

CD4⁺ T cells migrate from airway to bone marrow after antigen inhalation in rats

Susumu Isogai, MD,^a Shigeru Miyata, MD,^a Rame Taha, MD,^a
Yasuyuki Yoshizawa, MD,^b James G. Martin, MD,^a and Qutayba Hamid, MD, PhD^a
Montreal, Quebec, Canada, and Tokyo, Japan

Background: IL-5-producing T lymphocytes increase in rat bone marrow after inhalational challenge with allergen.

Objective: To test the hypothesis that T cells migrate from the airways to the marrow, we examined the trafficking of T cells in Brown Norway rats after sensitization and challenge with ovalbumin.

Methods: Purified CD4⁺ T cells, harvested from cervical lymph nodes of naive and ovalbumin-sensitized donors, were labeled with carboxy fluorescein diacetate succinimidyl ester; 20 × 10⁶ cells were placed in the trachea of naive or sensitized recipients under anesthesia, and 18 hours later, animals were challenged with inhaled ovalbumin. Cells were harvested 24 hours later from the bone marrow, bronchoalveolar lavage fluid, lungs, the lung blood pool of cells, lung draining lymph nodes, peripheral blood, and spleen.

Results: The number of carboxy fluorescein diacetate succinimidyl ester-positive cells, measured by fluorescence-activated cell sorter, in the bone marrow of ovalbumin sensitized, primed T-cell recipients was higher than either the sham-sensitized, primed T-cell recipients or sham-sensitized, naive T-cell recipients (*P* < .05). The number of eosinophils in both bone marrow and bronchoalveolar lavage fluid was increased in ovalbumin-sensitized, primed T-cell recipients. The expression of the T-cell chemoattractants eotaxin and IL-16, evaluated by immunohistochemistry, was higher in the bone marrow of ovalbumin-sensitized, primed T-cell recipients.

Conclusions: CD4⁺ T cells travel from airway to bone marrow after antigen inhalation. The homing of the CD4⁺ T cells might be facilitated by eotaxin and IL-16 expression in the bone marrow and might contribute to the stimulation of eosinophilopoiesis after airway allergen exposure. (*J Allergy Clin Immunol* 2004;113:455-61.)

Key words: T-cell trafficking, CFSE, bone marrow, lung, eotaxin, interleukin-16, eosinophilia

Abbreviations used

BAL: Bronchoalveolar lavage
BM: Bone marrow
CFSE: Carboxy fluorescein diacetate succinimidyl ester
MBP: Major basic protein
MIP: Macrophage inflammatory protein
OVA: Ovalbumin

Asthma is characterized by chronic airway inflammation, often as a consequence of repeated allergic airway responses. T cells and eosinophils are prominent among the many cells that are present in the airways.¹ Eosinophils contribute to the pathophysiology of asthma through the release of pro-inflammatory cytokines and chemokines, as well as cytotoxic proteins, released during the degranulation process in the airways in asthma.² Airway hyperresponsiveness and airway narrowing are potentially caused by eosinophil products such as major basic protein (MBP), eosinophilic cationic protein, and cysteinyl leukotrienes.³⁻⁵ Allergen exposure is an important inducer/trigger of asthma, and eosinophils accumulate in the airway within a short time after allergen challenge.⁶ Vascular cell adhesion molecule-1⁷ and eotaxin-3,⁸ which are synthesized by activated endothelial cells as a result of their stimulation by IL-4, might promote the efflux of eosinophils from the vessels and their influx into the tissues of the airway. Lipid-derived mediators such as the cysteinyl-leukotrienes also participate in the recruitment process.⁹

Eosinophils rapidly increase in number in both the airway tissue and bone marrow (BM) after airway allergen challenge.¹⁰⁻¹² IL-5 has an important function in both of these phenomena through its support of the differentiation and proliferation of eosinophil progenitors in BM.¹³ The number of CD34⁺ progenitor cells expressing the IL-5 receptor in the BM is also increased in asthma.¹⁴ Although the airway is of importance as a source of IL-5 in persons with asthma,^{15,16} some investigators have postulated that other sources of IL-5 might be present. The number of CD3⁺ T cells expressing IL-5 mRNA is increased in BM after allergen challenge in mice¹⁷ and in humans,¹⁸ suggesting that these CD3⁺ T cells might contribute to eosinophil differentiation and maturation. The source of the increase in BM T cells after allergen challenge is still unclear. We hypothesized that these T cells

From ^aMeakins Christie Laboratories, Department of Medicine, McGill University, Montreal, Quebec, ^bDepartment of Pulmonary Medicine, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo

Supported by the Canadian Institutes of Health Research and GlaxoSmith-Kline Canada.

Received for publication July 15, 2003; revised November 5, 2003; accepted for publication November 13, 2003.

Reprint requests: Dr Qutayba Hamid, Meakins Christie Laboratories, Department of Medicine, McGill University, 3626 St Urbain, Montreal, Quebec, H2X 2P2, Canada.

