

Progenitor egress from the bone marrow after allergen challenge: Role of stromal cell-derived factor 1 α and eotaxin

Sandra C. Dorman, PhD, Irene Babirad, Julia Post, BSc, Rick M. Watson, RT, BSc, Ronan Foley, MD, Graham L. Jones, MD, Paul M. O'Byrne, MD, FRCPI, FRCPC, FCCP, and Roma Sehmi, PhD *Hamilton, Ontario, Canada*

Background: CCR3 expression on CD34⁺ cells mediates migration to eotaxin *in vitro*. CXCR4 and stromal cell-derived factor (SDF)-1 α are important for stem cell homing to hemopoietic compartments.

Objective: To study chemokine-mediated progenitor cell traffic in allergic inflammation.

Methods: Bone marrow (BM) aspirates were obtained at baseline from normal subjects; atopic subjects without asthma; and subjects with asthma before, 5 hours after, and 24 hours after allergen inhalation (dual and early responders). Changes in chemokine receptor expression and migration were assessed. **Results:** Expression of CXCR4, but not CCR3, on BM CD34⁺ cells was greater in normal subjects compared with atopic subjects with asthma. Likewise, SDF-1 α , but not eotaxin, stimulated a greater migrational response by BM CD34⁺ cells from normal subjects compared with subjects with asthma. For all subjects, a positive correlation was found between intensity of CXCR4 expression and magnitude of CD34⁺ cell response to SDF-1 α . Allergen inhalation attenuated both intensity of CXCR4 expression and SDF-1 α levels in marrow from dual compared with early responders 24 hours postallergen. In contrast, the intensity of CCR3 expression on BM CD34⁺ cells increased in dual compared with early responders at 24 hours postallergen. In addition, an increase in migrational responsiveness of BM CD34⁺ cells to eotaxin and a decrease to SDF-1 α 24 hours postallergen was found in dual responder subjects with asthma.

Conclusion: After allergen inhalation in subjects with asthma, a downregulation in CXCR4 intensity on BM CD34⁺ cells and a reduction in BM SDF-1 α levels may reduce progenitor retention to marrow stroma promoting peripheral egress, possibly mediated by the CCR3/eotaxin axis. (*J Allergy Clin Immunol* 2005;115:501-7.)

Key words: CD34, progenitor cell mobilization, asthma, SDF-1 α -CXCR4, eotaxin-CCR3

Abbreviations used

BM: Bone marrow
FACS: Fluorescence-activated cell sorting
FITC: Fluorescein isothiocyanate
HPSC: Hematopoietic stem cell
rmANOVA: Repeated measures ANOVA
SDF: Stromal cell-derived factor
SMFI: Specific mean fluorescence intensity
WBC: White blood cell

Activation of hemopoietic events in the bone marrow (BM) is associated with the onset and maintenance of airway inflammation in asthma.^{1,2} The release of primitive and lineage-committed progenitors into the circulation may also be related to this event.³⁻⁶ Allergic subjects with asthma can be classified into 2 groups: early and dual responders. The difference in BM responses between these groups suggests that substantial progenitor cell egress from the BM occurs only in the dual responders.⁴⁻⁷

Homing and retention of hemopoietic stem cells (HPSCs) in the BM is orchestrated by chemokines—in particular, stromal cell-derived factor (SDF)-1 α .⁸⁻¹⁰ Interfering with interactions of SDF-1 α and its ligand, CXCR4, blocks the *in vivo* homing of HPSCs to the BM and mobilizes stem cells for release into the circulation.⁹⁻¹¹ HPSCs can also be mobilized from the BM after stimulation with various agents, including hemopoietic growth factors, inflammatory cytokines, chemokines, and complement components.^{10,12} Eotaxin, an important inflammatory chemokine upregulated in asthma, has been shown to stimulate selectively the directional migration of progenitors *in vitro*.¹³ In addition, we have shown that progenitors express CCR3, which is upregulated in dual responders after allergen challenge.¹⁴

The roles of CCR3/eotaxin and CXCR4/SDF-1 α axis have not been investigated in the context of progenitor cell mobilization during an allergic inflammatory response in subjects with asthma. The purpose of this study was 2-fold: first, to enumerate the chemokine receptor expression levels on BM progenitor cells and to relate this to the magnitude of migrational responsiveness of these cells *in vitro*; and second, to investigate the effect of allergen challenge on chemokine production and fluctuations in receptor expression on progenitor cells.

From the Asthma Research Group, Firestone Institute for Respiratory Health, St Joseph's Healthcare, and the Department of Medicine, McMaster University.

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Reprint requests: Roma Sehmi, PhD, Assistant Professor, Firestone Institute for Respiratory Health, St Joseph's Hospital, Luke Wing, Rm L314-6, McMaster University, 50 Charlton Ave East, Hamilton, Ontario, Canada, L8N 4A6. E-mail: sehmi@mcmaster.ca.

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METHODS

Study design

Subjects were screened and classified as normal, atopic non-asthmatic, early responders, or dual responders. Normal and atopic subjects visited the laboratory once, when skin prick test sensitivity and spirometry were performed and medical history and BM aspirate samples were taken. Early and dual responders attended the laboratory on 4 occasions. On visit 1, 1 week before allergen challenge, skin prick test sensitivity and spirometry were performed, and medical history and blood and BM aspirate samples were taken. On visit 2, 1 day before allergen challenge, the methacholine inhalation test was performed. On visit 3, the allergen challenge was performed, and blood and BM aspirate were taken at 5 hours postchallenge. On visit 4, 24 hours postchallenge, methacholine inhalation challenge was performed, and blood and BM aspirate samples were taken.

