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Airway exposure to staphylococcal enterotoxin A potentiates allergen-induced bone marrow eosinophilia and trafficking to peripheral blood and airways

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Squebola-Cola DM, Mello GC, Pissinatti L, Schenka AA, Anhê GF, DeSouza IA, Condino-Neto A, Antunes E. Airway exposure to staphylococcal enterotoxin A potentiates allergen-induced bone marrow eosinophilia and trafficking to peripheral blood and airways. *Am J Physiol Lung Cell Mol Physiol* 304: L639–L645, 2013. First published March 8, 2013; doi:10.1152/ajplung.00025.2013.—Bone marrow (BM) eosinopoiesis is a common feature during allergen exposure in atopic individuals. Airway exposure to staphylococcal superantigens aggravates allergic airway disease and increases the output of BM eosinophils. However, the exact mechanisms regulating eosinophil mobilization and trafficking to the peripheral circulation and airways remain to be elucidated. Therefore, this study aimed to investigate the mechanisms determining the BM eosinopoiesis in allergic mice under exposure to staphylococcal enterotoxin A (SEA). Ovalbumin (OVA)-sensitized male BALB/C mice were intranasally exposed to SEA (1 µg), and at 4, 12, 24, and 48 h later animals were challenged with OVA (10 µg, twice a day). Measurement of IL-5, eotaxin, and granulocyte-macrophage colony-stimulating factor (GM-CSF) levels, flow cytometry for CCR3⁺, VLA4⁺, and CCR3⁺VLA4⁺, as well as adhesion assays to VCAM-1 were performed in BM. Prior airway exposure to SEA time dependently increased the BM eosinophil number in OVA-challenged mice. Eosinophils gradually disappear from peripheral blood, being recruited over time to the airways, where they achieve a maximal infiltration at 24 h. SEA exposure increased the levels of IL-5 and eotaxin (but not GM-CSF) in BM of OVA-challenged mice. Marked increases in CCR3⁺ and CCR3⁺VLA4⁺ expressions in BM eosinophils of OVA-challenged mice were observed, an effect largely reduced by prior exposure to SEA. Adhesion of BM eosinophils to VCAM-1 was increased in OVA-challenged mice, but prior SEA exposure abrogated this enhanced cell adhesion. Accumulation of BM eosinophils by airway SEA exposure takes place through IL-5- and CCR3-dependent mechanisms, along with downregulation of CCR3/VLA4 and impaired cell adhesion to VCAM-1.

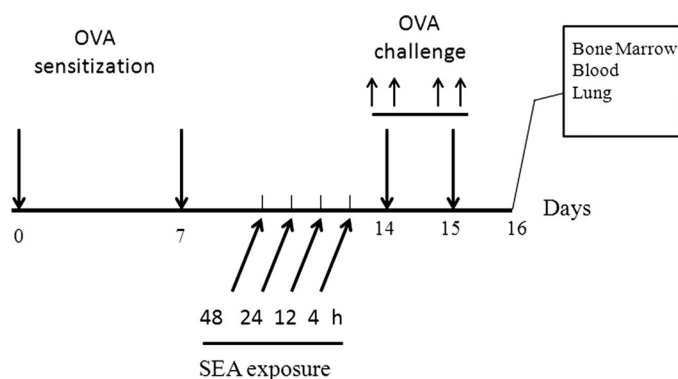
ovalbumin; eotaxin; IL-5; CCR3; VLA4; VCAM-1; asthma

SUPERANTIGENS ARE DERIVED from staphylococcal species and other bacteria (11). Staphylococcal superantigens bind to major histocompatibility complex class-II molecules without prior antigen processing by antigen-presenting cells, promoting im-

munostimulation (11). Clinical studies show that *Staphylococcus aureus* and its enterotoxins have been associated with aggravation of allergic diseases (1, 2, 4, 23, 42), including dermatitis, chronic rhinosinusitis, and asthma (26, 36, 42). Airway exposure to staphylococcal enterotoxin types A (SEA) and B (SEB) aggravates the pulmonary allergic inflammation, increases sensitization to inhaled allergens, and decreases T cell sensibility to steroids (10, 15). In rodent models of allergic diseases, airway exposure to SEA and SEB promotes leukocyte recruitment to the lung tissue by multiple inflammatory mechanisms (7, 8), with the potential involvement of mast cells and sensory fibers (27). Repeated intranasal application of SEB in mice causes a lymphocyte-dependent bronchial inflammation associated with airway hyperresponsiveness (17). Intranasal administration of SEA (29) and SEB (16, 18) also exacerbates the pulmonary eosinophilic inflammation in ovalbumin (OVA)-induced allergic airway disease. Interestingly, the pulmonary cell infiltration after instillation of SEA and SEB is accompanied by a late increase in bone marrow (BM) granulocyte number (7, 8, 29).