0091-6749/\$30.00

© 2004 American Academy of Allergy, Asthma and Immunology

doi:10.1016/j.jaci.2003.11.035

migrate from the airways to the BM after allergen challenge. To test this hypothesis carboxy fluorescein diacetate succinimidyl ester (CFSE)-labeled CD4⁺ T cells were instilled into the tracheas of recipient rats that then underwent allergen challenge. After allergen challenge, these cells were tracked by taking samples from airways, entire lung tissues, the lung marginal pool of blood, lung draining lymph nodes, spleen, and BM. Samples were analyzed by flow cytometry. Our results provide evidence in support of the migration of CD4⁺ T cells from airway to BM after allergen challenge. As a mechanism of the CD4⁺ T-cell accumulation in the bone marrow, we explored the possibility that the increase in T cells in BM was caused by an increase in the expression of the T-cell chemoattractants, IL-16^{19,20} and eotaxin.^{21,22} We assessed the expression of IL-16 and eotaxin by specific immunohistochemical staining.

METHODS

Animals and sensitization

Inbred male Brown Norway rats (5 to 6 weeks; 140 to 190 g) were purchased from Harlan Sprague Dawley (Blackthorn, UK). The animals were maintained in conventional animal facilities at McGill University (Montreal, QC, Canada). The animals were sensitized by a single subcutaneous injection of 1.0 mL PBS, containing 1 mg ovalbumin (OVA) (grade V, Sigma, St Louis, Mo) with 4.28 mg aluminum hydroxide gel (Anachemia Chemicals, Montreal, QC, Canada) as adjuvant. Simultaneously, 0.5 mL of *Bordetella pertussis* vaccine (kindly provided by Dr T. Issekutz, Dalhousie University, Halifax, NS, Canada) containing 1×10^9 heat-killed bacilli was injected intraperitoneally.

Preparation of the transferred cells

Cells were recovered by mincing the cervical lymph nodes of donors that were sensitized to OVA 2 weeks before or were naive. Cells were passed first through a nylon mesh and after red blood cell lysis, CD4⁺ T cells were purified by negative selection with magnetic cell sorting (Miltenyi Biotec Inc, Auburn, Calif). For this purpose, lymph node cells were incubated with primary antibodies (OX-8, anti-CD8; OX-33, anti-B cell; ED9, anti-myeloid cell; 10/78, anti-NK cell) for 30 minutes at 4°C. After washing, cells were incubated with magnetic cell sorting beads (anti-mouse IgG1 microbeads; Miltenyi Biotec Inc) for 20 minutes at 4°C. Unlabeled cells that passed through the magnetic cell sorting column were collected. The harvested cells were counted by light microscopy. The purity of the cells was assessed by flow cytometry (FACScan, Becton Dickinson, Mountain View, Canada). Purified cells were labeled with 10 μ mol/L CFSE for 15 minutes at 37°C.

Study protocol

Donor rats were sensitized subcutaneously with OVA on day 0 or were naive. Recipients rats were also sensitized with OVA or sham sensitized with PBS on day 0. On day 12, CFSE-labeled CD4⁺ T cells that were obtained from the donor rats were placed in the trachea of a recipient under anesthesia. Eighteen hours later, all recipients were challenged, while awake, with aerosolized OVA (5% in PBS) for 10 minutes in a chamber. All animals were killed 24 hours after the challenge, and cells were harvested from BM, bronchoalveolar lavage (BAL) fluid, lung, marginal blood pool of the lung, mediastinal lymph nodes, peripheral blood, and spleen.

We studied 3 groups of animals. As a test group, CD4⁺ T cells were obtained from OVA-sensitized donors, and recipients were

sensitized and challenged with OVA (OVA-OVA/OVA group). To assess whether systemic sensitization of the recipient animal was needed for the migration of cells, CD4⁺ T cells from OVA-sensitized donors were injected also into sham (PBS)-sensitized recipients. Animals were challenged with OVA (OVA-PBS/OVA group). As a control, CD4⁺ T cells from naive donors were injected to sham-sensitized recipients and challenged with OVA as well (naive-PBS/OVA group).

Cell harvest from BAL, lung, marginal blood pool of the lung, draining lymph nodes, peripheral blood, spleen and BM

Twenty-four hours after the OVA challenge, animals were killed and blood was taken from the inferior vena cava and kept in a heparinized tube. For BAL, 25 mL of PBS was instilled in 5-mL increments. Lymphocytes harvested from the lung marginal blood refers to the cells contained in the vascular space. To collect these cells from within the lung, 500 U heparin diluted in 30 mL PBS was injected into the right ventricle, and blood was collected from a window in the anterior wall of the left ventricle. Lymph nodes in the posterior mediastinum, at the main carina of the trachea and adjacent to the thymus, were collected and pooled. Cells from the left lung, spleen, and both femoral bones were also collected.

BAL

Total BAL cell counts were determined with a hemacytometer. BAL cytospin slides were prepared by use of a Cytospin model II (Shandon, Pittsburgh, Pa). The slides were Wright-Giemsa stained, and the percentage of eosinophils was assessed on a count of 300 cells.

Cell preparation for flow cytometric analysis

The left lung was weighed and minced with scissors. Minced lung cells were incubated in 0.2% collagenase 4 in 5 mL HBSS for 60 minutes at 37°C. After washing with HBSS, cells were filtered by nylon mesh. After red blood cell lysis (PharM Lyse, BD Bioscience, Mississauga, ON, Canada), the number of cells was determined using an automated cell counter (A^C•T diff Analyzer, Coulter Corp, Miami, Fla). The leukocyte numbers in the peripheral blood and marginal blood in the lung were counted (A^C•T diff Analyzer). Approximately 2 million leukocytes were retained. After red blood cell lysis, cells were washed and resuspended in 1% paraformaldehyde. The leukocytes from draining lymph nodes and spleen were also obtained by mechanical mincing and passing through nylon mesh. Red blood cells in all samples were also removed by lysis. All cells were fixed with 1% paraformaldehyde before fluorescence-activated cell sorting analysis.