Antibodies

The antibodies used in this study include: phycoerythrin-conjugated IgG₁ CD34 antibody (hematopoietic progenitor cell antigen 2 [HPCA-2]); fluorescein isothiocyanate (FITC)-conjugated IgG₁ CD45 antibody (anti-human leukocyte [HLE] antigen 1); phycoerythrin-conjugated isotype control antibody (ie, anti-IgG₁-phycoerythrin); and streptavidin-conjugated peridinin chlorophyll protein (Becton Dickinson, Oakville, Ontario, Canada). Anti-CXCR4, Anti-CCR3 mAb, IgG_{2a} isotype control, and IgG_{2b} isotype control (R&D, Minneapolis, Minn).

Subjects

Nine nonatopic subjects without asthma (determined by negative skin prick test result and PC₂₀ greater than 32 mg) and 5 asymptomatic atopic subjects without asthma (determined by clinical history, positive skin prick test result, and PC₂₀ greater than 32 mg) participated. In addition, 30 subjects with mild, stable, atopic asthma and baseline FEV₁ >70% predicted were enrolled. For postallergen comparisons, atopic subjects with asthma were broken into 2 groups: early and dual responders. Postallergen, early responders (n = 14) developed an early fall in FEV₁ >20% from baseline between 0 hours and 2 hours, whereas dual responders developed an early and late fall (FEV₁ >15%) from baseline between 3 hours and 7 hours (n = 16). Subjects were nonsmokers, were not on any asthma medication except for the use of inhaled β_2 -agonists intermittently (not used 8 hours before each visit), and had not had respiratory infection or been exposed to altered levels of allergen 2 weeks before allergen inhalation (see Table EI in the Journal's Online Repository at www.mosby.com/jaci). This study was approved by the Ethics Committee of McMaster University Health Sciences Centre, and subjects gave written informed consent before study entry.

Methacholine and allergen inhalation tests

Methacholine inhalation challenge was performed as described by Cockcroft.¹⁵

Allergen inhalation challenge was performed as described by O'Byrne et al¹⁶ using a Wright nebulizer (Roxon Medi-Tech Ltd, Montreal, Quebec, Canada). Allergen extract was selected and diluted for inhalation at a concentration determined from a formula using the results from the skin test and the methacholine PC₂₀.¹⁷

Isolation and immunofluorescence staining

Bone marrow aspiration and blood progenitor enumeration were performed according to Wood et al.⁶ Nonadherent mononuclear cells (containing progenitor cells and lymphocytes) were first stained with saturating amounts of biotin-conjugated anti-CXCR4, anti-CCR3, or the relevant biotin-conjugated isotype control antibody (deter-

mined in preliminary studies) in a final volume of 100 μ L ice-cold fluorescence-activated cell sorting (FACS) staining buffer (PBS plus 0.1% NaN₃; 2.5% each of mouse serum and human serum) for 30 minutes at 4°C. The cells were then washed with 3 mL PBS plus 0.1% NaN₃, resuspended in FACS staining buffer, and stained with streptavidin-conjugated peridinin chlorophyll protein together with saturating concentrations of FITC-conjugated IgG₁ CD45 antibody (anti-HLE1) and phycoerythrin-conjugated IgG₁ CD34 antibody (HPCA-2) for 30 minutes at 4°C. Lysis buffer (Becton Dickinson, Ontario, Canada) was then added to lyse red blood cells and incubated for 5 minutes, after which the cells were washed twice with 3 mL PBS plus 0.1% NaN₃ and fixed in 500 μ L PBS plus 1% paraformaldehyde. Cells were refrigerated until acquired by flow cytometry.

Cells were analyzed by using a FACScan flow cytometer (Oakville, Ontario, Canada) equipped with an argon ion laser (Becton Dickinson Instrument Systems). Progenitor cells were identified on the basis of their unique cell size, granularity, and immunofluorescence characteristics.¹⁸ Offline analysis was performed by using the PC lysis software as supplied by BDIS. True CD34⁺ blast cells were identified as cells with CD34^{high}/CD45^{dull} staining as previously described.¹⁸ Chemokine receptor expression with CD34^{high}/CD45^{dull} was expressed as either proportion of total progenitor cells (percent of CD34⁺45⁺ cells) as previously described¹⁴ or as the intensity of receptor expression (specific mean fluorescence intensity, SMFI) on 4000 to 5000 CD34⁺45⁺ cells.