Allergen exposure in atopic subjects promotes distinct phenotypic changes in BM progenitor cell population, leading to eosinopoiesis (20, 21, 37). This phenomenon requires IL-5 to stimulate the eosinophil development, providing proliferative and differentiation signals, as well as its release to peripheral circulation (3, 5, 14, 33). The CC-chemokine eotaxin in synergism with IL-5 stimulates the release of BM eosinophil progenitors (6, 41, 44), producing blood eosinophilia in allergen-challenged lung (5). It has been suggested that eosinophilic progenitor cells may undergo in situ lung differentiation in the presence of locally elaborated cytokines, providing an ongoing source for other inflammatory effector cells (41). However, the exact mechanisms underlying the communication of allergic airway inflammation and BM eosinopoiesis have been poorly investigated (5). Additionally, no study exists examining the mechanisms that regulate BM eosinophil mobilization and trafficking to the peripheral circulation in allergic conditions under airway exposure to staphylococcal superantigens. Therefore, this study was undertaken to investigate the mechanisms of BM eosinophil accumulation and cell trafficking to peripheral blood and lung tissue in allergic mice undergoing airway exposure to SEA. In the BM environment, we have further explored the contributions of IL-5, eotaxin, and

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Protocol

Sensitization	Pre-exposure	Challenge
OVA	PBS	PBS
OVA	SEA (4 h)	PBS
OVA	SEA (12 h)	PBS
OVA	SEA (24 h)	PBS
OVA	SEA (48 h)	PBS
OVA	PBS	OVA
OVA	SEA (4 h)	OVA
OVA	SEA (12 h)	OVA
OVA	SEA (24 h)	OVA
OVA	SEA (48 h)	OVA

Fig. 1. Experimental protocols for airways sensitization and challenge with ovalbumin (OVA) in mice preexposed to staphylococcal enterotoxin A (SEA). BALB/C mice were sensitized with OVA on days 0 and 7, and challenged on days 14 and 15 (2 challenges each day). Control mice were instilled with PBS. At time periods of 4 to 48 h prior to the first OVA challenge, mice were intranasally instilled with SEA (1 μ g / 50 μ l). Bone marrow, blood, and lung were collected on day 16.

granulocyte-macrophage colony-stimulating factor (GM-CSF) as well as the adhesion molecules VLA4 and VCAM-1 to the BM eosinophil accumulation.

MATERIALS AND METHODS

Animals. All animal care and experimental protocols were approved by the Ethical Principles in Animal Research adopted by the Brazilian College for Animal Experimentation and followed the Guide for the Care and Use of Laboratory Animals. Four-week-old male BALB/c mice were provided by the Central Animal House Services of State University of Campinas. The animals were housed three per cage on a 12-h light-dark cycle in temperature-controlled rooms and received water and food ad libitum until used.

Sensitization procedure and OVA challenge. Mice were actively sensitized with a subcutaneous injection (0.4 ml) of 100 μ g of OVA (grade V) mixed with 1.6 mg Al(OH)₃ in 0.9% NaCl (day 0). One week later (day 7), mice received a second subcutaneous injection of 100 μ g OVA (0.4 ml) (29). At days 14 and 15, sensitized mice were intranasally challenged with OVA (10 μ g/50 μ l) twice a day, thus resulting in four challenges (at day 14, the first challenge occurred at time zero and the second challenge 6 h later; at day 15, the third challenge occurred at time zero and the fourth challenge 6 h later). At 48 h after the first challenge, the mice were anesthetized with isoflurane and exsanguinated, after which BM, circulating blood, and lung were obtained (28).

Airway exposure to SEA. OVA-sensitized mice were intranasally exposed to SEA (1 μ g/50 μ l) or sterile PBS buffer (control group). At 4, 12, 24, and 48 h after SEA exposure, animals were challenged with OVA or instilled with PBS. Thus our experimental protocols resulted in 10 experimental groups of six mice each, as detailed in Fig. 1.

Blood leukocytes. Blood samples were obtained from the abdominal vena cava and were allowed to clot for 30 min at 37°C. The total counts were done (Neubauer), and blood smears were used for the differential counts (Diff-Quick stain) in which a minimum of 300 cells were counted and classified as eosinophils on normal morphological criteria.

Histopathology. Lungs were removed and postfixed by immersion for at least 24 h with 10% buffered formalin, after which they were macroscopically examined and cut transversally into slices of ~3 mm. Only the middle thirds of the caudal aspects of both lungs were sent to embedding in paraffin. Sections of these portions, 4–5 μ m thick, were stained with hematoxylin-eosin and evaluated for bronchiolitis under a Nikon Eclipse E200 microscope adapted to a Nikon Coolpix 995 camera (3 Mpixel). For each animal, the extent of the lung infiltrate was determined by establishing the percentage of compromised bronchioli within 30 of such structures, randomly selected at low-power fields (i.e., with a $\times 4$ objective). In addition, with the $\times 40$ objective, 18 random digital images per group ($n = 6$) were taken within areas of overt peribronchiolar inflammation. Total inflammatory and eosinophil cell counts were determined from these images, using the Imagemag Analysis software (version 2.4) (IMAGELAB 2000; Softium, São Paulo, Brazil) and expressed as number of cells per square millimeter. Such quantification was focused on peribronchiolar areas, provided these regions were the main sites of inflam-

