Title: Modulating progenitor accumulation attenuates lung angiogenesis in a mouse model of asthma

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Abstract

Asthmatic responses are associated with the lung-homing of bone marrow (BM)-derived progenitors implicated as effectors of disease pathology. Increases in lung-extracted vascular endothelial progenitors (VEPC) correlate with airway angiogenesis and declining lung function. We investigated the effect of modulating lung-homing of VEPC on tissue remodeling and airway hyperresponsiveness (AHR).

Balb/C mice were sensitized to ovalbumin (OVA), subjected to a chronic exposure protocol and given early concurrent or delayed treatment with a modulator of progenitor traffic AMD3100, (CXCR4 antagonist; inhibits chemotactic activity of SDF-1alpha on VEPC). Post-OVA challenge, early hemopoietic stem cells (HSC) and VEPC were enumerated as well as indices of airway inflammation, lung morphometry and AHR.

Following OVA challenge, there was decrease in BM and an associated increase in the lung tissue-extracted HSC and VEPC cells together with increases in airway eosinophilia, microvessel density and AHR. These outcomes were significantly inhibited by early concurrent treatment with AMD3100. Where lung disease was established, delayed treatment with AMD3100 significantly attenuated HSC numbers and lung angiogenesis but only partially reversed sustained AHR compared to untreated OVA exposed mice.

Progenitor lung-homing is associated with the development of asthma pathology and early modulation of this accumulation can prevent airway remodeling and lung dysfunction.
**Key words:**  Asthma, Angiogenesis, Bone Marrow Progenitors, Lung Remodeling, Stromal Derived Factor-1, Vascular Endothelial Progenitor Cells,

**Abbreviations:**

BM: Bone marrow

VEPC: Vascular endothelial progenitor cells

OVA: Ovalbumin

AHR: Airway hyperresponsiveness

MCh: Methacholine

MVD: Microvessel density

vWF: Von Willenbrand Factor

IP: intraperitoneal

SDF-1: Stromal cell derived factor-1

HSC: Early hemopoietic progenitor cells (Sca1+ c-kit+)

VEPC: Vascular endothelial progenitor cells (VEPC)
INTRODUCTION

Asthma is a chronic inflammatory disease characterized by remodeling of the airways, including goblet cell hyperplasia, thickening of the fibrous region beneath the basement membrane, smooth muscle hyperplasia/hypertrophy and airway angiogenesis (1;2). Although inflammation of the airway is an essential component of asthma exacerbations, current anti-inflammatory therapies have been unsuccessful in completely reversing sustained airway hyperresponsiveness (AHR) and this has initiated the perspective that remodeling of the lung tissue may have additional functional consequences to those produced by inflammation (3).

It is evident that an association exists between allergen-induced asthmatic responses and mobilization of hemopoietic progenitors from the bone marrow (BM) (4). These multipotent progenitors, once homed to the lung, can be manipulated by a spectrum of local cytokines and chemokines to differentiate in situ into a variety of inflammatory (eosinophils, basophils, mast cells) (5-7) and/or structural cells (fibrocytes, vascular endothelial cells) (8). Following allergen challenge in atopic asthmatics (6) and sensitized mice (7) increases in BM derived hemopoietic progenitors are detected in the lung which preceded the development of lung eosinophilia. In the latter study, Southam et al. showed that non-lineage committed primitive progenitors increased in the lung tissue of mice well after the resolution of airway eosinophilia suggesting that, in addition to contributing to early inflammatory events, progenitors could be involved in the initiation and/or maintenance of airway structural changes (7). Phenotypic analysis of primitive lung extracted progenitors showed a high level of expression for CXCR4 and a corresponding increase in the cognate ligand, stromal derived factor-1 (SDF-1) (7;9). Since SDF-1 is a potent progenitor chemoattractant, shown to be increased in the airways of asthmatics, it maybe involved in promoting the lung homing of progenitors in asthma (10-13).
A recent study reported an increase in lung vascular endothelial progenitor cells (VEPC) following allergen challenge in sensitized mice which was associated with increased lung angiogenesis, considered to be an early remodeling event in asthma (14;15). Interestingly, VEPC express CXCR4 (13), suggesting that SDF-1 may play a pivotal role in the lung homing of progenitors, specifically VEPC, to the asthmatic lung. We hypothesize that angiogenesis can be prevented by modulating lung homing of VEPC within the lung. This was tested in an established chronic allergen exposure mouse model, which characteristically develops both inflammatory and tissue remodeling changes associated with increased AHR (16). To prevent the migration of VEPC from the bone marrow to the lung, mice were treated with a CXCR4 antagonist, AMD3100 (17), previously shown to attenuate both eosinophilia and AHR in a mouse model of asthma (18). In this study AMD3100, given intranasally (i.n.) was administered either concurrently i.e. during the onset of disease or in a reversal/delayed treatment regimen i.e. once airway disease was established.