The detection of CFSE-positive cells by flow cytometry

Harvested cells were analyzed for CFSE with fluorescence-activated cell sorting analysis (FACScalibur, Becton Dickinson); 500,000 cells were counted for each sample. The total number of cells, excluding red blood cells and debris (R_{total}), and CFSE-positive cells (P_{CFSE}) in the lymphocyte gate (R_{lymph}) were measured. CFSE-positive cells were distinguished from autofluorescent cells. The total cell number of each sample, excluding red blood cells (C_{total}) was counted by automated cell counter (A^C•T diff Analyzer) or by hemacytometer. The calculation of the number of cells that migrated was as follows:

$$\text{The number of migrated cells} = C_{total} \times P_{CFSE}/R_{total}$$

In the case of peripheral blood, the number of positive cells in 1 mL blood was counted. In BM, the number of positive cells in both femora was counted.

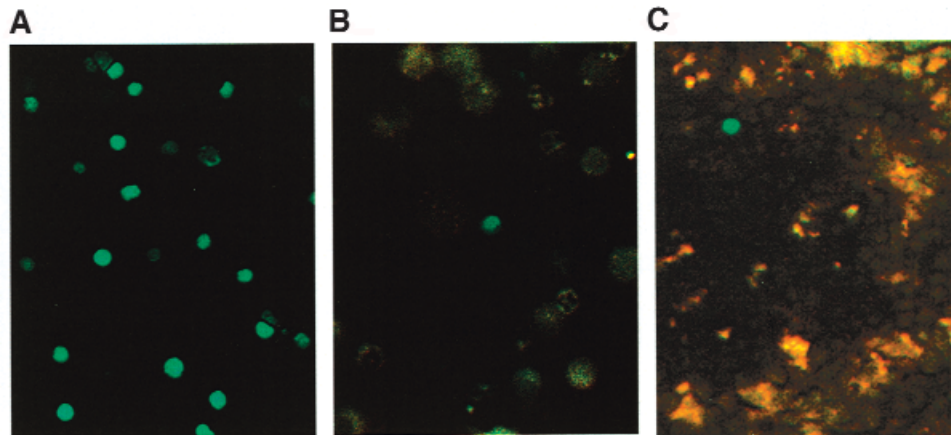


FIG 1. Fluorescence confocal microscopic images (magnification $\times 400$) showing representative examples of CFSE-stained CD4⁺ T cells before being placed in the trachea (**A**), CFSE-positive cells in the BAL (**B**) and in the spleen (**C**) 24 hours after OVA challenge of an OVA-sensitized rat.

CFSE-positive cells; detection by confocal microscopy

Sections of spleen and cytospin slides of BAL cells were prepared for fluorescent microscopic analysis. Slides were covered with PermaFluor mounting medium (Shandon, Pittsburgh, Pa) and evaluated at 660 $\times$ magnification with a fluorescence microscope (LSM410, Carl Zeiss, Jena, Germany).

Immunocytochemical evaluation of eosinophil-derived MBP expression, eotaxin, and IL-16 in BM

Cytospin slides of BM cells were fixed in acetone-methanol 60:40 and were stained with mouse anti-human MBP mAb (BMK-13) with the alkaline phosphatase anti-alkaline phosphatase method. Specimens were incubated with an unconjugated rabbit anti-mouse immunoglobulin secondary antibody (DAKO Corporation, Carpinteria, Calif), followed by incubation with the alkaline phosphatase anti-alkaline phosphatase mouse monoclonal complex (DAKO Corp). The slides were developed with an alkaline phosphatase substrate (naphthol phosphate, Sigma Chemical Co, St Louis, Mo) added to Fast Red (Sigma Chemical Co) as a chromogen and counterstained with hematoxylin.

Antibodies

Monoclonal antibody for eosinophil-derived MBP was provided by Dr R. Moqbel (University of Alberta, Edmonton, Canada). Polyclonal antibody against IL-16 (H-110, rabbit anti-human rat mouse polyclonal antibody, Santa Cruz Biotechnology, Santa Cruz, Calif) and polyclonal antibody against eotaxin (C-19, goat anti-human rat mouse polyclonal antibody, Santa Cruz Biotechnology) were used.

Statistical analysis

Data are presented as mean $\pm$ 1 standard error. Statistical comparisons were performed using 1-way ANOVA followed by Fisher least significant difference test. Data that were not normally distributed were analyzed by the Kruskal-Wallis and the Mann-Whitney *U* tests. *P* values $< .05$ were considered significant.

RESULTS

Sensitization and allergen challenge-enhanced CD4⁺ T-cell migration from airway to BM

The purity of the CD4⁺ T cells before their administration intratracheally was 99.4% by fluorescence-activated cell sorting analysis. CFSE-labeled CD4⁺ T cells before transfer are shown in Fig 1, A. Twenty-four hours after allergen challenge, CFSE-positive cells were still detectable in BAL fluid, and positive cells also appeared in the spleen. These findings on confocal microscopy are illustrated in Fig 1, B and C. The number of CFSE-positive cells within the lymphocyte cluster was quantified by flow cytometry. CFSE-positive cells were detected in BAL, tissues of the left lung, marginal blood pool of the lung, and mediastinal lymph nodes (Fig 2), but there were no significant differences in the numbers of these cells among the 3 groups of animals. In peripheral blood and spleen, there were also no significant differences among groups, although there was a trend for an increase in cells in the spleen in the OVA-OVA/OVA group (data not shown). However, the number of CFSE-positive cells in the BM in OVA-OVA/OVA animals was significantly increased compared with those of OVA-PBS/OVA group and naive-PBS/OVA group (Fig 2; *P* $< .005$), indicating BM recruitment of these cells after allergen challenge of sensitized animals that were also recipients of allergen-primed CD4⁺ cells.