Transmigration assay

The migrational response of progenitors *in vitro* was assessed by using transwell chambers (24-well cell clusters with 5- μ m pore polycarbonate filters, Costar, Cambridge, Mass) as previously described with minor modifications.⁸ Bone marrow CD34⁺ cells were enriched (purity >85%) by magnet-activated cell sorting. Cells were loaded into each filtered transwell insert (1 $\times$ 10⁵/100 μ L). These transwell inserts were then placed in a larger well containing 600- μ L dilutions of the chemoattractant SDF-1 α (25, 50, 100, or 200 ng/mL), eotaxin (50, 100, 200, or 500 ng/mL), or diluent in serum-free medium, previously incubated for 15 minutes at 37°C in 5% CO₂. After 4 hours, the transwell insert was removed, and the cells in the bottom chamber (representing migrated cells) were aspirated. These cells were resuspended in FACS staining buffer and stained with phycoerythrin-conjugated CD34 mAb and FITC-conjugated CD45 mAb as described. To obtain absolute values of migratory cells, flow-cytometric counts for each sample were obtained during a constant predetermined period. Because a constant number of CD34⁺45⁺ cells was added to the top well for all migrational assays performed, the migrational data for responding progenitor cells are presented as total CD34⁺45⁺ cell numbers counted in the lower well.

Serum and bone marrow supernatant

Serum and BM supernatant were stored at -70°C. Eotaxin and SDF-1 α were quantified by using standard ELISA (R&D). The lower limits of detection were 5 and 18 pg/mL, respectively.

Statistical analysis

Results are expressed as means $\pm$ SEMs. The difference between groups at baseline was analyzed by using a repeated measures ANOVA (rmANOVA; between-group: normal vs atopic without asthma vs dual responder vs early responder). The effect of allergen on methacholine PC₂₀, CD34⁺45⁺ cell numbers, and proportion of CD34⁺45⁺ cells expressing CXCR4⁺ and CCR3⁺ were analyzed by using a rmANOVA (between-group: early vs dual responders; within-group: pre vs 5 hours and 24 hours). Transmigration data were log₁₀-transformed and analyzed by using a rmANOVA (between-treatment: diluent vs various concentrations of chemokines; between-group:

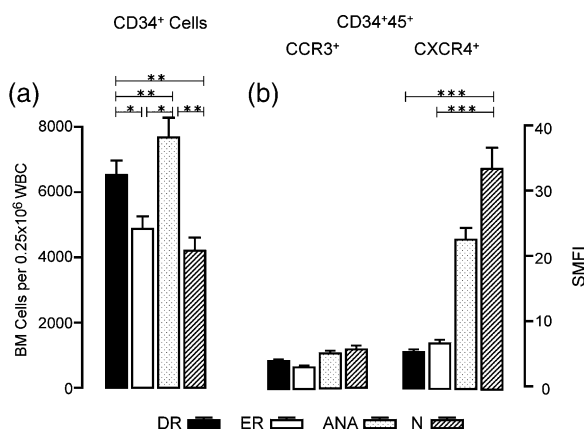


FIG 1. Progenitor cells in BM aspirates from dual responders (DR; n = 16), early responders (ER; n = 14), atopic nonasthmatics (ANA; n = 5), and normals (N; n = 9). Data are expressed as means $\pm$ SEMs of cells per 0.25×10^6 WBCs for CD34⁺45⁺ cells and as SMFI for chemokine receptor enumeration on CD34⁺45⁺ cells. Difference between groups: * $P < .05$; ** $P < .01$; *** $P < .001$.

normal vs atopic without asthma vs early and dual responders). Comparisons of migrational responses before versus 24 hours after allergen were analyzed by using a Student *t* test for dependent samples. Cytokine levels were analyzed by using nonparametric testing (Kruskal-Wallis between-group: early vs dual responders; Wilcoxon within-group: pre vs 5 hours and 24 hours; Statistica version 6.0 [Statsoft, Tulsa, Okla]). Statistical significance was accepted as $P < .05$.

RESULTS

Chemokine receptor expression on progenitor cells

In agreement with previous studies, we found that greater numbers of CD34⁺45⁺ cells were detected in the BM of atopic subjects compared with normal subjects^{14,18,19} (Fig 1, A). More specifically, greater CD34⁺45⁺ cell numbers were detected in BM aspirates from atopic subjects without asthma compared with both dual and early responder subjects with asthma and normal subjects. In addition, greater numbers of CD34⁺45⁺ cells from BM were detected in dual responders compared with early responders ($P < .05$) and normal subjects ($P < .01$) but not between early responders and normal subjects (atopic without asthma: 7285 ± 1727 ; dual responders: 6532 ± 435.7 ; early responders: 4945 ± 389.5 ; and normals: 4206 ± 402 CD34⁺45⁺ cells/ 0.25×10^6 white blood cells [WBCs]; Fig 1, A).

A comparison of various classes of chemokine receptors on BM progenitors showed that the intensity of CXCR4 expression on CD34⁺45⁺ cells was higher in normal subjects (33.9 ± 6.5) compared with dual responders (5.7 ± 0.6 ; $P < .001$) and early responders (6.9 ± 1.0 ; $P < .001$) but not atopic subjects without asthma (22.7 ± 4.5 ; Fig 1, B). By comparison, there was no difference in the intensity of CCR3 expression on CD34⁺45⁺ cells be-

