugated IgG_{2b}, CD45 mAb (30-F11), purified rat antimouse CD16/CD32 (Fc γ III/II receptor) mAb (2.4G2), biotin-conjugated rat IgG₁, monoclonal immunoglobulin isotype standard (R3-34; Fc block), and streptavidin-conjugated peridinin chlorophyll protein (PerCp) were

purchased from Becton Dickinson (Mississauga, Ontario, Canada). Biotin-conjugated rat IgG₁ antimouse IL-5 receptor α mAb (T21) was purchased from Wako Chemicals USA (Richmond, Va).

Lung cell immunofluorescence staining

Samples of 0.75 to 1 $\times$ 10⁶ nonadherent mononuclear cells (NAMNCs) were washed in 2 mL ice-cold PBS containing 0.1% NaN₃ (PBS/NaN₃) and centrifuged at 319g at 4°C for 5 minutes. Samples were incubated with saturating concentrations of Fc block at 4°C for 20 minutes. Optimal concentrations of either IL-5R α or IgG₁ mAb were added to samples and incubated at 4°C for 40 minutes. Cells were washed in 2 mL ice-cold PBS/NaN₃ and centrifuged at 319g at 4°C for 5 minutes. Cells were simultaneously incubated with Fc block and stained with saturating concentrations of anti-CD34-FITC, anti-CD45-phycoerythrin, and streptavidin-conjugated peridinin chlorophyll protein at 4°C for 30 minutes. Samples were washed with 2 mL PBS/NaN₃ and fixed in 400 μ L of PBS plus 1% paraformaldehyde (BDH Laboratory Supplies, Mississauga, Ontario, Canada) and stored at 4°C until ready for analysis.

Flow cytometry

Flow cytometry was performed on the basis of protocols previously described.^{1,7,15} Briefly, stained cells were analyzed by using a FACS Caliber flow cytometer equipped with an 488-nm argon ion laser (Becton Dickinson Instrument Systems, Mississauga, Ontario, Canada). Five data parameters were acquired and stored in list-mode files: linear forward light scatter, linear side-angle light scatter, log FITC, log phycoerythrin, and log PerCp fluorescence; each measurement contained 100,000 events. Compensation settings were established by using NAMNC stained with (1) anti-CD34-FITC and anti-IL-5R α -PerCp or (2) anti-CD45-PE. Offline analysis was performed by using Cellquest software package (BD Biosciences, Oakville, Ontario, Canada). The use of a multiparameter sequential gating strategy was adapted and performed on the basis of protocols previously described.^{1,7,15}

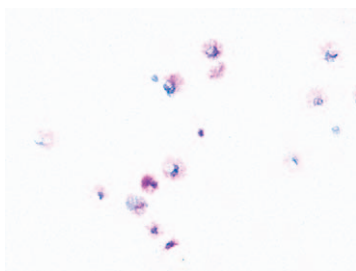


FIG 3. Eosinophils plucked from colony-forming units (CFUs) plated from 1.4×10^5 lung cells from saline-perfused mouse lung tissue extracted 6 hours after brief ovalbumin sensitization and challenge protocol and incubated for 10 days with 10 ng/mL IL-5.

Statistical analysis

Reported values are expressed as means and SEMs. Comparisons between saline control mice and mice receiving allergen exposure, with respect to BAL cell counts, were made by using independent *t* tests because of heteroscedasticity of variances. Bonferroni correction was performed for the 8 comparisons, with a *P* value < .00625 considered significant. Comparisons between saline control mice and mice receiving allergen exposure with respect to (1) BAL IL-5, SDF-1 α , and eotaxin measurements and (2) CD34⁺CD45⁺ and CD34⁺CD45⁺IL-5R α ⁺ cell counts were made by using ANOVA. Post hoc multiple comparison testing was performed by using the Duncan test. Comparisons using ANOVA and post hoc analysis were 2-tailed, and *P* values < .05 were considered significant.