The number of eosinophils in BM and BAL cells is increased in the actively sensitized and challenged group

The number of eosinophils in the BAL and BM was evaluated 24 hours after challenge. Because immature

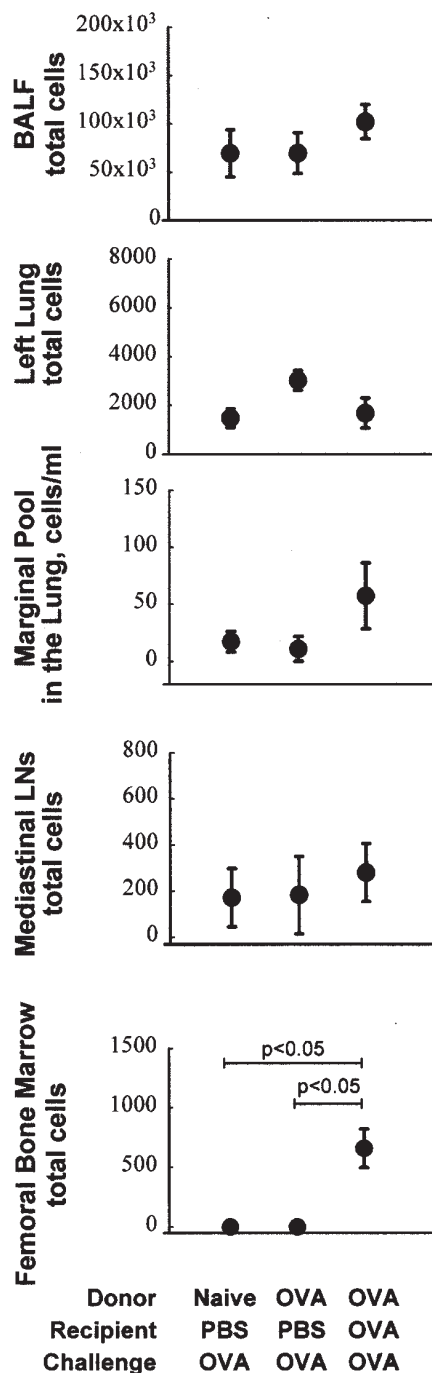


FIG 2. The number of CFSE-positive CD4⁺ T cells in BAL, left lung, marginal blood pool of the lungs, draining lymph nodes of the lungs, peripheral blood, and bone marrow compartments of recipients. Primed T-cell transferred, OVA-sensitized, OVA-challenged group (OVA-OVA/OVA), primed T-cell transferred, sham-sensitized, OVA-challenged group (OVA-PBS/OVA), and naive T-cell transferred, sham-sensitized, OVA-challenged group (naive-PBS/OVA) are shown. The means and standard errors are shown. Significant differences are indicated.

and mature eosinophils are mixed in bone marrow, the morphologic recognition of the eosinophil might be difficult. So we determined the number of eosinophils in the

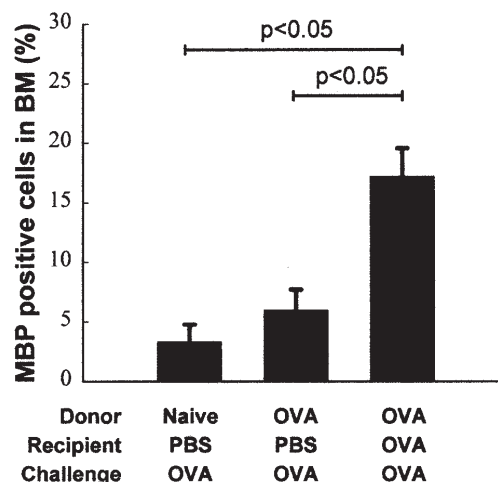


FIG 3. The number of MBP-positive cells in bone marrow was determined by immunocytochemistry. The means and standard errors are shown. Significant differences are indicated.

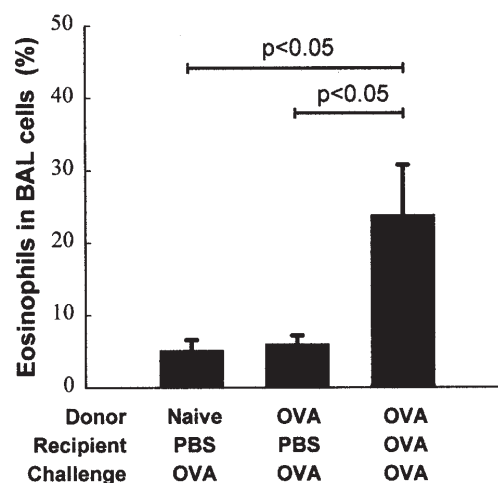


FIG 4. The number of eosinophils in BAL was evaluated by Wright-Giemsa staining. The means and standard errors are shown. Significant differences are indicated.