Our data show that localized treatment with AMD3100 given concurrently significantly attenuated the accumulation of primitive hemopoietic progenitor cells (HSC) and VEPC (delete lung-homing), vascularization of lung tissue, airway eosinophilia and AHR. In established disease, AMD3100 treatment attenuated accumulation (delete “lung homing”) of HSC and lung vascularization but only partially reversed sustained AHR. This suggests that progenitor cell increases within the lung (delete “homing and differentiation”) are associated with (delete “necessary for initiating”) early angiogenic remodeling events and the development of AHR, and modulating progenitor activity or reversing lung angiogenesis can partially resolve previously established airway dysfunction.
MATERIALS AND METHODS

Animals

Female BALB/c mice, 8 to 10 weeks of age, (Charles River Laboratories, Saint-Constant, PQ, Canada) were maintained in a pathogen-free environment. All procedures were reviewed and approved by the Animal Research Ethics Board at McMaster University (Hamilton ON, Canada).

Allergen Exposure – Chronic Exposure Protocol

Mice (n=10/group) were sensitized and challenged as previously described and shown in Figure 1. Outcomes were made on day 91 (24hrs post-OVA) and where mentioned, on day 118 (4 weeks post-OVA).

Treatment Intervention

Mice were given AMD3100 i.n., a CXCR4 antagonist (19) (Sigma). Optimal dosing of the drug (15mg/ Kg) was established in pilot experiments as the lowest dose capable of preventing allergen-induced increases in airway vascularity in a brief allergen exposure protocol (see supplemental data, Figure S1). Secondary outcome measures for this pilot study included airway inflammation and progenitor cells from lung-extracted tissue Intervention with AMD3100 consisted of i) concurrent treatment: drug given 4hrs before each allergen challenge during the 18-90 days of exposure (Figure 1A), or ii) reversal treatment: drug given on two consecutive days/ week during four weeks (90-118 days) after cessation of the allergen exposure (Figure 1B). Control mice received OVA plus saline vehicle instead of drug.
**Airway Responsiveness**

Airway responsiveness to intravenous methacholine was measured based on the response of total respiratory system resistance using the FlexiVent ventilator system (SCIREQ, Montreal Canada) as previously described in detail (20).

**Bronchoalveolar Lavage**

Bronchial alveolar lavage was performed as described previously in detail (21). VEGF levels in BAL fluid were assessed using a Sandwich ELISA kit (R&D Systems Minneapolis, MN).

**Progenitor Cell Isolation, Immunofluorescence Staining and Flow cytometry**

Mice were sacrificed by exsanguinations via cardiac puncture and lungs perfused clear of blood with saline and removed from the thoracic cavity as described previously (7). Lung tissue-associated cells were extracted from the right lobe by mincing and enzymatic digestion as previously described (7). BM samples were flushed from the femur and tibia and mononuclear cells were collected following density gradient centrifugation over Accu-Prep™ (400 g, 30mins) (Accurate Chemicals, NY, USA). Cells were immunostained with Sca-1-FITC, c-kit-PE (BD Bioscience, Oakville, ON, Canada) and VEGF2R-APC (eBioscience) or isotype control antibodies (40 mins, 4°C) and fixed in PBS with 1% paraformaldehyde (BDH Lab. Supplies, Mississauga, ON) and cell data (100,000 events in the lymphomononucelar region) was acquired using a FACS Caliber flow cytometer equipped with a 488-nm argon ion laser (BD Instrument Systems, Mississauga, ON, Canada). Primitive progenitor cells (Sca1<sup>+</sup>c-kit<sup>+</sup>) and lineage-committed VEPC (Sca1<sup>+</sup>c-kit<sup>+</sup>VEGF2R<sup>+</sup>) were enumerated using Cellquest software package.
Details of data analyses outlined in supplemental data (Figure S2). Absolute numbers of cells were calculated using the percentage of population positivity obtained from FACS analysis and the total white cell counts calculated after separation on Accu-Prep™.

