

Recruitment of T cells to the lung in response to antigen challenge

Qutayba A. Hamid, MD, PhD, and Lisa A Cameron, PhD *Montreal, Quebec, Canada*

T-cell recruitment to the lungs is thought to represent a key step in airway allergic inflammation. T cells coordinate and amplify effector functions of antigen-specific and nonspecific proinflammatory cells, such as B cells and eosinophils. The T_H2 cell, in particular, promotes allergic inflammation through the expression of IL-4, IL-5, and IL-13, proinflammatory cytokines that are important in the induction of B-cell switching and the promotion of eosinophil proliferation and survival. This cytokine profile has been implicated in asthma; elevations in bronchoalveolar lavage IL-4 and IL-5 levels have been observed in asthmatic patients. The recruitment of T_H cells to the site of allergic inflammation (lung) is the subject of this review. (*J Allergy Clin Immunol* 2000;106:S227-34.)

Key words: Asthma, cytokines, recruitment, T cells

As key cells in the adaptive immune response, T cells coordinate and amplify the effector functions of antigen-specific and nonspecific inflammatory cells such as B cells and eosinophils. T cells have been divided into 2 distinct subsets according to effector function. The CD4 T cells, termed T_H cells, are involved in humoral immunity, whereas CD8 T cells are referred to as T-suppressor cells and drive the cell-mediated response. The CD4 T cells are particularly important in the regulation of antigen-driven inflammatory processes, although reports have indicated a possible role for the CD8 T cell as well.¹ Through antigen-specific T-cell receptors, CD4 T cells are capable of recognizing processed foreign antigen in association with major histocompatibility complex class II on specialized antigen-presenting cells, playing a pivotal role in initiating and sustaining chronic allergic inflammation. We focus on CD4 T-cell recruitment to the site of allergic inflammation in response to antigen challenge.

ROLE OF T CELLS IN ASTHMA

Evidence for the involvement of T cells in asthma came from early studies on postmortem lungs that linked the abnormality of the lung with the presence of chronic inflammatory infiltrate that consisted mostly of lymphocytes and eosinophils.² The use of fiberoptic bronchoscopy has allowed bronchial biopsy specimens and

Abbreviations used

BAL: Bronchoalveolar lavage
LAR: Late airway response

lavage fluid to be examined in patients with less severe asthma and normal subjects and the kinetics of cell recruitment in response to antigen challenge to be examined. The studies have shown that the T cell is the predominant cell in both normal and asthmatic airway mucosa, accounting for approximately 50% to 60%² of the cells present. In asthmatic subjects, the total number of inflammatory cells is elevated, with the eosinophil exhibiting the greatest percentage increase. However, the percentage of T cells is also enhanced, which translates into a significant increase in the absolute number of T cells, given the large number of these cells present at baseline.³ More recently, lung resection studies have also demonstrated an increase in the number of T cells and eosinophils in the airways of asthmatic subjects compared with nonasthmatic control subjects who were matched for age, sex, smoking history, lung function, and airway size.⁴ The presence of these inflammatory cells in larger airways is consistent with the increase observed in bronchoscopic biopsy specimens of asthmatic airways.^{5,6} We have recently reported a similar but more severe inflammatory process in the peripheral airways and in the airway wall external to the smooth muscle layer, both of which are not routinely biopsied with fiberoptic bronchoscopy.⁴

T-CELL RECRUITMENT TO THE LUNGS

There are a number of reports that provide evidence to support the concept that T cells are recruited to the airways in response to specific antigen challenge and that this recruitment may be inhibited by anti-inflammatory agents.^{7,8} In 1984, Gerblich et al⁷ showed that the number of CD4⁺ T cells was reduced in the peripheral blood of asthmatic subjects immediately after antigen challenge. They also demonstrated that the activation status of these T cells was increased after antigen challenge. Metzger et al⁸ confirmed this by showing that the number of CD4⁺ cells are increased in bronchoalveolar lavage (BAL) 48 hours after challenge and remain elevated even at 98 hours. In 1991, Gerblich et al⁹ demonstrated that reduction in CD4⁺ in the peripheral blood coincided with an increase in BAL CD4⁺ cells after antigen challenge, which further supported the concept that there is T-cell recruitment from the blood to the lung after specific antigen challenge.