BM on the basis of MBP immunoreactivity. Representative MBP stains are shown in Fig 5, A and B. MBP-positive cells were increased in the OVA-OVA/OVA group compared with the naive-PBS/OVA and OVA-PBS/OVA groups (Fig 3; $P < .05$).

The BAL cell profile was evaluated with Wright-Giemsa staining. The total number of cells was not significantly different among naive-PBS/OVA, OVA-PBS/OVA, and OVA-OVA/OVA groups (6.9 ± 1.6 , 11.37 ± 3.37 , $11.36 \pm 1.47 \times 10^6$ cells, respectively). However, the OVA-OVA/OVA group had more eosinophils in the BAL fluid than did the OVA-PBS/OVA and naive-PBS/OVA groups (Fig 4; $P < .05$).

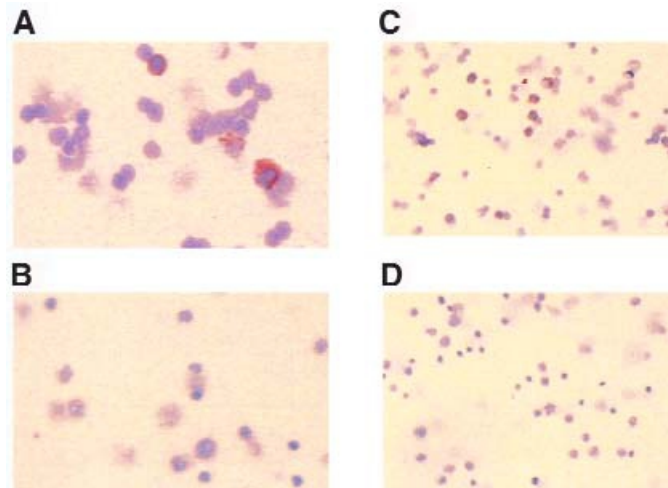


FIG 5. MBP-positive cells in bone marrow of OVA-sensitized, OVA-challenged recipients of primed T cells were evaluated by immunocytochemistry by means of the APAAP technique and Fast Red staining (A) and in PBS-sensitized and OVA-challenged recipients of primed T cells (B) (magnification $\times 400$). IL-16-positive cells in bone marrow of OVA-sensitized, OVA-challenged recipients of primed T cells (C) and in PBS-sensitized and OVA-challenged recipients of primed T cells (D) (magnification $\times 200$).

Eotaxin and IL-16-expressing cells are increased after active sensitization and challenge

The cells expressing the T-cell chemoattractants eotaxin and IL-16 in the BM were visualized by immunocytochemistry. Representative IL-16 stains are shown in Fig 5, C and D. The OVA-PBS/OVA group showed higher numbers of eotaxin-positive cells than did the naive-PBS/OVA group (Fig 6, A, $P < .05$). The OVA-OVA/OVA group also had a higher number of eotaxin-positive cells than the OVA-PBS/OVA group ($P < .05$). IL-16-positive cells were increased in the OVA-OVA/OVA group compared with the naive-PBS/OVA group ($t = 6$, B ; $P = .05$) but not compared with the OVA-PBS/OVA group.

DISCUSSION

Animals that are actively sensitized to ovalbumin and subsequently challenged by way of the airways show both an airway and BM eosinophilia. We were prompted to examine the distribution of CD4⁺ T cells placed in the bronchoalveolar compartment in various distal sites because of the reported appearance of IL-5-synthesizing T cells in the marrow.^{17,18} We placed fluorescently labeled purified CD4⁺ T cells in the tracheobronchial tree, and we examined different tissue compartments for evidence of these cells. The sites examined were the lung, marginal blood pool within the lung, lung draining lymph nodes, spleen, and femoral BM. We also studied the influence of allergen sensitization and challenge on the T-cell distributions. In the femoral marrow, the number of labeled CD4⁺ T cells was significantly higher in the animals that received cells harvested from sensitized animals and that were, themselves, actively OVA-sensitized and challenged. There was no increase in marrow T

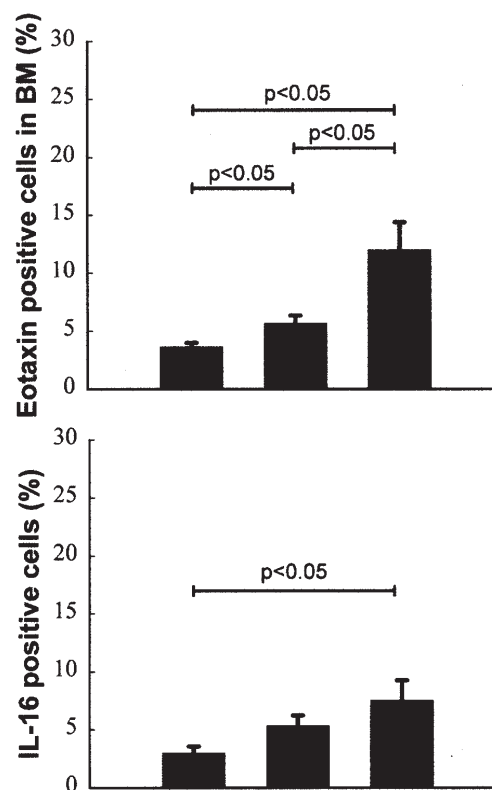


FIG 6. The expression of eotaxin (upper panel) and the expression of IL-16 (lower panel) in primed T-cell transferred, OVA-sensitized, OVA-challenged group (OVA-OVA/OVA), primed T-cell transferred, sham-sensitized, OVA-challenged group (OVA-PBS/OVA), and naive T-cell transferred, sham-sensitized, OVA-challenged group (naive-PBS/OVA).

cells when recipients were unsensitized. In contrast, we did not find any significant differences among the various other organs and tissues in the number of labeled

CD4⁺ T cells, whether actively sensitized or not. These results suggest a possible role for CD4⁺ T cells migrating from the airway to the marrow in promoting marrow response to allergen challenge.