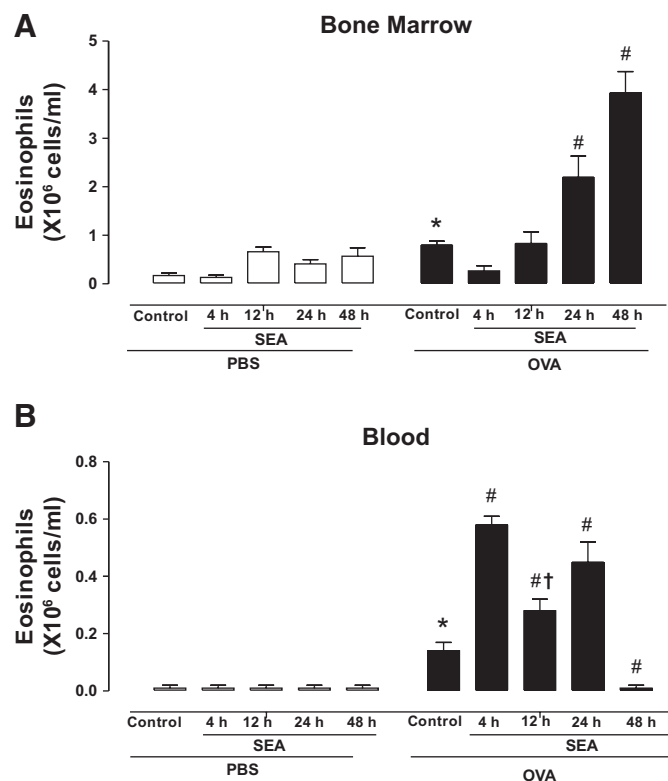


Fig. 2. Effect of airway preexposure to SEA on eosinophil number in bone marrow (A) and peripheral blood (B) of OVA-sensitized and challenged mice. Animals were exposed to SEA (1 μ g) at 4, 12, 24, and 48 h prior to OVA challenge. Control animals were instilled with phosphate-buffered saline (PBS; 50 μ l). Results are mean values $\pm$ SE from 6 mice for each group. * $P < 0.05$ compared with control values in PBS group; # $P < 0.05$ compared with control mice in OVA group; † $P < 0.05$ compared with SEA-OVA.

matory reaction. Parenchymal inflammation, represented by extension of the peribronchiolar infiltrates to alveoli, was mild/focal and only detected in few animals; thus parenchymal infiltrates were not assessed quantitatively.

BM preparation. The mice femurs were removed immediately after killing, and the epiphyses were cut transversely. BM cells were collected and subsequently placed in plates (100 mm $\times$ 20 mm dish) for 30 min (37°C, 5% CO₂) to allow the macrophages to adhere. The cell supernatants were collected, and the plates were washed again with 2 ml of RPMI 1640 medium. The supernatant was centrifuged (500 g for 10 min at 4°C), and the cell pellets were resuspended in 2.5 ml of RPMI. The total (Neubauer) and differential (Diff-Quick stain) cell counts were done. A minimum of 300 cells were counted. The cell number for each assay described below was adjusted to 4×10^6 cells/ml.

Eosinophil adhesion by measurement of eosinophil peroxidase activity. The adhesion assays were carried out in 96-well plates precoated with recombinant mouse VCAM-1 (2.5 μ g/ml) for 30 min at 37°C, 5% CO₂. After incubation, nonadherent cells were removed and the remaining cells were washed twice with PBS. Fifty microliters of varying concentrations of the original cell suspension in minimum essential medium were added to empty wells to form a standard curve. Eosinophil adhesion was calculated by measuring residual eosinophil

peroxidase activity of adherent cells. Fifty microliters of eosinophil peroxidase substrate (1 mM H₂O₂, 1 mM *o*-phenylenediamine and 0.1% Triton X-100 in Tris-buffer pH 8.0) were added to each well. After 30 min of incubation at room temperature, 25 μ l of 4 M H₂SO₄ were added to each well to stop the reaction and absorbance was measured at 490 nm with a microplate reader (Versamax, Molecular Devices, Sunnyvale, CA). Adherence was calculated by comparing absorbance of unknowns to that of the standard curve.