Lung Histology and Morphometry

The left lung lobe was perfused with saline, formalin fixed, embedded in paraffin and cut in 3µm sections. Lung vascularity was identified by staining with polyclonal rabbit anti-human von Willenbrand factor (vWF) (Dako, Carpinteria, CA, USA) which cross-reacts with mouse antigen (22). Slides were analyzed using a customized digital image analyses system (Northern Eclipse; Empix Imaging, Mississauga ON Canada). Vessels within a 50µm bandwidth from the main airway and ≤10µm in diameter were included in microvessel quantification. Microvessel density (MVD) was calculated as total positive vessels per bandwidth area.

Lung sections were also stained with picrosirius red (PSR) for collagen deposition (Clone 1a4; Dako). Tissue was examined within a 20µm peribronchial bandwidth around the main airway and percent positive stain was analyzed by Northern Eclipse software.

Statistical Analysis

In the text and figure legends, data are presented as mean ± SEM. Data analysis was completed using STATISTICA software (Statsoft, Tulsa, OK, USA) to perform ANOVAs; post-hoc analyses for between groups comparisons were performed using Duncan’s test. Alpha was set at 0.05.
RESULTS

Progenitor Cells: Early concurrent treatment with AMD3100 inhibited allergen-induced lung-homing of primitive and vascular endothelial progenitor cells

Using a chronic allergen exposure model in Balb/C mice, early hemopoietic progenitor cells (HSC; Sca1^+c-kit^+) and lineage-committed vascular endothelial progenitors (VEPC; Sca-1^+c-kit^+VEGF2R^+) were enumerated in BM and lung-extracted cells 24h post-challenge with allergen (OVA) or saline (SAL) (Figure 2). For both HSC and VEPC levels, compared to SAL, there was a significant decrease in the BM and a corresponding increase in the lung in the OVA group (Figure 2A & B, Figure 2C & D, respectively).

Intranasal treatment with AMD3100 given concurrently with allergen challenge caused a reduction in BM HSC compared to OVA and SAL groups, respectively, 24hrs post-allergen (Figure 2A). In the lung, concurrent treatment with AMD3100 significantly attenuated the lung-homing of HSC compared to OVA group (Figure 2B). Although the drug did not cause a significant mobilization of VEPC from the BM levels compared to the OVA group (Figure 2C), there was a significant attenuation in the lung-homing of VEPC compared to the OVA group (Figure 2D).

To confirm a selective effect of AMD3100 on progenitor cells, total cell counts of the MNC population were enumerated from BM and lung-extracted cell samples. The cell counts showed that in the treatment group, the drug did not significantly lower total cell numbers compared to OVA group in either of the compartments (Supplemental data; Table S1). In addition, the effect of AMD3100 on the directional migrational responses of mouse BM derived HSC was demonstrated in vitro. The results show that AMD3100 selectively attenuated the directional migration of HSC to SDF-1 but not VEGF, in vitro (Supplemental data; Table S2).
**Airway Remodeling:** concurrent treatment with AMD3100 significantly attenuated allergen-induced airway angiogenesis but not collagen deposition

Formation of new blood vessels was assessed by staining for vWF in mouse lung slices (Figure 3A). Enumeration of microvessel density (MVD) showed a significant increase in OVA compared to SAL group, 24hrs post-allergen. Concurrent treatment with AMD3100 significantly attenuated MVD levels when compared to the OVA but were not completely reversed as they remained significantly higher than SAL.