Memory T cells and activated T cells have been shown to be preferentially distributed to nonlymphoid organs, such as the lung, after their injection to recipient animals.²³ However, whether they have any tendency to home to the BM does not seem to have been investigated. Our study suggests that such a mechanism exists, and the expression of the T-cell chemoattractants, eotaxin and IL-16, in the BM in association with active sensitization and challenge suggests that these chemokines might provide an explanation for CD4⁺ T-cell retention in the BM.

Brown Norway rats have been frequently used to model allergic asthma. These animals show early airway responses, late airway responses, and eosinophilia in the airways after active OVA sensitization and inhalation challenge.²⁴ Adoptive transfer has been used in the same rat strain to demonstrate that CD4⁺ T cells are sufficient for the transfer of allergen sensitivity to naive recipient rats.²⁵⁻²⁷ Intraperitoneally injected, 2×10^6 CD4⁺ T cells from sensitized animals transferred late airway responses and BAL fluid eosinophilia to naive recipients after the challenge²⁶ and 20×10^6 cells intravenously injected induced airway hyperresponsiveness.²⁷ Although in this study 20×10^6 cells were placed in the trachea, BAL fluid eosinophilia was not observed in the OVA-PBS/OVA group, indicating that this is not an optimal route for the delivery of T cells for the adoptive transfer of sensitivity to allergen. One reason might be a lesser efficiency of cell migration from the bronchoalveolar space into the airway tissues because of the epithelial barrier or the lack of chemotactic signals for this retrograde migration. Even though it has been shown that the T cells might migrate from the bronchoalveolar space into the bloodstream or airway tissues across the alveolar or airway epithelium,^{28,29} the number of cells that ultimately reaches the airway tissues might be small. In contrast, injected memory T cells have been shown to home to mucosal surfaces.²³ Another possibility is that the number of T cells might have been reduced by mucociliary clearance. Nonetheless, the number of CD4⁺ T cells placed in the trachea was sufficient to permit the detection of a significant increase of labeled cells in the BM in animals that were actively sensitized and allergen challenged. However, it seems likely that T cells within the airway tissues, after the process of antigen presentation, would migrate with greater ease to the marrow than those within the bronchoalveolar compartment, which have an additional tight epithelial barrier to cross.

We chose to perform the OVA challenge 18 hours after the instillation of CD4⁺ T cells into the trachea and to examine the distribution of cells after a further 24 hours. Although the choice of these times was somewhat arbitrary, we based this timing on studies showing that lymphocytes placed in bronchoalveolar space migrate and already appear in the draining lymph nodes 6 hours later.²⁹ We anticipated that time would be required for

these cells to circulate and to accumulate in certain sites if stimuli for such accumulation were present. In addition, BM eosinophilia is evident from 2 to 24 hours after challenge¹² and eosinophilia in BAL occurs as early as 2 hours and lasts at least 72 hours after allergen challenge.³⁰

Naive T cells traffic mostly through lymphoid tissue by crossing high endothelial venules, whereas memory T cells traffic preferentially through tissues and inflammatory sites and re-enter the lymph stream by way of the afferent lymphatics.³¹ The CFSE-positive cells in the lymph nodes draining the lung likely contained both naive and memory cells, and the total numbers of these cells were not different among groups. There were no significant differences among the groups in T cells in other organs and tissues, with the exception of the BM. This also might be because naive cells represent most CFSE-labeled T cells in the body, and there is no mechanism for the selective accumulation of these cells in the tissues. As a corollary, it is likely that the increase in BM cells is caused by the appearance of labeled effector or memory cells. The number of BM eosinophils was greater in the OVA-OVA/OVA group than the other groups. We speculate that the CFSE-labeled T cells that homed to the BM might have contributed, directly or indirectly, to eosinophil differentiation by expressing IL-5.^{17,18}

The mechanism of the increase in marrow CD4⁺ T cells might be caused by retention of the cells within the marrow as these cells come under the influence of chemotactic gradients as they circulate within the marrow. Many chemokines and cytokines have been reported as T cell chemoattractant factors and include IL-16,^{19,20} eotaxin,²¹ RANTES, macrophage inflammatory protein (MIP)-1 α , MIP-1 β , and monocyte chemoattractant protein-1.³² We limited our study to 2 of these chemokines, namely IL-16 and eotaxin. IL-16 is expressed by CD34⁺ cells,³³ and the number of CD34⁺ cells increases after OVA challenge in OVA-immunized mice.¹⁷ In our study, IL-16-expressing cells in the BM of the OVA-OVA/OVA group were increased over those of naive-PBS/OVA group. We postulate that IL-16 expressed by CD34⁺ cells after allergen challenge might contribute to the migration of CD4⁺ T cells into BM. IL-16 expressed by T cells themselves after their migration to the marrow is another potential mechanism that might contribute to their chemoattraction,³⁴ thereby providing a feed-forward mechanism for further T-cell recruitment to the marrow.