RESULTS

Lung cell kinetics

CD34⁺CD45⁺ progenitor cells increased in the lung after allergen challenge (Fig 2). At 24 and 48 hours after allergen challenge, a greater number of CD34⁺CD45⁺ (1069 cells/10⁶ NAMNC, SEM 93; and 1057 cells/10⁶ NAMNC, SEM 78; *P* < .05, respectively) were observed in the lungs compared with saline control (726 cells/10⁶ NAMNC, SEM 81). By 7 days, the number of CD34⁺CD45⁺ returned to preallergen levels, before a subsequent increase to greater levels by 14 days (1398 cells/10⁶ NAMNC, SEM 190; *P* < .05).

CD34⁺CD45⁺IL-5R α ⁺ progenitor cells were transiently elevated in the lung after allergen challenge (Fig 2). At 6 hours after allergen challenge, increased CD34⁺CD45⁺IL-5R α ⁺ (44 cells/10⁶ NAMNC, SEM 14; *P* < .05) were observed compared with saline control (10 cells/10⁶ NAMNC, SEM 3). By 12 hours, the number of CD34⁺CD45⁺IL-5R α ⁺ returned to preallergen levels, before a subsequent increase by 14 days (44 cells/10⁶ NAMNC, SEM 17; *P* < .05).

To investigate changes in CD34⁺CD45⁺IL-5R α ⁺ progenitor cells in relation to changes in total CD34⁺CD45⁺ progenitor cells, we enumerated the percentage change in detection of these cells. The data indicated a significant expansion of the CD34⁺CD45⁺IL-5R α ⁺ cells population at 6 hours after allergen challenge (9.5%; SEM 2.5%; *P* < .05) compared with saline control (1.4%; SEM 0.3%).

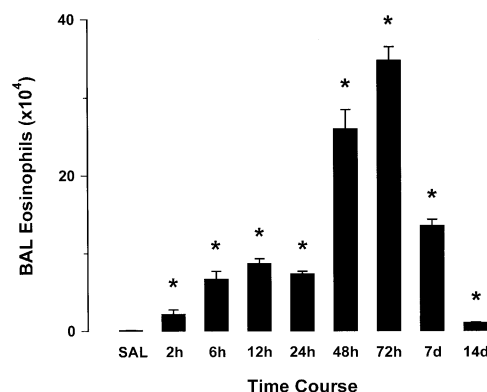


FIG 4. BAL eosinophils after airway ovalbumin challenge (mean $\pm$ SEM). *n* = 4-11/group. **P* < .00625 compared with saline (SAL) control at 24-hour time point.

In contrast, there was no increase in the proportion of CD34⁺CD45⁺IL-5R α ⁺ cells per CD34⁺CD45⁺ (2.8%; SEM 0.8%) observed at 14 days after allergen challenge. These data suggest that at 6 hours after allergen challenge, there is a selective eosinophil lineage commitment of resident CD34⁺CD45⁺ progenitor cells, compared with 14 days, at which time a noneosinophil-specific increase in progenitor cells is seen.

We observed a selective increase in Eo-CFU numbers from ovalbumin-challenged mice 6 hours after second ovalbumin exposure (11.6, SEM 1.5) compared with saline-challenged mice (0.0, SEM 0.0; *P* < .05) after incubation with 10 ng/mL IL-5 but not diluent. These cells were concluded to be eosinophils on the basis of morphologically homogenous appearance and positive stain with Diff-Quik (Fig 3).

Bronchoalveolar lavage eosinophils were elevated in the lung after allergen challenge compared with saline control and remained markedly increased until at least 14 days (Fig 4). The increase in BAL eosinophils was evident at 2 hours after allergen challenge (2.16, SEM 0.58×10^4 , *P* < .00625), peaking by 72 hours (34.80, SEM 1.69×10^4 ; *P* < .00625), and declining by 14 days (1.08, SEM 0.06×10^4 ; *P* < .00625). Other cell types measured from BAL are outlined in Table I.

Cytokines

Compared with saline control (12.3 pg/mL, SEM 1.0), there was an elevation in the level of BAL IL-5 at 2 hours (200.8 pg/mL, SEM 44.2; *P* < .05) after allergen challenge, which peaked by 12 hours (316.4 pg/mL, SEM 52.8; *P* < .05) before returning to preallergen levels by 48 hours (Fig 5).