CCR3 and VLA4 surface content and colocalization in BM. To examine the surface content and colocalization of CCR3 and VLA4, 1×10^5 BM cells (100 μ l) were washed and preincubated for 20 min in PBS containing 1% BSA. Next, cells were washed with PBS and incubated with AlexaFluor 647-conjugated anti-mouse CCR3 and phycoerythrin (PE)-conjugated anti-mouse VLA-4 for 20 min at 4°C for subsequent analysis using a Becton-Dickinson FACSCalibur Cytometer (San Jose, CA) with CellQuest Pro software. CCR3 and VLA-4 fluorescent signals were acquired in the FL3 and FL2 channels, respectively. Prior to fluorescence acquisition, an independent group of cells was incubated with 5 μ l of both AlexaFluor-conjugated mouse IgG2a monoclonal isotype control and PE-conjugated mouse IgGk monoclonal isotype control to set autofluorescence in FL3 and FL2 channels, respectively. Surface content of CCR3 and VLA-4 were expressed by the mean fluorescence obtained from a histogram

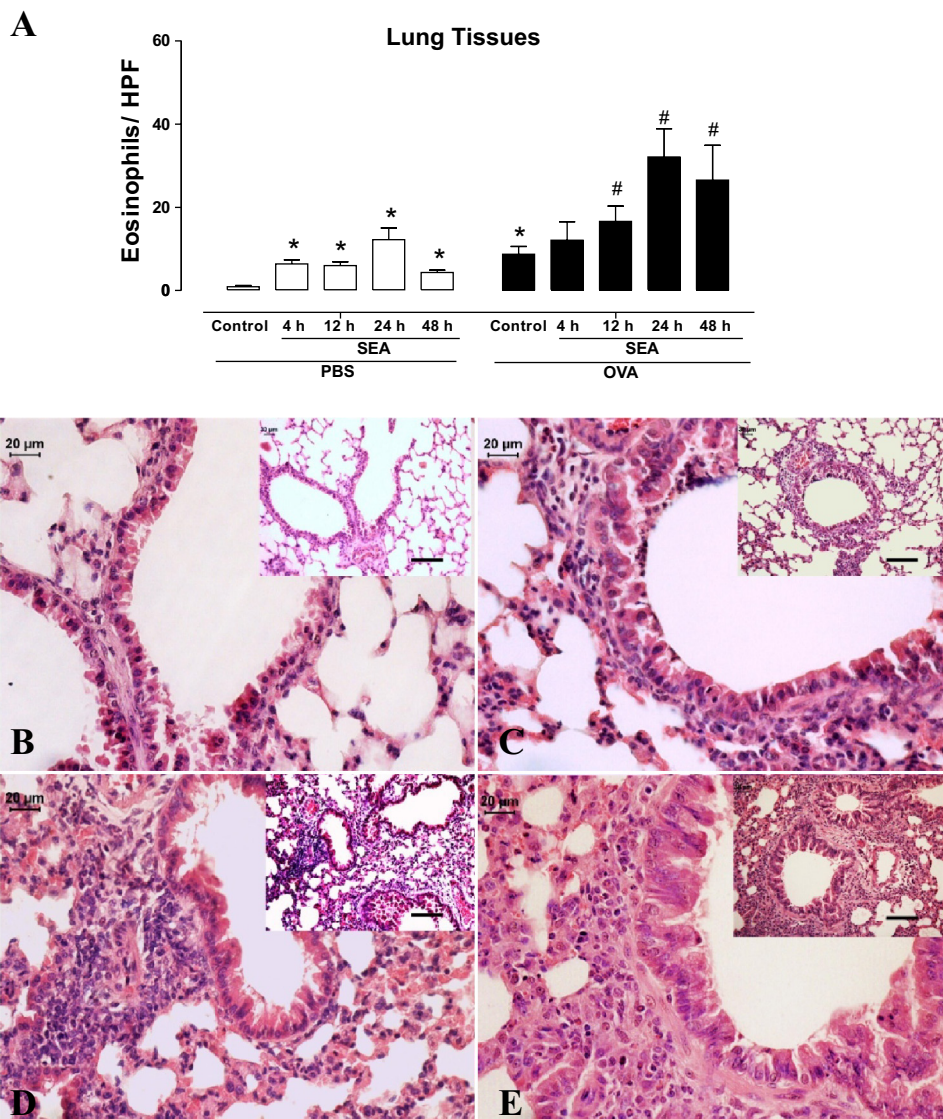


Fig. 3. Effects of prior SEA on the eosinophil infiltration in lung tissue surrounding the bronchial and bronchiolar segments of OVA-sensitized and challenged mice. A: number of eosinophils per higher power field (HPF) in PBS-instilled and OVA-challenged mice, previously exposed to SEA (1 μ g) for 4, 12, 24, and 48 h. Control animals were exposed to saline (50 μ l). Each column represents mean $\pm$ SE from 6 mice for each group. * P < 0.05 compared with control values in PBS group; # P < 0.05 compared with control mice in OVA group. B–E: representative images at 12 h from PBS+PBS (B), SEA+PBS (C), PBS+OVA (D), and SEA+OVA (E) groups. Note the marked peribronchiolar infiltrate in OVA-challenged mice preexposed to SEA (E). Hematoxylin and eosin; scale bars = 20 μ m (main panels) or 30 μ m (insets).

graph of a 10,000-event acquisition. CCR3 and VLA-4 colocalization was determined by the percentage of events localized in the upper quadrant of a dot-plot graph (FL2 vs. FL3) of a 10,000-event acquisition. FL2 and FL3 channels were compensated prior to double-staining analysis. FL2 compensation was performed by adjusting the region of AlexaFluor 647-stained cells out of the right quadrants. FL3 compensation was performed by adjusting the region of PE-stained cells out of the upper quadrants.