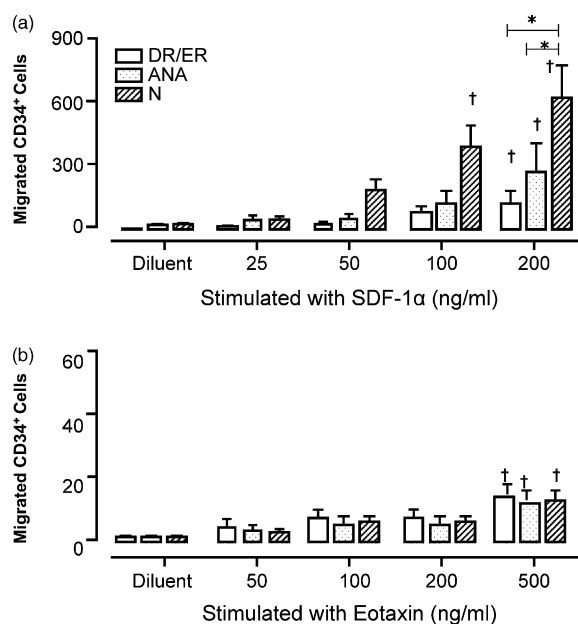


FIG 2. Transwell migration assay of BM progenitor cells from subjects with asthma (dual and early responders, DR/ER; n = 9), atopic nonasthmatics (ANA; n = 5), and normals (N; n = 9). SDF-1α stimulated a dose-dependent migrational response of CD34⁺ cells optimal at 200 ng/mL for all groups. The magnitude of transmigration was greatest in normal subjects. Difference within group: † $P < .05$; difference between groups: * $P < .05$.

tween any of the subject groups (normal subjects: 6.8 ± 1.7 ; dual responders: 4.8 ± 0.9 ; early responders: 3.8 ± 0.5 ; atopic without asthma: 5.5 ± 1.5). Similar findings were obtained when we analyzed the data as the percentage of BM CD34⁺45⁺ cells that expressed either CXCR4 (normal subjects: $34\% \pm 5.0\%$; vs dual responders: $13\% \pm 3.0\%$, $P < .05$; early responders: $7\% \pm 1.1\%$, $P < .05$; atopic without asthma: $27\% \pm 4.5\%$ CD34⁺45⁺ cells) or CCR3 (normal subjects: $1.4\% \pm 0.2\%$; dual responders: $1.8\% \pm 0.4\%$; early responders: $1.3\% \pm 0.1\%$; atopic without asthma: $1.1\% \pm 0.3\%$ CD34⁺45⁺ cells; see Figs E1 and E2 in the Online Repository at www.mosby.com/jaci).

Baseline transmigration of progenitor cells

To investigate the relationship between levels of chemokine receptor expression and functional responsiveness, we enumerated the migrational responses of BM-derived progenitor cells to SDF-1α and eotaxin *in vitro*. SDF-1α stimulated a dose-dependent migrational response of CD34⁺45⁺ cells from all subject groups, optimal at 200 ng/mL (Fig 2, A). For between-group comparisons, the number of CD34⁺45⁺ cells transmigrating toward SDF-1α was greater in normal subjects compared with atopic subjects without asthma and subjects with asthma at optimal concentrations (200 ng/mL; 609 ± 173 , 279 ± 64 , and 111 ± 55 CD34⁺45⁺ cells, respectively; $P < .05$; Fig 2, A). No difference in the magnitude of migrational response to any dose of SDF-1α tested was detected in comparisons between dual and early

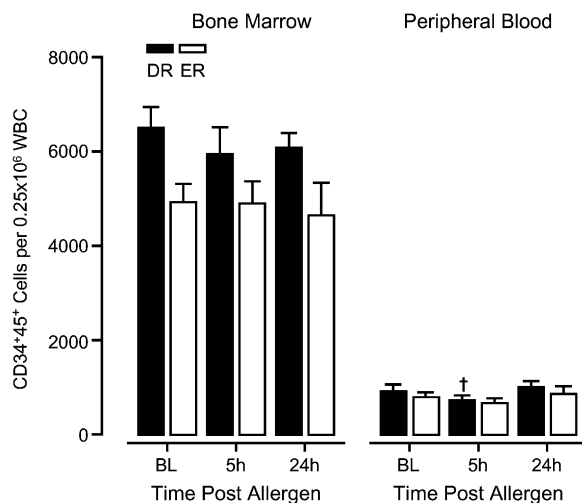


FIG 3. CD34⁺45⁺ cells in BM and peripheral blood from dual responders (DR; n = 16) and early responders (ER; n = 14). Peripheral CD34⁺45⁺ cells decreased at 5 hours followed by an increase at 24 hours postallergen in DR only. No change in blood progenitors was detected in ER at any time point after allergen challenge. Difference over time: †P < .05. BL, Baseline.

responder subjects with asthma (data not shown). For all subjects, a significant positive correlation was observed between the proportion of CD34⁺45⁺ cells expressing CXCR4 and the magnitude of progenitor cell migrational response to SDF-1 α at 200 ng/mL ($r = 0.33$; $P < .05$) and 100 ng/mL ($r = 0.43$; $P < .05$).

Eotaxin stimulated a weak migrational response for BM CD34⁺45⁺ cells that was greater than diluent only at 500 ng/mL for all groups of subjects ($P < .05$; Fig 2, B).

Allergen-induced airway responses

Dual responders had a greater fall in FEV₁ between 3 hours and 7 hours after inhaled allergen and a significant decrease in methacholine PC₂₀ 24 hours after inhaled allergen ($P < .05$), which was not demonstrated in isolated early responders (see Table E2 in Online Repository at www.mosby.com/jaci).