Eotaxin is an essential modulator of eosinophil trafficking.³⁵ However, eotaxin receptor CCR-3 is expressed not only on eosinophils³⁶⁻³⁹ but also T_H2 cells,²¹ and eotaxin can induce the recruitment of T_H2 cells.²² Other T-cell chemokines that have been reported as T-cell chemoattractant factors such as MIP-1 α , MIP-1 β and RANTES are also expressed by CD34⁺ T cells³³ and are also possible candidates. However, how these BM cells are stimulated after antigen challenge is still unclear.

We have shown that CD4⁺ T cells migrate from within the airway compartment to the BM after antigen inhalation. Cells harvested from sensitized donors and administered to sensitized and challenged recipients were most

likely to appear in the marrow, suggesting that antigen specificity, changes in the recipient animal after sensitization, and signals resulting from airway challenge are necessary for homing to the marrow to occur. Eotaxin and IL-16 expression in the bone marrow might contribute to the homing of the CD4⁺ T cells and the stimulation of eosinophilopoiesis after airway allergen exposure. The extent to which CD4⁺ T cells migrating from the airways to BM are important in signaling the BM after allergen challenge requires further study.

REFERENCES

- Walker C, Kaegi MK, Brauer P, Blaser K. Activated T cells and eosinophilia in bronchoalveolar lavages from subjects with asthma correlated with disease severity. *J Allergy Clin Immunol* 1991;88:935-42.
- Gleich GJ, Motojima S, Frigas E, Kephart GM, Fujisawa T, Kravis LP. The eosinophilic leukocyte and the pathology of fatal bronchial asthma: evidence for pathologic heterogeneity. *J Allergy Clin Immunol* 1987;80(3 Pt 2):412-5.
- Hamid Q, Song Y, Kotsimbos TC, Minshall E, Bai TR, Hegele RG, et al. Inflammation of small airways in asthma. *J Allergy Clin Immunol* 1997;100:44-51.
- Venge P, Bystrom J, Carlson M, Hakansson L, Karawaczyk M, Peterson C, et al. Eosinophil cationic protein (ECP): molecular and biological properties and the use of ECP as a marker of eosinophil activation in disease. *Clin Exp Allergy* 1999;29:1172-86.
- Weller PF. Cytokine regulation of eosinophil function. *Clin Immunol Immunopathol* 1992;62(1 Pt 2):S55-9.
- Metzger WJ, Richerson HB, Worden K, Monick M, Hunninghake GW. Bronchoalveolar lavage of allergic asthmatic patients following allergen bronchoprovocation. *Chest* 1986;89:477-83.
- Fukuda T, Fukushima Y, Numao T, Ando M, Arima M, Nakajima H, et al. Role of interleukin-4 and vascular cell adhesion molecule-1 in selective eosinophil migration into the airways in allergic asthma. *Am J Respir Cell Mol Biol* 1996;14:84-94.
- Cuvelier SL, Patel KD. Shear-dependent eosinophil transmigration on interleukin 4-stimulated endothelial cells: a role for endothelium-associated eotaxin-3. *J Exp Med* 2001;194:1699-709.
- Eum SY, Maghni K, Hamid Q, Campbell H, Eidelman DH, Martin JG. Involvement of the cysteinyl-leukotrienes in allergen-induced airway eosinophilia and hyperresponsiveness in the mouse. *Am J Respir Cell Mol Biol* 2003;28:25-32.
- Kung TT, Stelts D, Zurcher JA, Watnick AS, Jones H, Mauser PJ, et al. Mechanisms of allergic pulmonary eosinophilia in the mouse. *J Allergy Clin Immunol* 1994;94(6 Pt 2):1217-24.
- Lefort J, Bachelet CM, Leduc D, Vargaftig BB. Effect of antigen provocation of IL-5 transgenic mice on eosinophil mobilization and bronchial hyperresponsiveness. *J Allergy Clin Immunol* 1996;97:788-99.
- Gaspar Elsas MI, Joseph D, Elsas PX, Vargaftig BB. Rapid increase in bone-marrow eosinophil production and responses to eosinopoietic interleukins triggered by intranasal allergen challenge. *Am J Respir Cell Mol Biol* 1997;17:404-13.
- Yamaguchi Y, Suda T, Suda J, Eguchi M, Miura Y, Harada N, et al. Purified interleukin 5 supports the terminal differentiation and proliferation of murine eosinophilic precursors. *J Exp Med* 1988;167:43-56.
- Sehmi R, Wood LJ, Watson R, et al. Allergen-induced increases in IL-5 receptor alpha-subunit expression on bone marrow-derived CD34⁺ cells from asthmatic subjects. A novel marker of progenitor cell commitment towards eosinophilic differentiation. *J Clin Invest* 1997;100:2466-75.
- Hamid Q, Azzawi M, Ying S, Moqbel R, Wardlaw AJ, Corrigan CJ, et al. Interleukin-5 mRNA in mucosal bronchial biopsies from asthmatic subjects. *Int Arch Allergy Appl Immunol* 1991;94(1-4):169-70.
- Tang C, Rolland JM, Ward C, Quan B, Walters EH. Allergen-induced airway reactions in atopic asthmatics correlate with allergen-specific IL-5 response by BAL cells. *Respirology* 1997;2(1):45-55.