Collagen deposition, as a marker of airway fibrosis, was detected by staining with picrosirius red (PSR) (Figure 3B). The level of collagen deposition was assessed at 4wks post challenge as this has previously been show to be the optimal time point for detecting allergen-induced airway fibrosis (16). (The cellular inflammation at earlier time points interferes with the accurate immunostaining for fibrosis). In the OVA group, collagen deposition was significantly elevated when compared to SAL. Concurrent drug treatment had no effect on collagen levels compared to the OVA group and these levels were significantly greater than SAL (Figure 3B).

**Airway Inflammation:** Concurrent treatment with AMD3100 significantly attenuated allergen-induced airway inflammation

There was a significant increase in inflammatory cells in the BAL including eosinophils in the OVA compared to SAL group, 24hrs post-allergen challenge (Table 1). Mice treated concurrently with AMD3100 showed a significant attenuation in total cells including neutrophils, eosinophils and macrophages compared to the OVA group (Table 1).
Airway Responsiveness: *Concurrent treatment with AMD3100 significantly attenuated allergen-induced airway hyperresponsiveness*

Airway responsiveness was assessed by measuring the maximum respiratory system resistance to incremental doses of intravenous methacholine (MCh) (Figure 4A). A significant increase in maximum resistance to MCh was observed 24hrs post-allergen in the OVA compared to SAL group. In the drug treatment group there was a significant attenuation in resistance compared to OVA group (Figure 4B).

**Reversal Treatment Regimen**

We studied the effect of modulating progenitor cell activity in a model where indices of allergic airway disease were already established. The effective of modulating progenitor cell activity during a 4 week period following 90 days of allergen challenge, at which time point it has previously been shown that despite the subsidence of inflammation i.e. airway eosinophilia, airway remodeling and AHR persist (16;23) was investigated.

**Progenitor Cells: Delayed treatment with AMD3100 inhibited allergen-induced lung-homing of primitive progenitor cells**

Following the reversal treatment regimen with AMD3100 (Figure 1B), HSC and VEPC were enumerated 4 weeks post-challenge (Figure 5). Within the BM, compared to SAL, the absolute numbers of HSC were significantly lower in the in the OVA group and drug treatment did not further reduce these numbers (Figure 5A). Within the lung, HSC numbers remained higher in the OVA compared to SAL group 4 weeks post-allergen and delayed treatment with AMD3100 significantly reduced these levels compared to untreated OVA (Figure 5B).
Conversely, in the VEPC populations in both the BM and the lung compared to SAL there was no significant difference in the OVA group and treatment with AMD3100 during the 4 weeks post challenge did not affect these levels (Figure 5C & D).

**Tissue Remodeling and Airway Function:** *Delayed treatment with AMD3100 significantly attenuated allergen-induced airway angiogenesis (delete “and AHR”)*

Significantly greater levels of vWF staining were found in the OVA compared to SAL groups 4 weeks after allergen challenge (Figure 6A) and drug treatment significantly reduced MVD levels compared to OVA group.

Collagen deposition in the OVA exposed mice was significantly greater than in the SAL group, 4 weeks post challenge. Drug treatment had no effect on the fibrosis levels compared to the OVA group and these levels remained significantly greater than SAL group.

Corresponding to primitive progenitor cell numbers in the lung and measures of lung vascularity, there was a sustained increase in airway resistance in the OVA group 4wks after allergen challenge (Figure 6C). Delayed treatment with AMD3100 reduced methacholine airway resistance to levels comparable with SAL group but not significantly lower than the OVA group (p=0.054).

**BAL VEGF levels:** *Allergen-induced Increases in BAL VEGF levels were not affected by treatment with AMD3100*

A significant increase in VEGF levels were detected in OVA compared to SAL group 24h post Ag- challenge and drug treatment had no effect (Table 2). In the reversal treatment group, BAL VEGF levels measured 4 weeks after allergen challenge in the OVA group and drug treatment groups had returned to SAL levels (Table 2).
DISCUSSION

Asthma has a systemic component involving the mobilization and lung-homing of BM derived progenitors that may contribute to inflammation and tissue remodeling including angiogenesis (4;15). We hypothesize that modulating lung homing of VEPC can prevent the increased bronchial vascularization and in turn modulate lung function in a mouse model of asthma. Our data show that (a) in allergic inflammatory responses both primitive and lineage committed progenitors migrate from the BM to the lung, (b) modulating progenitor cell accumulation (delete “traffic”) during the early onset of disease prevents increased vascularization of airway tissue and the development of AHR, and (c) in an established disease, treatment with AMD 3100 attenuated HSC levels in the lung and airway vascularity and only partially resolved sustained AHR.