Allergen-induced changes in progenitors

BM. In accordance with previous studies, no change in the number of BM CD34⁺45⁺ cells was detected after allergen in either group of subjects with asthma^{6,14,18} (Fig 3). No change in the proportion of BM CD34⁺45⁺ cells expressing CXCR4 or CCR3 was detected after allergen in either group of subjects with asthma (Fig 4). Similarly, no allergen-induced change in the intensity of CXCR4 or CCR3 on BM CD34⁺45⁺ cells was detected (Table I).

However, lower intensity of CXCR4 expression on progenitor cells was detected in dual compared with early responders at 24 hours postallergen ($P < .05$; Table I). In contrast, higher intensity of CCR3 expression on CD34⁺45⁺ cells was detected in the BM of dual compared with early responders at 24 hours postallergen ($P < .05$; Table I).

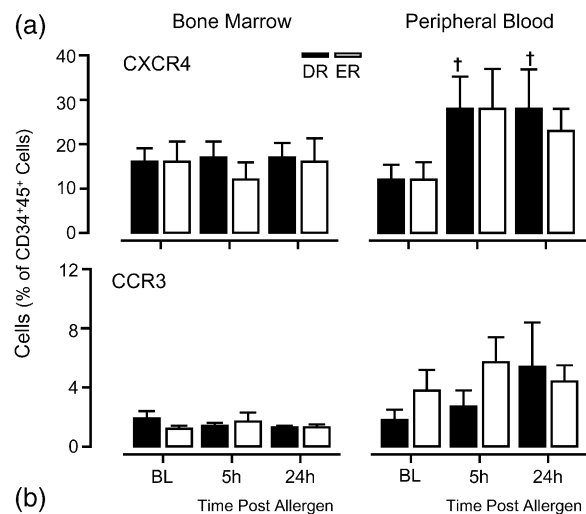


FIG 4. CXCR4 and CCR3 expression on CD34⁺45⁺ cells from BM and peripheral blood from dual responders (DR; n = 16) and early responders (ER; n = 14). Peripheral CD34⁺45⁺ cells expressing CXCR4 increased at 5 hours and 24 hours postallergen in DR only. Difference within group: †P < .05. BL, Baseline.

TABLE I. Fluctuations in chemokine receptor expression on BM progenitor cells after allergen challenge

Receptor expression on BM CD34 ⁺ 45 ⁺ cells	Preallergen	5 Hours postallergen	24 Hours postallergen
Dual responders (n = 16)			
CCR3 (SMFI)	4.8 ± 0.9	4.9 ± 0.87	6.13 ± 0.9*
CXCR4 (SMFI)	5.7 ± 1.9	9.02 ± 5.26	2.9 ± 1.4
Early responders (n = 14)			
CCR3 (SMFI)	3.8 ± 0.5	3.9 ± 2.21	4.4 ± 1.4
CXCR4 (SMFI)	6.9 ± 1.0	10.5 ± 5.02	8.9 ± 2.4*

Difference between groups: *P < .05.

Blood changes. After allergen inhalation, dual responders developed a decrease in the number of CD34⁺45⁺ cells at 5 hours compared with baseline and compared with 24-hour levels (Fig 3). The numbers of hemopoietic progenitors decreased from 931 ± 139.8 cells preallergen to 740 ± 103.4 cells at 5 hours ($P < .05$) and subsequently increased to 1015 ± 125.8 cells at 24 hours ($P < .05$) post-allergen. In contrast, in early responders, no change in circulating CD34⁺45⁺ cell numbers at any time point was found (Fig 3).

After allergen inhalation, dual responders developed an increase in the proportion of CD34⁺45⁺ cells expressing CXCR4 at 5 hours and 24 hours compared with baseline. Cell numbers increased from 12% ± 3.4% preallergen to 28% ± 7.3% at 5 hours ($P < .05$) and to 28% ± 8.9% of CD34⁺45⁺ cells at 24 hours postallergen ($P < .05$; Fig 4, A). In early responders, there was a trend for a change in the proportion of circulating CD34⁺45⁺ cells expressing CXCR4 at 5 hours ($P = .07$; Fig 4, A).

For both groups of subjects with asthma, there was no allergen-induced change in the proportion of circulating CD34⁺45⁺ cells expressing CCR3 (Fig 4, B).

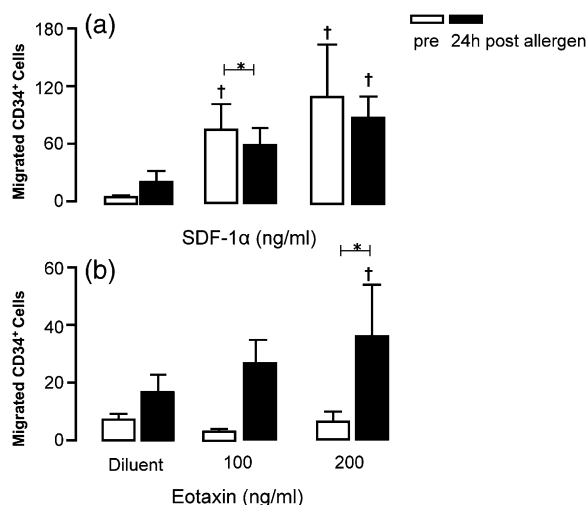


FIG 5. Transwell migration assay of BM progenitors before and after allergen inhalation in dual responders ($n = 6$). Twenty-four hours postallergen, migrational responses to SDF-1 α (100 ng/mL) decreased compared with preallergen. In contrast, migrational responses to eotaxin (200 ng/mL) increased. SDF-1 α and eotaxin stimulated an increased migration compared with diluent. Difference with diluent: $\dagger P < .05$; difference over time: $*P < .05$.