Measurement of BM cytokines. The cytokines IL-5, eotaxin, and GM-CSF were measured in BM cell supernatants. All measurements were carried out with commercially available ELISA kits (Mouse DuoSet ELISA Development System), following the instructions of the manufacturer (R&D, Minneapolis, MN).

Drugs. OVA grade V and SEA were purchased from Sigma Aldrich (St. Louis, MO). CCR3 (CD193) AlexaFluor 647 anti-mouse and VLA-4 (CD49d) PE anti-mouse were obtained from BD Biosciences Pharmingen (San Jose, CA).

Statistical analysis. Data were presented as the mean values $\pm$ SE and were analyzed by ANOVA for multiple comparisons followed by Bonferroni posttest, by using a program package for statistical analysis (GraphPad software, version 5.00; San Diego). Significance was defined by a P value of 0.05.

RESULTS

Airway exposure to SEA increases BM eosinophil number in OVA-challenged mice. In the control group, challenge with OVA significantly increased ($P < 0.05$) the BM eosinophil number, as expected (Fig. 2A). Prior airway exposure to SEA markedly enhanced ($P < 0.001$) the BM eosinophils in OVA-challenged mice, particularly at 24 and 48 h of preexposure (Fig. 2A). Exposure to SEA alone did not change the number of BM eosinophils.

Eosinophil counts in peripheral blood decays in OVA-challenged mice exposed to SEA. In peripheral blood, challenge with OVA in control mice significantly increased the number of eosinophils compared with PBS-instilled mice (control group; $P < 0.05$; Fig. 2B). Prior airway exposure to SEA markedly enhanced blood eosinophilia in OVA-challenged mice, particularly at 4 h ($P < 0.001$), decaying significantly at 12 h, but still greater than control mice in OVA group (Fig. 2B). At 24 h of SEA exposure in the OVA challenged group, blood eosinophilia significantly increased compared with 12 h, and it disappeared after 48 h of SEA preexposure, a time by which blood eosinophil counts did not differ from control group in PBS-instilled mice (Fig. 2B). Exposure to SEA alone did not change the number of peripheral blood eosinophils compared with respective control group.

Airway exposure to SEA promotes time-dependent eosinophil infiltration into the lung tissue of OVA-challenged mice. Histological examination of the lungs from PBS-instilled mice showed normal tissue, with no significant amount of inflammatory cells throughout the lung. However, OVA challenge itself produced a significant influx of eosinophils into the connective tissue surrounding the bronchial and bronchiolar segments compared with PBS-instilled mice (Fig. 3). Exposure to SEA alone also promoted an infiltration of eosinophils in the lung tissue that peaked at 24 h of exposure. Prior airway exposure to SEA in OVA-challenged mice markedly enhanced the eosinophil recruitment in the lung tissue with maximal infiltration at 24 h, remaining elevated at 48 h of preexposure (Fig. 3). For the other experiments, the time points of 12 and 24 h of preexposure to SEA were routinely used.

Levels of IL-5, eotaxin, and GM-CSF in BM of OVA-challenged mice. Figure 4 shows levels of IL-5, eotaxin, and GM-CSF in supernatants of BM eosinophils after PBS instillation or OVA challenge, in mice preexposed or not with SEA. Airways preexposure to SEA alone (12 h) did not significantly change the levels of IL-5 and eotaxin. Similarly, in OVA-challenged mice, no significant increases in the levels of IL-5 and eotaxin were observed, compared with PBS-instilled mice ($P < 0.05$). However, SEA preexposure markedly increased the levels of IL-5 and eotaxin in the OVA-challenged mice. The levels of GM-CSF remained unchanged in all experimental groups (Fig. 4).