- Minshall EM, Schleimer R, Cameron L, Minnicozzi M, Egan RW, Gutierrez-Ramos JC, et al. Interleukin-5 expression in the bone marrow of sensitized Balb/c mice after allergen challenge. *Am J Respir Crit Care Med* 1998;158:951-7.
- Wood LJ, Sehmi R, Dorman S, Hamid Q, Tulic MK, Watson RM, et al. Allergen-induced increases in bone marrow T lymphocytes and interleukin-5 expression in subjects with asthma. *Am J Respir Crit Care Med* 2002;166:883-9.
- Center DM, Cruikshank W. Modulation of lymphocyte migration by human lymphokines. I. Identification and characterization of chemoattractant activity for lymphocytes from mitogen-stimulated mononuclear cells. *J Immunol* 1982;128:2563-8.
- Cruikshank W, Center DM. Modulation of lymphocyte migration by human lymphokines. II. Purification of a lymphotactic factor (LCF). *J Immunol* 1982;128:2569-74.
- Sallusto F, Mackay CR, Lanzavecchia A. Selective expression of the eotaxin receptor CCR3 by human T helper 2 cells. *Science* 1997;277:2005-7.
- Gerber BO, Zanni MP, Uguccioni M, Loetscher M, Mackay CR, Pichler WJ, et al. Functional expression of the eotaxin receptor CCR3 in T lymphocytes co-localizing with eosinophils. *Curr Biol* 1997;7:836-43.
- Tietz W, Hamann A. The migratory behavior of murine CD4⁺ cells of memory phenotype. *Eur J Immunol* 1997;27:2225-32.
- Renzi PM, Olivenstein R, Martin JG. Inflammatory cell populations in the airways and parenchyma after antigen challenge in the rat. *Am Rev Respir Dis* 1993;147:967-74.
- Watanabe A, Mishima H, Renzi PM, Xu LJ, Hamid Q, Martin JG. Transfer of allergic airway responses with antigen-primed CD4⁺ but not CD8⁺ T cells in brown Norway rats. *J Clin Invest* 1995;96:1303-10.
- Mishima H, Hojo M, Watanabe A, Hamid QA, Martin JG. CD4⁺ T cells can induce airway hyperresponsiveness to allergen challenge in the brown Norway rat. *Am J Respir Crit Care Med* 1998;158:1863-70.
- Haczku A, Macary P, Huang TJ, Tsukagoshi H, Barnes PJ, Kay AB, et al. Adoptive transfer of allergen-specific CD4⁺ T cells induces airway inflammation and hyperresponsiveness in brown-Norway rats. *Immunology* 1997;91:176-85.
- Pabst R, Binns RM. Lymphocytes migrate from the bronchoalveolar space to regional bronchial lymph nodes. *Am J Respir Crit Care Med* 1995;151(2 Pt 1):495-9.
- Lehmann C, Wilkening A, Leiber D, Markus A, Krug N, Pabst R, et al. Lymphocytes in the bronchoalveolar space reenter the lung tissue by means of the alveolar epithelium, migrate to regional lymph nodes, and subsequently rejoin the systemic immune system. *Anat Rec* 2001;264:229-36.
- Inman MD, Ellis R, Wattie J, Denburg JA, O'Byrne PM. Allergen-induced increase in airway responsiveness, airway eosinophilia, and bone-marrow eosinophil progenitors in mice. *Am J Respir Cell Mol Biol* 1999;21:473-9.
- Young AJ, Hay JB, Mackay CR. Lymphocyte recirculation and life span in vivo. *Curr Top Microbiol Immunol* 1993;184:161-73.
- Olson TS, Ley K. Chemokines and chemokine receptors in leukocyte trafficking. *Am J Physiol Regul Integr Comp Physiol* 2002;283:R7-28.
- Majka M, Janowska-Wieczorek A, Ratajczak J, Ehrenman K, Pietrzowski Z, Kowalska MA, et al. Numerous growth factors, cytokines, and chemokines are secreted by human CD34(+) cells, myeloblasts, erythroblasts, and megakaryoblasts and regulate normal hematopoiesis in an autocrine/paracrine manner. *Blood* 2001;97:3075-85.
- Krug N, Cruikshank WW, Tschernig T, Erpenbeck V, Balke K, Holfeld J, et al. Interleukin-16 and T-cell chemoattractant activity in bronchoalveolar lavage 24 hours after allergen challenge in asthma. *Am J Respir Crit Care Med* 2000;162:105-111.
- Rothenberg ME. Eotaxin. An essential mediator of eosinophil trafficking into mucosal tissues. *Am J Respir Cell Mol Biol* 1999;21:291-5.
- Combadiere C, Ahuja SK, Murphy PM. Cloning and functional expression of a human eosinophil CC chemokine receptor. *J Biol Chem* 1995;270:16491-4.
- Ponath PD, Qin S, Post TW, Wang J, Wu L, Gerard NP, et al. Molecular cloning and characterization of a human eotaxin receptor expressed selectively on eosinophils. *J Exp Med* 1996;183:2437-48.
- Daugherty BL, Siciliano SJ, DeMartino JA, Malkowitz L, Sirotna A, Springer MS. Cloning, expression, and characterization of the human eosinophil eotaxin receptor. *J Exp Med* 1996;183:2349-54.
- Zimmermann N, Bernstein JA, Rothenberg ME. Polymorphisms in the human CC chemokine receptor-3 gene. *Biochim Biophys Acta* 1998;1442(2-3):170-6.