Allergen-induced changes in transmigration of progenitor cells

To investigate the effect of allergen inhalation on functional responsiveness of progenitor cells, comparisons of the chemokine-stimulated migrational responses before and 24 hours after allergen were performed in dual responder subjects with asthma. After allergen challenge, there was a reduction in the stimulated migrational response of BM CD34⁺45⁺ cells to a suboptimal concentration of SDF-1 α at 100 ng/mL (Fig 5, A). In contrast, the stimulated migrational response to a suboptimal concentration of eotaxin (200 ng/mL) was greater when preallergen levels were compared with postallergen levels ($P < .05$; Fig 5, B).

Allergen-induced changes in SDF-1 α and eotaxin

SDF-1 α . Dual responders developed a decrease in SDF-1 α at 24 hours postallergen inhalation compared with baseline in the BM. Protein levels were 1666 ± 61.8 pg/mL preallergen, 1630 ± 69.9 pg/mL at 5 hours, and 1460 ± 79.2 pg/mL at 24 hours postallergen ($P = .05$). In early responders, there was no change in SDF-1 α levels postallergen inhalation. Between-group comparisons showed that at 24 hours postallergen, lower levels of SDF-1 α were detected in dual compared with early responders ($P < .05$; Fig 6, A). There was no change in the amount of circulating SDF-1 α over time in either group (Fig 6, A).

Eotaxin. Dual responders developed an increase in the amount of circulating eotaxin 5 hours postallergen compared with baseline. Protein levels increased from baseline: 81 ± 11.2 pg/mL to 101 ± 9.9 pg/mL at 5 hours ($P < .05$) and 96.6 ± 12.1 pg/mL at 24 hours postallergen. By contrast, there was no change in circulating eotaxin levels in early responders. Between-group comparisons showed that at 5 hours postallergen, higher levels of eotaxin were detected in dual compared with early responders ($P < .05$). There was no change in BM eotaxin levels over time in either group (Fig 6, B).

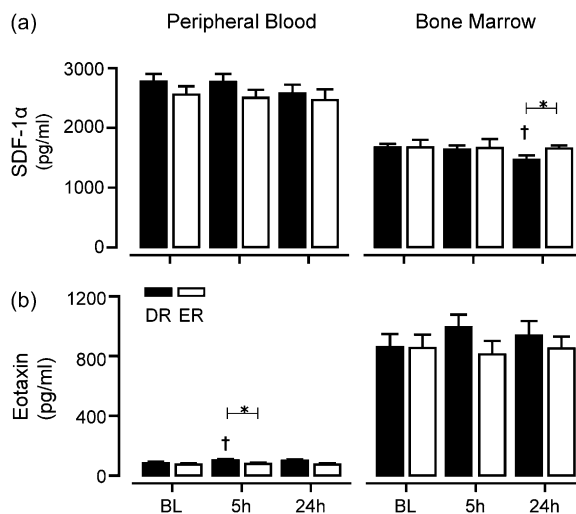


FIG 6. SDF-1 α and eotaxin levels from BM supernatants and plasma taken from dual responders (DR; $n = 16$) and early responders (ER; $n = 14$) at baseline (BL), 5 hours, and 24 hours postallergen inhalation. BM SDF-1 α levels decreased at 24 hours, whereas circulating eotaxin levels increased at 5 hours in DR only. Difference within group: $\dagger P < .05$; difference between groups: $*P < .05$.

DISCUSSION

The distinctive findings of this study are that in the BM, (1) at baseline, there are differences in the level of CXCR4 but not CCR3 expression on progenitors from different groups, (2) a correlation exists between the magnitude of the migrational response and the level of CXCR4 expression on progenitors, (3) there is a downregulation of CXCR4 expression on CD34⁺45⁺ cells from dual compared with early responders at 24 hours postallergen, and (4) there is a reduction in migrational responsiveness to SDF-1 α *in vitro* when preallergen is compared with 24 hours postallergen levels in dual responders. In addition, eotaxin in the blood and SDF-1 α in the marrow fluctuate in response to allergen inhalation at times that are consistent with progenitor movement. Together, these data support the hypothesis that after allergen inhalation in subjects with asthma, downregulation of SDF-1 α in the BM and increased eotaxin in the circulation, with relevant fluctuations of receptors on CD34⁺45⁺ cells, facilitate chemokine-mediated egress of progenitors from the BM to the blood.

We have recently shown increases in sputum CD34⁺45⁺ cells in the airways of dual responder subjects

with asthma at 7-hour and 24-hour postallergen inhalation.²⁰ Now we have shown decreases in the number of circulating CD34⁺45⁺ cells at 5 hours in dual responders only, a time point preceding the 7-hour increases previously seen. This suggests that during an asthmatic exacerbation, BM progenitors are both activated and released into the circulation, where they may home to the airways.