Similarly, the level of eotaxin observed in BAL fluid was transiently increased during the first 48 hours after allergen challenge (Fig 5). Compared with saline control (0.1 pg/mL, SEM 0.2), eotaxin was elevated from 2 hours to 48 hours (10.9 pg/mL, SEM 2.7, to 10.0 pg/mL, SEM 2.6, respectively; *P* < .05), peaking at 24 hours

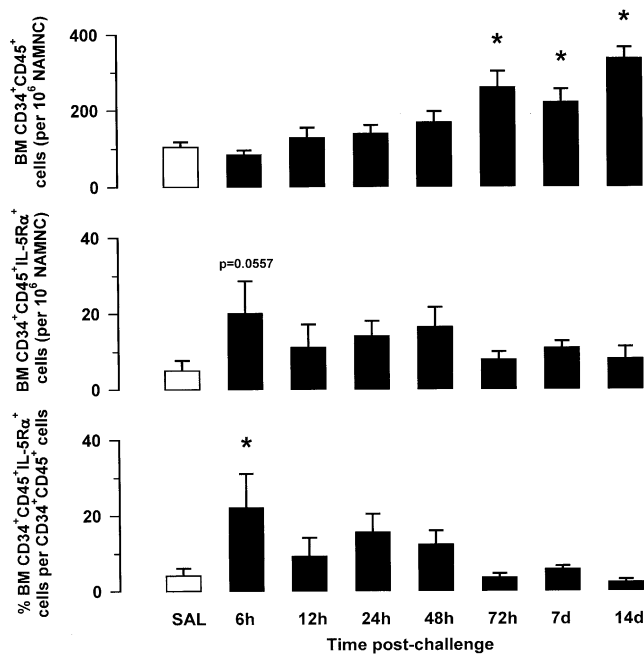


FIG 5. IL-5, eotaxin, and SDF-1α ELISA of BAL fluid at various time points after airway ovalbumin challenge (mean ± SEM). n = 7-10/group. **P* < .05 compared with saline (SAL) control at 24-hour time point.

TABLE I. Cellular contents of BAL fluid at indicated time points after final intranasal challenge of sham/sham (saline) or ovalbumin/ovalbumin mice*

Time	Eosinophils	Neutrophils	Mononuclei	Macrophages
Saline 24 h (n = 11)	0.01 (0.00)	0.14 (0.05)	0.01 (0.01)	2.69 (0.30)
2 h (n = 4)	2.16† (1.16)	0.13 (0.05)	0.22† (0.05)	3.36 (0.53)
6 h (n = 6)	6.66† (2.25)	7.50† (1.04)	0.41† (0.06)	2.94 (0.64)
12 h (n = 10)	8.69† (1.93)	5.80† (0.81)	0.60† (0.13)	3.79 (0.84)
24 h (n = 10)	7.36† (0.99)	0.11 (0.04)	0.37† (0.04)	4.29 (0.36)
48 h (n = 9)	26.03† (7.38)	0.06 (0.05)	0.84† (0.25)	6.75 (1.30)
72 h (n = 10)	34.80† (5.34)	0.04 (0.02)	1.62† (0.31)	9.36† (0.99)
7 d (n = 9)	13.57† (2.40)	0.05 (0.04)	1.15† (0.21)	4.54 (0.69)
14 d (n = 8)	1.08† (0.17)	0.00 (0.00)	0.15 (0.06)	2.29 (0.31)

*Numbers in each column are means (SEMs).
†Different from saline at 24-hour time point (*P* < .00625) as per Bonferroni correction.

(25.7 pg/mL, SEM 5.0; *P* < .05), before returning to preallergen levels by 72 hours.