Our data showed a rapid and sustained allergen-induced lung-homing of VEPC which correlated with the level of lung vascularity ($r^2=0.46$, $p<0.005$) and airway resistance ($r^2=0.71$, $p<0.005$) in a mouse model of asthma, in agreement with previous studies (14;24). Lung-extracted VEPC can differentiate into endothelial cells with the capacity to form tube like structures resembling vasculature (25;26). In this study, levels of lung-extracted VEPC were elevated in the lungs of allergen challenged mice, at 24h post OVA but not 4 weeks post OVA (Fig 2D & 5D) whilst primitive progenitor cells and vascularity were increased 24 hours post OVA (Figure 2B & 3A) and remained elevated up to 4 weeks post OVA compared to SAL (Figures 5B and 6A). This suggests that homing and rapid terminal differentiation of lung-extracted VEPC cells is limited to the early onset of the disease and during an active inflammatory process. The sustained increase in lung levels of HSC suggest that these cells maybe involved in both, maintaining established tissue remodeling and contributing to a priming
mechanism for future inflammatory responses (27). Studies of tumor vasculature have also shown that whilst BM-derived VEPC contribute to the early angiogenic switch, once the cancer is established the traffic and migration of these cells is limited and that extramedullary processes perpetuate the vascularization of the tumor (25;28).

Recent studies have shown that despite the capacity of lung-extracted VEPC to form tube like structures in culture, few GFP labeled VEPC actually incorporate into the vascular structures within the lung following allergen challenge in sensitized mice (14;25;29). Instead, these progenitors home to perivascular sites and secrete pro-inflammatory and pro-angiogenic factors that have the potential to orchestrate the inflammatory response and tissue remodeling changes associated with asthma (24;30). Regardless of the exact role of these cells, our study shows that that modulation of lung-homing of BM-derived progenitor cells during disease onset attenuated lung vascularity, and the subsequent development of airway inflammation and AHR (2).

In this study, AMD3100 was administered i.n. to focus its effect on the lung. Despite the localized delivery, AMD3100 had a systemic effect, seen as increased mobilization of primitive progenitors from the BM likely due to drug-mediated destabilization of the SDF-1/CXCR4 axis within the stroma (31). Analyses of the target organ however, showed that AMD3100 given by the concurrent treatment regimen inhibited the lung-homing of both HSC and VEPC in response to allergen challenge. Treatment with AMD3100 also significantly prevented airway angiogenesis, BAL eosinophilia and AHR; supporting the proposal that by preventing progenitor cell lung-homing, precursors for new blood vessels were attenuated and thus increased lung vascularization was inhibited. Reduced vascular activity could inturn attenuate oedema formation and the resultant airway thickening and could be manifest as a decrease in airway resistance (32).
In the reversal treatment regimen, AMD3100 showed a reduction in the total lung-extracted HSC only and an associated reversal in the level of airway vascularization and sustained AHR. It is likely that longer duration of treatment and higher doses of AMD3100 may have attenuated all the above outcomes to a greater extent. Interestingly, since these results were taken once the inflammation (airway eosinophilia) had subsided, these data indicate that the ability of AMD 3100 to decrease angiogenesis and AHR may be independent of its anti-inflammatory properties.