SDF-1 α mediates its effects through ligation of CXCR4.²¹ SDF-1 α is produced by stromal cells and is bound within the marrow by heparans and proteoglycans. Therefore, ligation of CXCR4 by SDF-1 α is a migrational stimulus and causes retention of progenitors.²² Petit et al¹² have proposed that manipulation of SDF-1 α -CXCR4 interactions may be a way to control the navigation of progenitor cells between BM and blood. They demonstrated that the mobilization of HPSCs was a result of both the loss of chemotactic responsiveness of CXCR4 to SDF-1 α ¹¹ and the proteolytic degradation of SDF-1 α .¹² Because both SDF-1 α and CXCR4 are essential for the homing and retention of HPSCs in the BM, disruption of binding may represent a critical step in the release of HPSCs into the circulation.¹¹ The current study is consistent with these findings, showing a reduction in BM SDF-1 α levels at 24 hours, a decrease in the intensity of CXCR4 expression on BM CD34⁺45⁺ cells, and an increase in circulating CD34⁺45⁺CXCR4⁺ cells at 5 hours and 24 hours after allergen inhalation. The increase in circulating CD34⁺45⁺CXCR4⁺ cells before the measured decrease in SDF-1 α presumably reflects the initial cleavage process in which cells become nonresponsive and are released into the circulation. This is supported by the decrease in the number of BM CD34⁺45⁺ cells transmigrating toward SDF-1 α at 24 hours compared with baseline.

Interestingly, SDF-1 α ligation has also been shown to arrest progenitor cell cycling, promoting a transition into quiescence.²³ In the current study, the decrease in SDF-1 α occurs at a time that has previously been shown to be associated with an increase in eosinophilopoiesis in dual responders only.⁶ Therefore, SDF-1 α , in addition to allowing the egress of progenitor cells from the BM, may contribute indirectly to the increased production of inflammatory cells in this site. This is in agreement with our findings of a significant increase in IL-5-responsive eosinophil/basophil colony-forming units detected in dual responders.⁷

Eotaxin mediates its effect through ligation of CCR3.²⁴ CCR3 expression on progenitor cells has now been shown by several groups.^{14,25,26} We have previously shown the ability of these cells to transmigrate toward eotaxin, which was abrogated by mAbs against CCR3. In the same study, we found significant increases in CCR3 expression on BM CD34⁺ cells from dual responders 24 hours after allergen inhalation.¹⁴ In the current study, we confirm the expression of CCR3 on CD34⁺45⁺ cells and show that after allergen challenge, there was an increase in the intensity of CCR3 expression on CD34⁺45⁺ cells from BM of dual responder subjects with asthma.

Using an *in situ* arterial perfusion system with guinea pig femoral BM, eotaxin infusion stimulated a release of eosinophil colony-forming progenitor cells.¹³ In the current study, we have shown for the first time increases in circulating eotaxin levels at 5 hours in dual responders only. The inability to detect similar increases in circulating CD34⁺CCR3⁺ cells at 5 hours may reflect the speed of homing of the progenitors or the dilution effect of the blood.

Preallergen, CCR3 expression on CD34⁺45⁺ cells was not different between groups. Similarly, there were no differences between groups in the ability of the CD34⁺45⁺ cells preallergen to transmigrate toward eotaxin. In contrast, the difference in CXCR4 expression between groups was associated with increases in the number of CD34⁺45⁺ cells transmigrating toward SDF-1 α . Together, these results suggest that chemokine receptor expression reflects the ability of cells to respond to stimulus. This is further supported by our findings of a positive correlation between the intensity of CXCR4 expression on progenitor cells and the magnitude of the migrational response to SDF-1 α .

Although some of the differences measured in this study are modest and deserve careful examination, we believe that these differences are important because they were detectable in a very small subset of patients examining a rare cell population (see Figs E3 and E4 in the Online Repository at www.mosby.com/jaci).

Normally, the BM acts as a site for the turnover and traffic of mature leukocytes to the blood. However, in inflammatory conditions such as atopic asthma, an increased turnover of BM and blood progenitors also occurs. Although the precise mechanisms controlling progenitor cell traffic are not understood, we demonstrate that fluctuations in CXCR4 and CCR3 expression on BM CD34⁺45⁺ cells together with changes in SDF-1 α and eotaxin levels contribute to the release of progenitors from the BM in allergic asthma. These results also indicate that BM activation may have to be abrogated if asthma is to be fully controlled.

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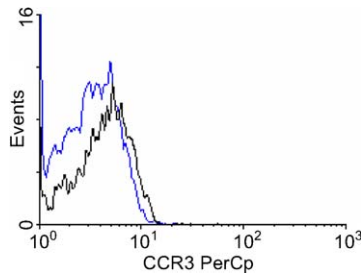


FIG E1. A representative histogram showing a unimodal positive shift in the fluorescence intensity of CCR3 on BM CD34⁺ cells from a dual responder asthmatic preallergen (blue line) compared with 24 hours postallergen (black line). Mean fluorescence intensity values for 90% of the area under each histogram are 6.85 vs 8.71, respectively. *PerCp*, Peridinin-chlorophyll-protein complex.