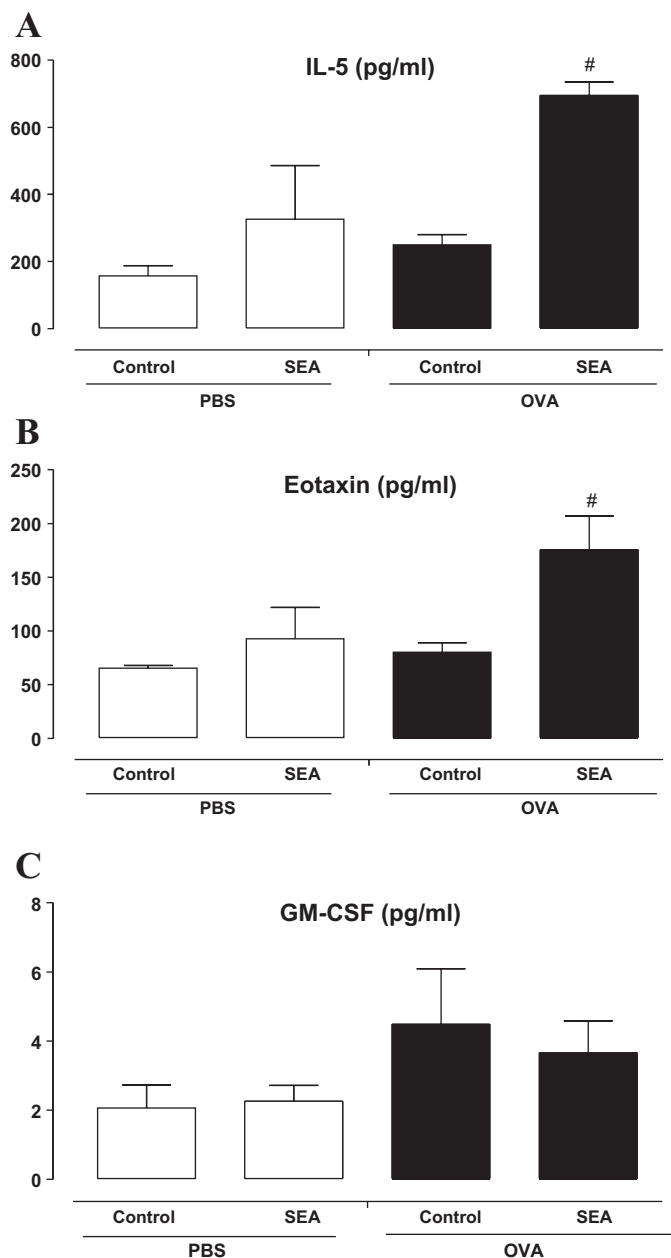


Fig. 4. Effects of SEA preexposure (12 h) on the levels of IL-5 (A), eotaxin (B), and granulocyte-macrophage colony-stimulating factor (GM-CSF; C) in bone marrow cells of OVA-challenged mice. Control animals were instilled with PBS (50 μ l). Results are mean values $\pm$ SE from 6 mice for each group. $\#P < 0.05$ compared with control mice in OVA group.

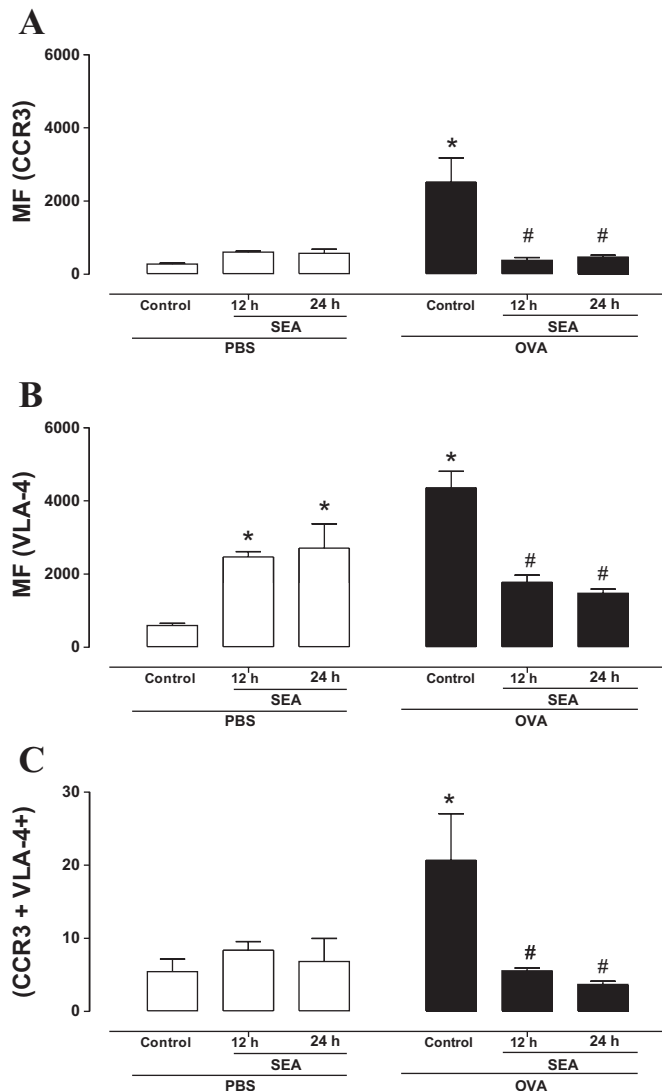


Fig. 5. Effect of airway preexposure to SEA on CCR3⁺ (A), VLA-4⁺ (B), and CCR3⁺/VLA-4⁺ (C) expression in bone marrow eosinophils of OVA-challenged mice. Animals were exposed to SEA (1 μ g) at 12 and 24 h prior to OVA challenge or PBS instillation. Eosinophils were gated for CCR3 population. Results are mean values $\pm$ SE from 6 mice for each group. * P < 0.05 compared with control value in PBS group; # P < 0.05 compared with control value in OVA group. MF, mean fluorescence.