The amount of SDF-1α observed in BAL fluid was elevated during the first 12 hours after allergen challenge; however, the pattern of elevation was biphasic. Compared with saline control (10.5 pg/mL, SEM 2.1), there was an increase in the level of SDF-1α at 2 hours (42.1 pg/mL, SEM 8.8; *P* < .05) after allergen challenge, remaining elevated at 12 hours (48.3 pg/mL, SEM 11.0; *P* < .05) before returning to preallergen levels by 24 hours and 48 hours (Fig 5). Subsequently, there was a second elevation in SDF-1α at 72 hours (38.6 pg/mL, SEM 6.4; *P* < .05) and 7 days (38.0 pg/mL, SEM 10.9; *P* < .05) before again returning to preallergen levels by 14 days.

BM cell kinetics

Total progenitor cells (CD34⁺CD45⁺) were increased in the BM after allergen challenge (Fig 6). There was a nonsignificant increase in CD34⁺CD45⁺ cells from 6 hours to 48 hours (83 cells/10⁶ NAMNC, SEM 12, to 167 cells/10⁶ NAMNC, SEM 28, respectively) compared with saline control (104, SEM 13; *P* > .05). However, between 72 hours and 14 days after allergen challenge, a significant increase in CD34⁺CD45⁺ cells (259 cells/10⁶ NAMNC, SEM 43, to 335 cells/10⁶ NAMNC, SEM 30; *P* < .05, respectively) was observed in the BM compared with saline control.

The CD34⁺CD45⁺IL-5Rα⁺ cells exhibited a transient elevation in the BM after allergen challenge (Fig 6). At

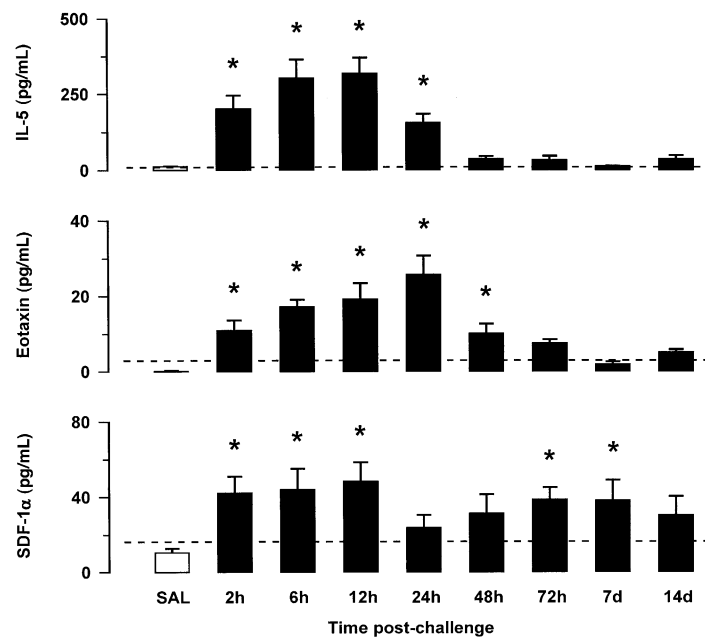


FIG 6. Flow-cytometric enumeration of the time course of the absolute number of BM extracted total ($CD34^+CD45^+$) progenitor cells, eosinophil-lineage committed ($CD34^+CD45^+IL-5R\alpha^+$) progenitor cells, and proportion of BM extracted $CD34^+CD45^+IL-5R\alpha^+$ per $CD34^+CD45^+$ after airway ovalbumin challenge (mean $\pm$ SEM). $n = 8-10/\text{group}$. * $P < .05$ compared with saline (SAL) control at 24-hour time point.

6 hours after allergen challenge, a greater number of $CD34^+CD45^+IL-5R\alpha^+$ cells (20 cells/ 10^6 NAMNC, SEM 8; $P = .055$) was observed compared with saline control (5 cells/ 10^6 NAMNC, SEM 3). By 12 hours, the number of $CD34^+CD45^+IL-5R\alpha^+$ returned to preallergen levels.

The time course of the proportion of $CD34^+CD45^+IL-5R\alpha^+$ cells per $CD34^+CD45^+$ in the BM indicated an expansion of the $CD34^+CD45^+IL-5R\alpha^+$ cell population at 6 hours after allergen challenge (22.0%, SEM 9.0%; $P < .05$) compared with saline control (4.1%, SEM 2.0%). No further increase in the proportion of $CD34^+CD45^+IL-5R\alpha^+$ cells per $CD34^+CD45^+$ in the BM was observed.