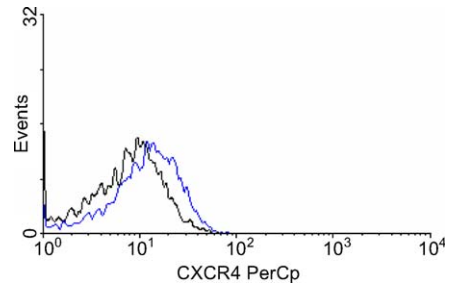


FIG E2. A representative histogram showing a unimodal downward shift in the fluorescence intensity of CXCR4 on bone marrow CD34⁺ cells from a dual responder asthmatic preallergen (blue line) compared with 24 hours postallergen (black line). Mean fluorescence intensity values for 90% of the area under each histogram are 16.6 vs 12.31, respectively. *PerCp*, Peridinin-chlorophyll-protein complex.

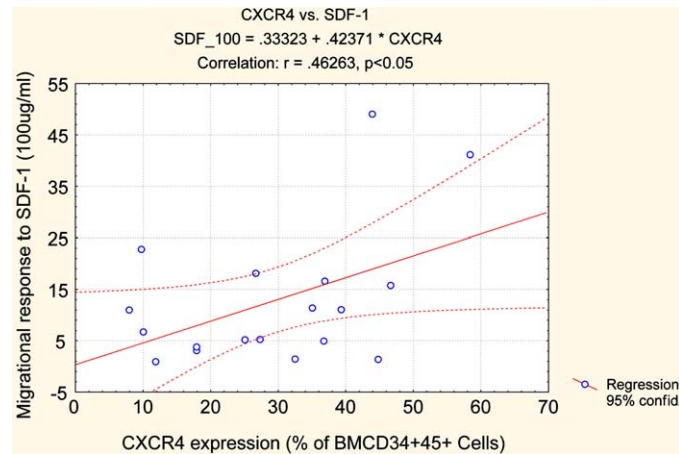


FIG E3. The graph shows a positive correlation between the proportion of BM CD34⁺ cells expressing CXCR4 and the migrational response of CD34⁺ cells to SDF-1 α (100 ug/mL; ie, numbers of CD34⁺ cells counted in the lower transwell chamber). The graph shows the data collected from normal subjects, atopic subjects, and atopic subjects with asthma (both dual and early responders).

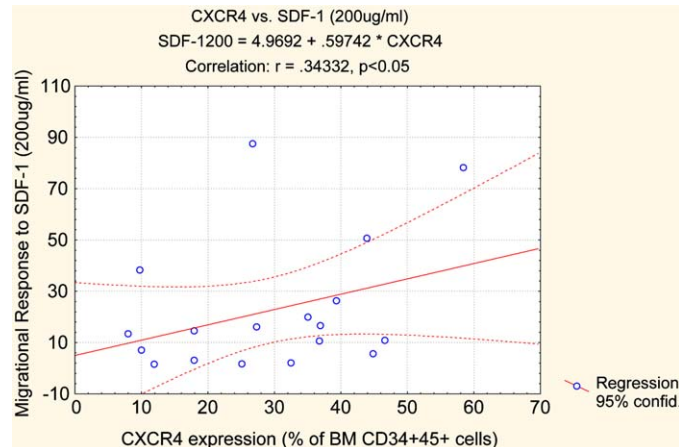


FIG E4. The graph shows a positive correlation between the proportion of BM CD34⁺ cells expressing CXCR4 and the migrational response of CD34⁺ cells to SDF-1 α (200 ug/mL; ie, numbers of CD34⁺ cells counted in the lower transwell chamber). The graph shows the data collected from normal subjects, atopic subjects, and atopic subjects with asthma (both dual and early responders).

TABLE E1. Subject characteristics

Early responders	Age (y)	Sex	Allergen	Dual responders	Age (y)	Sex	Allergen
1	29	F	Cat	1	26	F	Ragweed
2	24	F	Cat	2	23	F	HDM
3	30	F	HDM	3	26	M	Cat
4	26	M	Ragweed	4	24	M	Ragweed
5	24	F	Grass	5	23	F	HDM
6	23	M	Cat	6	52	M	Cat
7	51	F	Cat	7	33	M	Grass
8	21	F	Cat	8	40	M	HDM
9	27	F	Grass	9	32	M	Ragweed
10	19	F	HDM	10	41	M	Cat
11	23	M	HDM	11	19	F	Ragweed
12	39	M	HDM	12	27	M	HDM
13	27	F	Cat	13	26	F	HDM
14	20	F	Ragweed	14	23	F	HDM
				15	28	F	HDM
				16	20	F	Ragweed
Mean $\pm$ SEM	28 $\pm$ 2				31 $\pm$ 2		

HDM, House dust mite.

TABLE E2. Allergen-induced airway responses

	Early responder subjects with asthma	Dual responder subjects with asthma
% Fall in FEV ₁		
Early airway response	21.9% $\pm$ 1.54%	28.3% $\pm$ 1.65%
Late airway response	5.4% $\pm$ 1.03%	20.5% $\pm$ 2.04% [‡]
Methacholine PC ₂₀		
Preallergen	3.3 mg/mL (% SEM, 1.5)	2.4 mg/mL (% SEM, 1.3)
Postallergen	2.2 mg/mL (% SEM, 1.5)	0.7 mg/mL (% SEM, 1.6) ^{†‡}

*Blood, BM, and airway eosinophil numbers for a subset of these patients have been reported.³⁴

Difference within group: [†] $P < .05$; and difference between groups: [‡] $P < .05$.