Airway SEA exposure downregulates CCR3 and VLA-4 expressions in BM of OVA-challenged mice. Airway preexposure to SEA alone did not significantly affect the expression of CCR3⁺, as detected at 12 and 24 h of preexposure (Fig. 5A). In OVA-challenged mice, a marked increase in the expression of CCR3⁺ was observed, compared with PBS-instilled mice (P < 0.05). However, prior exposure to SEA suppressed the enhanced expression of CCR3⁺ in OVA-challenged mice. Figure 5B shows that airway exposure to SEA alone (12 and 24 h) significantly enhanced (P < 0.05) the VLA-4⁺ expression. A marked increase in the expression of VLA4⁺ was also observed in the OVA-challenged group (P < 0.05). Prior exposure to SEA significantly attenuated the enhanced VLA4⁺ expression in OVA-challenged mice. Airway exposure to SEA alone (12 and 24 h) did not significantly affect the expression of CCR3⁺VLA-4⁺ (Fig. 5C). In OVA-challenged mice, a

marked increase in the expression of CCR3⁺VLA-4⁺ was observed, compared with PBS-instilled mice (P < 0.05). Prior exposure to SEA suppressed the enhanced expression of CCR3⁺VLA-4⁺ in OVA-challenged mice.

SEA preexposure impairs adhesion of BM eosinophils to VCAM-1 in OVA-challenged mice. To determine whether airway SEA exposure influences the function of VLA-4 (CD49d/CD29) after OVA challenge, we investigated the adhesive properties of BM eosinophils using VCAM-1-coated plates. Preexposure to SEA alone had no effect in the cell adhesion (Fig. 6). A significant elevation of adhesion of BM eosinophils to VCAM-1 was observed in OVA-challenged mice compared with the PBS group. Prior exposure to SEA (12 and 24 h) abrogated the enhanced adhesion to VCAM-1 in OVA-challenged mice (Fig. 6).

DISCUSSION

In the present study, we have demonstrated that airway exposure to SEA in allergic mice time dependently (within 48 h) increases the BM eosinophil number that is accompanied by higher levels of IL-5 and eotaxin, downregulation of CCR3 and VLA-4 protein expression, and impaired cell adhesion to VCAM-1. Eosinophils gradually disappear from peripheral blood, being recruited over time to the airways, where they achieve a maximal infiltration at 24 h.

Eosinophils exert critical roles in allergic responses, and pulmonary allergen exposure increases the output of eosinophils from hemopoietic tissues and increased migration of these cells to the lung (37, 41). The eosinophil progenitors are currently defined as CD34⁺ cells that coexpress IL-5 receptor alpha (IL-5R α) on their surface (5, 19, 35, 40). IL-5 itself upregulates its receptor (IL-5R α) on CD34⁺ progenitor cells acting in an autocrine manner (13, 21, 30). Allergic stimulation elevates the serum levels of IL-5 that further enhances the expression of IL-5 receptor (IL-5R1), expanding to 10- to 20-fold (or even greater) the BM eosinophil progenitor, thus promoting their differentiation into mature cells for release into the peripheral blood (41). Furthermore, increased number of CD34⁺/IL-5R α ⁺ and CD34⁺ cells expressing the eotaxin receptor CCR3 (CD34⁺/CCR3⁺ cells) was observed in BM during allergen challenge in mice (9, 40, 39). Eotaxin is a CC-chemokine responsible for selective eosinophil chemotaxis and transendothelial migration in airways of allergic subjects

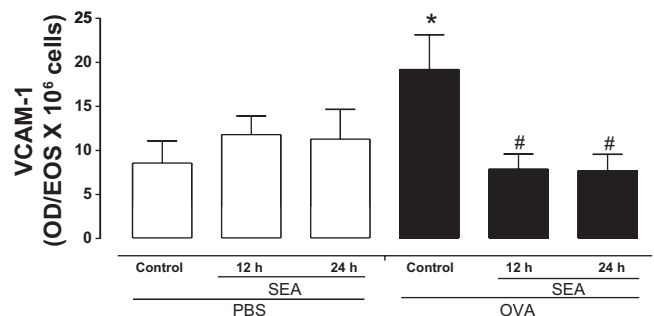


Fig. 6. Adhesion of bone marrow eosinophils to VCAM-1-coated plates. Eosinophils were obtained from bone marrow of mice preexposed to SEA (1 μ g) at 12 and 24 h prior to OVA challenge. Control animals were instilled with phosphate-buffered saline (PBS; 50 μ l). Results are mean values $\pm$ SE from 6 mice for each group. * P < 0.05 compared with control value in PBS group; # P < 0.05 compared with control value in OVA group.

(34, 39). CCR3 behaves as a functional receptor responsible for transducing the chemotactic responses after binding to eotaxin (12). Eotaxin increases the expression of IL-5R on CD34⁺45⁺ progenitor cells, priming these cells to respond to IL-5, which in turn may prime CD34⁺45⁺ progenitor cells to respond to eotaxin, presumably by the upregulation of CCR3 (23). Studies have shown that IL-5 and eotaxin are strong and specific stimuli for BM eosinophil differentiation (24, 25, 31).