A second receptor for SDF-1, CXCR7, has recently been discovered (33) and although signaling through this new receptor is still controversial, recent studies suggest that decoy and scavenger activity of CXCR7 might be important for the fine tuning of the mobility of progenitors in the bone marrow and in the lymphoid organs (34). The scavenger activity of CXCR7 towards SDF-1 is thought to generate guidance cues for CXCR4-dependent migration. In the adult mammal CXCR7 appears to be strongly expressed in many tumours being seen both on the emerging neovasculature and tumour cells where it appears to function by blocking apoptosis and thus allowing cellular proliferation (35). Although the current study did not target this second receptor for SDF-1, the contribution of this receptor to SDF-1 mediated VEPC lung accumulation and angiogenesis following allergen challenge may explain why the delayed treatment with AMD3100 did not completely reverse the level of lung extracted HSC, tissue vascularity or sustained AHR.

Despite an attenuation of progenitor cell influx and angiogenesis, collagen deposition remained unchanged. Since fibrocytes express CXCR4+ (36) it was postulated that treatment with AMD3100 would attenuate airway fibrosis. It is possible that other compensatory mechanism may have responded to allergen challenge despite the ability of AMD3100 to
potentially prevent lung-homing of fibrocytes, although this was not measured in the current study. The activation of local fibroblasts to differentiate into myofibroblasts or the trans-differentiation of epithelial cells may have contributed to increased collagen deposition in the absence of a systemic component (8). A multi-pronged approach to treating tissue remodeling that reverses collagen deposition may be an additional therapeutic target for the optimal treatment of airway dysfunction in asthma.

VEGF is a potent angiogenic factors found in BAL of asthmatics and capable of stimulating increase lung neoangiogenesis through local over expression (37). In the OVA group, increased levels of BAL VEGF were detected 24h post challenge which returned to saline levels in the following 4 weeks post challenge. This is contrary to the level of MVD which was increased 24h post challenge in the OVA group and was maintained even 4 weeks post challenge. It has been proposed that a balance between pro- and anti-angiogenic factors control the local level of angiogenesis. As anti-angiogenic factors such as angiopoietin were not measured in this study, it remains unclear as to whether an imbalance between the pro- and anti-angiogenic factors permitted lower levels of VEGF to maintain increased vascularization of the lung tissue.

In summary, we report that during disease onset, modulating progenitor cell accumulation in (delete “trafficking to”) the lung prevents airway angiogenesis and the development of AHR. Further, in established disease, this treatment reversed indices of airway angiogenesis but only partially resolved sustained AHR. These findings suggesting that during early onset of disease modulating progenitor cell homing to the lung may be a viable therapy for preventing the development of airway dysfunction but that in established disease this therapy alone may not be effective.
ACKNOWLEDGEMENTS

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Reference List


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FIGURE LEGENDS

Figure 1: Chronic sensitization and drug treatment protocol: Mice Balb/C mice were sensitized to ovalbumin (OVA; 80μg in 200μl Saline) (i.p) on day 1 and 11 and challenged i.n. (100μg OVA in 25μl Saline) on day 11. Mice were then got six-two-day i.n. OVA or saline (SAL) challenges through the 18-90 day period. Drug intervention protocols (A) concurrent or (B) reversal regimens. Administration of AMD3100 (15 mg/ Kg) or vehicle (IN) prior to OVA or SAL challenge occurred on days as indicated. Outcomes were made 24h (concurrent outcomes-A) or 4 weeks (reversal outcomes-B) post- final allergen exposure.

Figure 2A-D: Enumeration of primitive and VEPC from BM and lung-extracted cells by flow cytometry. Samples were taken 24hrs post-challenge: saline (SAL), allergen (OVA) or OVA+AMD3100 group (n=10 mice/group). * p<0.05 compared to SAL and # p<0.05 compared to OVA. Compared to SAL, a significant egress from BM and influx into the lungs was detected in the OVA group for all progenitors. Concurrent treatment with AMD3100 further mobilized progenitors from the BM but attenuated the lung homing of these cells.