DISCUSSION

We have previously reported that $CD34^+45^+$ and $CD34^+45^+IL-5R\alpha^+$ progenitor cells are detected in elevated levels in both the BM and peripheral blood of atopic patients with asthma and suggested that $CD34^+45^+IL-5R\alpha^+$ progenitor cells may be the phenotype of the earliest eosinophil lineage committed progenitor cells.^{6,7} Here we describe for the first time the detailed kinetics of true $CD34^+45^+$ and $CD34^+45^+IL-5R\alpha^+$ progenitor cells isolated from mouse lungs and BM during an allergen-induced airway eosinophilia. The increase in $CD34^+45^+IL-5R\alpha^+$ progenitor cells coincided with the maximal increase in BAL IL-5 and

preceded the increase in both lung $CD34^+45^+$ progenitor cells and maximal BAL eosinophils. We also observed that cells isolated from the lungs of allergic but not control mice gave rise to eosinophil colonies. These data suggest that changes in local eosinophil numbers after allergen challenge may be caused, at least in part, by local lineage commitment and subsequent differentiation of progenitor cells via an IL-5-dependent mechanism.

Our findings demonstrate a rapid and transient increase in numbers of lung-extracted $CD34^+45^+IL-5R\alpha^+$ progenitor cells at 6 hours during an allergen-induced airways eosinophilia, which preceded the increase in both $CD34^+45^+$ progenitor cells and maximal BAL eosinophils (Figs 2 and 4). Although previous findings have demonstrated a selective expansion of $CD34^+45^+IL-5R\alpha^+$ cells at sites distal to airway inflammation, such as the BM, the current study shows for the first time that true $CD34^+45^+IL-5R\alpha^+$ progenitor cells can be isolated from lung tissue and fluctuate relative to an allergen-induced eosinophilia. Contrary to a recent report in which no changes in $CD34^+IL-5R\alpha^+$ immunopositive cell numbers from BM, blood, or BAL were detected 24 hours after allergen challenge,¹⁶ our findings show a rapid increase in $CD34^+45^+IL-5R\alpha^+$ progenitor cells in the lungs at 6 hours in the absence of a change in $CD34^+45^+$ cells. This suggests that a resident population of $CD34^+45^+$ progenitor cells may undergo *in situ* eosinophil lineage commitment. Despite this evidence, we cannot rule out the possibility that the early increase in $CD34^+CD45^+IL-5R\alpha^+$ cells is BM-derived.

We observed a decline in IL-5R α expression on CD34⁺ cells between 6 and 12h after allergen challenge. These findings are in agreement with Cameron and colleagues¹³ who have previously reported a rapid decline (within 6-12 hours) in the numbers of CD34⁺ cells expressing mRNA for IL-5R α detected in an *ex vivo* challenge model of nasal biopsy tissue after exposure to allergen. This decline was shown to be IL-5-driven and correlated with an increase in the numbers of MBP-positive cells (ie, eosinophils). These data may indicate that in the presence of increasing amounts of IL-5, differentiation of eosinophil lineage-committed progenitors occurs with a resultant decrease in the numbers of CD34⁺CD45⁺IL5R α ⁺ progenitor cells.

Here we report the growth of Eo-CFU from lung cells 10 days after extraction from saline perfused mouse lung tissue 6 hours after final ovalbumin challenge (11.6, SEM 1.5) incubated with 10 ng/mL IL-5 but not diluent (Fig 3). These data suggest that progenitor cells may undergo *in situ* differentiation and develop into active eosinophils. Similarly, it has been shown in mice that CD34⁺ cells isolated from BAL can be cultured to form eosinophil colonies, providing evidenced that true blast cells are present in the airways.¹⁶

It is conceivable that the early increase in lung-extracted CD34⁺CD45⁺IL-5R α ⁺ progenitor cells is caused by *in situ* proliferation of BM-derived CD34⁺CD45⁺IL-5R α ⁺ progenitor cells. Although there was no significant increase in the absolute number of CD34⁺CD45⁺IL-5R α ⁺ progenitor cells in the BM at 6 hours ($P = .055$), there was an increase in the proportion of CD34⁺CD45⁺IL-5R α ⁺ progenitor cells per CD34⁺CD45⁺ progenitor cells (Fig 6). Thus, the increase in CD34⁺CD45⁺IL-5R α ⁺ progenitor cells detected in the lungs at 6 hours may be a result of an increase in CD34⁺CD45⁺IL-5R α ⁺ progenitor cells in the BM, followed by migration and homing to the lung tissue undergoing an allergic inflammatory response.