In our study, OVA challenge significantly increased the BM eosinophil number that was markedly potentiated by SEA, particularly at later times of preexposure (24 to 48 h). Our findings that SEA concomitantly increases the levels of IL-5 and eotaxin in BM of OVA-challenged mice strongly suggest that the local eosinophil accumulation may be caused, at least in part, by local lineage commitment and subsequent differentiation of progenitor cell via IL-5 and eotaxin-dependent mechanisms acting in a synergistic fashion. Interestingly, the higher BM levels of IL-5 and eotaxin in mice preexposed to SEA and challenged with OVA were accompanied by downregulation of CCR3⁺ expression. This is consistent with a previous study showing downregulation of CCR3 by eotaxin in human eosinophils (38, 45). Moreover, CCR3 can be rapidly internalized into the early endosome compartment after eotaxin stimulation, leading to actin polymerization and cellular shape change (45).

The recruitment of BM eosinophils to blood and blood into tissue in allergic conditions requires a series of cell adhesion molecule-mediated interactions involving selectins, integrins, and molecules from the immunoglobulin gene superfamily. However, the mechanisms regulating the expression of the adhesion molecules are still a matter of debate. Selective activation of VLA-4 ($\alpha_4\beta_1$ integrin) by eotaxin has provided an efficient mechanism for recruitment of eosinophils into the lungs (22). VLA-4 mediates both the initial tethering, as well as the firm adhesion of eosinophils to the walls of inflamed vessels through interaction with VCAM-1 (5, 32, 43). It has been suggested that allergen-evoked integrin VLA-4 downregulation results in decreased BM progenitor cell adhesiveness, facilitating the egress to the peripheral blood (5). In OVA-sensitized mouse models, local intrapulmonary blockade of VLA-4 by intranasal administration of anti-CD49d monoclonal antibody inhibited all signs of lung inflammation, IL-5 release, and hyperresponsiveness to methacholine (32). Our data showed that OVA challenge markedly increased the BM expressions of CCR3⁺ and CCR3⁺VLA4⁺. Interestingly, SEA preexposure suppressed the enhanced CCR3⁺VLA4⁺ expression in OVA-challenge mice. Moreover, OVA challenge significantly increased the eosinophil adhesion to VCAM-1, an effect suppressed by prior exposure to SEA. Altogether, our findings are consistent with the suggestion that downregulation by SEA exposure of CCR3/VLA4 expressions and impaired adhesion of eosinophils to VCAM-1 in allergic mice contribute to eosinophil retention in BM, at least at prolonged periods of SEA exposure. Interestingly, SEA alone (with no OVA challenge) significantly increased the eosinophil influx into the lung section, as observed at 4 to 48 h preexposure. This is consistent with previous studies showing the presence of pulmonary inflammatory infiltrates in animals exposed to SEA alone (17, 29, 30). It is intriguing, however, that SEA exposure alone promotes eosinophil infiltration into the lung with no accompanying changes in BM and circulating blood. We may

not exclude that pulmonary cell infiltration by SEA takes place at earlier times prior to 4 h preexposure.

In BM, immunopathological correlations between GM-CSF and eosinophil activation have been reported (34, 35). In addition to its critical role in eosinophil survival, GM-CSF initiates a priming state whereby eosinophils become responsive to chemotactic, which may be accompanied by upregulation of adhesion molecules in cell surface (35). In our study, BM levels of GM-CSF remained unchanged in all experimental groups, excluding that this cytokine participates in the eosinophil accumulation in OVA-challenged mice previously exposed to SEA.

In conclusion, airway exposure to SEA produces a marked eosinophil recruitment to the lung tissue of allergic mice that is clearly detected at prolonged times of this enterotoxin exposure (24 and 48 h before OVA challenge). Eosinophils accumulate in BM at these same time periods of SEA exposure through IL-5- and CCR3-dependent mechanisms, along with downregulation of CCR3/VLA4 and impaired cell adhesion to VCAM-1. On the other hand, eosinophils time dependently disappear from circulating blood after SEA exposure. It is likely that alterations of BM function are an early step for the progression of asthmatic disease and exacerbation by staphylococcal enterotoxin. Understanding the mechanisms that regulate BM eosinophil mobilization and its trafficking to the peripheral circulation and airways in allergic animals may be important for the development of effective asthma therapies in conditions of staphylococcal superantigen exposure.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

D.M.S.-C. and E.A. conception and design of research; D.M.S.-C., G.C.M., L.P., and G.F.A. performed experiments; D.M.S.-C., G.C.M., L.P., A.A.S., G.F.A., I.A.D., and E.A. analyzed data; D.M.S.-C., L.P., A.A.S., G.F.A., I.A.D., A.C.-N., and E.A. interpreted results of experiments; D.M.S.-C. prepared figures; D.M.S.-C., A.C.-N., and E.A. drafted manuscript; A.C.-N. and E.A. edited and revised manuscript; E.A. approved final version of manuscript.

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