Figure 3: (A) Microvessel density assessed by immunostaining for von Willenbrand factor was measured in lung slices taken 24h post-challenge and (B) collagen deposition in lung slices taken 4 weeks post challenge. Calibration bar (——) represents 50 μm * p <0.05 compared to SAL and # p < 0.05 compared to OVA, (n=10 mice/group). There was a significant increase in both MVD and collagen deposition in OVA group compared to SAL. Concurrent treatment with AMD3100 significantly attenuated MVD but not the level of collagen deposition compared to OVA group.
Figure 4: Airway responses were measured as (A) maximum resistance to intravenous methacholine (g/kg) in mice 24hrs post-challenge (n=10 mice/ group). * p <0.05 compared to SAL and # p < 0.05 compared to OVA. (B) In response to 100 (g/kg) methacholine i.v. there was a significant increase in AHR in OVA group compared to SAL and this was significantly attenuated by concurrent treatment with AMD3100.

Figure 5: Enumeration of progenitors in bone marrow and lung-extracted cell samples. Using the reversal treatment regimen (A), primitive (Sca1+c-Kit+) (B&C) and vascular endothelial progenitors (Sca1+c-Kit+VEGFR2+) (D&E) were measured by flow cytometry 4 weeks post-challenge. * p <0.05 compared to SAL and # p < 0.05 compared to OVA (n=10 mice/group).

Figure 6: Effect of modulating progenitor cell traffic on reversing established markers of airway remodeling: (A) Microvessel density, (B) Collagen deposition and (C) Airway resistance to methacholine (C). Outcomes were measured in mice from the reversal treatment regimen at 4 wks (d118)-post challenge in the saline (SAL), allergen (OVA) or OVA+AMD3100 (n=8-10 mice/ group). * p <0.05 compared to SAL and # p < 0.05 compared to OVA. Compared to SAL there was a sustained increase in AHR in the OVA group which was reduced to SAL levels following delayed treatment with AMD3100.
Figure 1

A

Preventative Treatment
AMD 3100 15mg/kg

B

Reversal Treatment
AMD 3100 15mg/kg

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A

Sca-1+ c-kit+ cells (Absolute no.)

B

Lung

C

Sca-1+ c-kit+VEGF2R+ cells (Absolute no.)

D

Lung

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Figure 2
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Figure 4

A

Maximal Resistance (cmH$_2$O/ml/s)

B

Maximal Resistance (cmH$_2$O/ml/s)

- SAL
- OVA
- AMD3100
Figure 5

Doyle et al.

A. BM Sca-1+ c-kit+ cells (Absolute no.)
- SAL
- OVA
- AMD3100

B. Lung Sca-1+ c-kit+ cells (Absolute no.)
- SAL
- OVA
- AMD3100

C. BM Sca-1+ c-kit+VEGF2R+ cells (Absolute no.)
- SAL
- OVA
- AMD3100

D. Lung Sca-1+ c-kit+VEGF2R+ cells (Absolute no.)
- SAL
- OVA
- AMD3100

Legend:
* p < 0.05 compared to SAL
# p < 0.05 compared to OVA

Note: BM = Bone Marrow

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Legend: Sca-1+ c-kit+ cells
SAL = Saline
OVA = Ovalbumin
AMD3100 = AMD3100

Legend: BM Sca-1+ c-kit+VEGF2R+ cells
SAL = Saline
OVA = Ovalbumin
AMD3100 = AMD3100
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Figure 6
Table 1: Airway Inflammation

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Table 1: Total and Differential cell counts in bronchoalveolar lavage samples (x10^2 deleted and added to table). TCC is total cell count. Cells were counted in BAL collected from mice (n=10/group) sensitized by the chronic exposure protocol with concurrent treatment of drug. Measurements were made at 24hrs post-: saline (SAL), ovalbumin challenge (OVA) or OVA+AMD3100. Data are presented as mean ± SEM.* indicates p <0.05 compared to SAL and # indicates p < 0.05 compared to OVA.
Table 2: Levels of VEGF were determined in bronchoalveolar lavage samples (n=10 mice per group) by ELISA at 24h post challenge in the concurrent (early) treatment group and 4 weeks post challenge in the reversal (delayed) treatment group. Data are presented as mean ± SEM pg/ml, * indicates p <0.05 compared to SAL (delete “and # indicates p < 0.05 compared to OVA”). Treatment with AMD3100 had no significant effect on VEGF levels compared to OVA group.

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<th>SAL</th>
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Table 2: BAL VEGF Levels