Interestingly and unexpectedly, we observed a non-specific increase in CD34⁺CD45⁺ progenitor cells at 14 days during allergen-induced airway eosinophilia (Fig 2). According to the cellular contents of BAL (Table I), this nonspecific increase in CD34⁺CD45⁺ progenitor cells does not appear to be contributing to a specific inflammatory cell. It is well established that the immune system may acquire a long-lived memory of previously exposed antigen.¹⁷ In concert with this phenomenon, we hypothesize that the increase in CD34⁺CD45⁺ progenitor cells may correspond to a type of immunological memory, allowing the immune system to make a more rapid or robust inflammatory response to a subsequent allergen exposure to a novel antigen. This is also in agreement with observation of increased CD34⁺CD45⁺ cells in the peripheral blood and lung of patients with asthma versus control subjects without asthma.^{4,6}

We observed peak in IL-5 levels and a corresponding increase in CD34⁺CD45⁺IL-5R α ⁺ progenitor cell numbers at 6 hours after allergen challenge. Robinson et al⁴ have shown that IL-5 upregulates its own receptor on CD34⁺ cells, suggesting that this early event may be mediated by

IL-5. In contrast, maximal production of eotaxin coincided with the maximal increase in lung-extracted CD34⁺CD45⁺ progenitor cells at 24 to 48 hours after allergen challenge. We have recently shown that eotaxin, but not IL-5, stimulates progenitor cell migrational responses¹ and that this is primed by IL-5.⁷ The similar time course observed for IL-5 and eotaxin levels supports the hypothesis of an apparent synergistic role of these mediators; however, this study was not designed to investigate the role of chemokines in progenitor cell recruitment, making the determination of their precise role difficult. Recently, it has been suggested that IL-5 may prime CD34⁺CD45⁺ progenitor cells to respond to eotaxin or RANTES, presumably by upregulation of CCR3.¹¹ However, these authors also suggest that eotaxin may induce the expression of IL-5R on CD34⁺CD45⁺ progenitor cells and prime these cells to respond to IL-5. To date, it is not clear at which stage of eosinophils differentiation/recruitment IL-5 and eotaxin exert their effects on CD34⁺CD45⁺ progenitor cells.

It should be noted that all time points discussed in this study are relative to the second of 2 daily allergen challenges. Therefore, the 6-hour time point is after the second allergen challenge, and also 30 hours after the first allergen challenge. It is therefore possible that these time points are not necessarily reflective of that actual time course of airway eosinophilia. However, we do not think that this interferes with the main purpose of this study, which was to compare the kinetics of several hematopoietic and inflammatory events in an attempt to support or refute hypotheses related to mechanisms of inflammation.

In summary, we have isolated, for the first time, true CD34⁺CD45⁺IL-5R α ⁺ progenitor cells from the lungs during allergen-induced airway eosinophilia. These CD34⁺CD45⁺IL-5R α ⁺ progenitor cells were significantly elevated at 6 hours after allergen challenge and preceded the increase in CD34⁺CD45⁺ progenitor cells and maximal BAL eosinophils. In addition, cells isolated from the lungs of mice 6 hours after allergen challenge, but not control mice, gave rise to eosinophil colonies. The increase in CD34⁺CD45⁺IL-5R α ⁺ progenitor cells also coincided with a rapid increase in IL-5 and eotaxin. In conclusion, these findings suggest that CD34⁺CD45⁺IL-5R α ⁺ progenitor cells may undergo *in situ* differentiation and play an early role in the development of airways eosinophilia.

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