From the Meakins-Christie Laboratories, McGill University.

Supported in part by a grant from MRC Canada/Glaxo Wellcome.

Dr. Hamid has no significant financial interest or relationship with Schering-Plough.

Reprint requests: Qutayba A. Hamid, MD, PhD, Professor of Medicine and Pathology, Meakins-Christie Laboratories, McGill University, 3626 Urbain St, Montreal, Quebec H2X 2P2, Canada.

Copyright © 2000 by Mosby, Inc.

0091-6749/2000 \$12.00 + 0 10/110161

doi:10.1067/mai.2000.110161

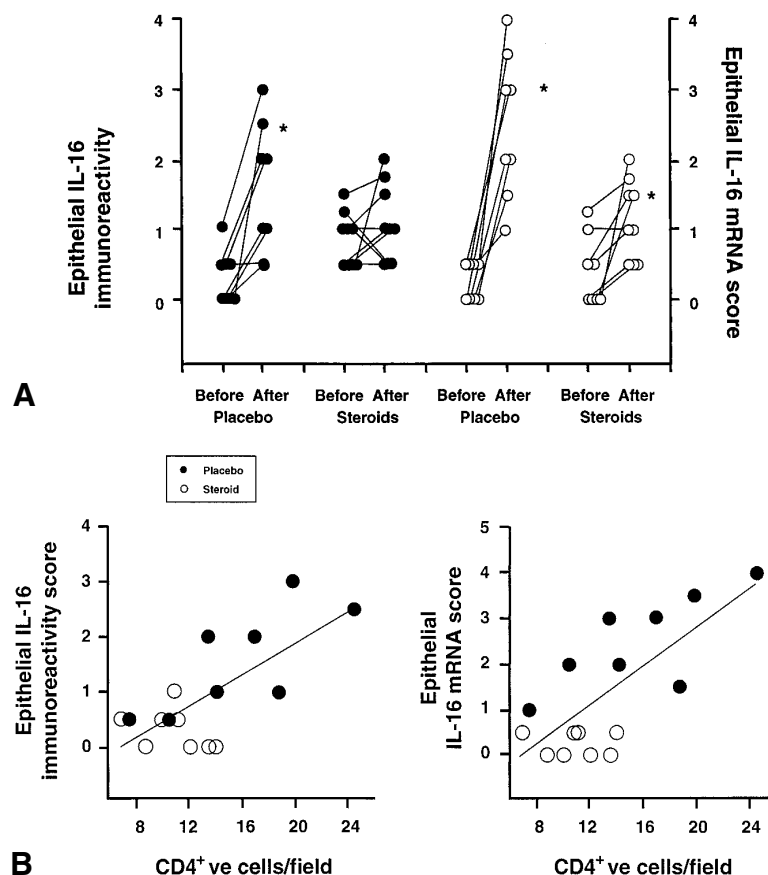


FIG 1. A, The effect of an allergen challenge on epithelial IL-16 immunoreactivity (●) and mRNA (○) expression in nasal biopsy specimens from placebo- and steroid-treated subjects with allergic rhinitis. Biopsy specimens were obtained at baseline (before) and 24 hours after allergen challenge. Scores are expressed as a percentage of positive cells per total cells (score range, 0 to 4). **B,** Within the placebo-treated group (●), there were significant correlations between epithelial IL-16 immunoreactivity and mRNA ($r = 0.75$) and the number of CD4+ cells ($r = 0.76$, $r = 0.75$, respectively). There were no significant correlations in steroid-treated patients (○). *Statistical difference of $P < .05$ for within-group comparisons with the use of Wilcoxon matched pairs signed ranks test. (Fig 1A reproduced with permission from Laberge S, Durham SR, Ghaffar O, et al. Expression of IL-16 in allergen-induced late-phase nasal responses and relation to topical glucocorticosteroid treatment. *J Allergy Clin Immunol* 1997;100:569-74.)

In contrast with these studies, there are also a number of recently published reports that provide evidence against the concept that there is T-cell recruitment after allergen challenge.¹⁰⁻¹⁴ Montefort et al¹⁰ demonstrated that after endobronchial challenge, CD3+ cells, but not CD4+ or CD8+ cells, were increased in biopsy specimens obtained at 6 hours. Gratziou et al^{11,12} showed fewer CD3+ cells, an increased CD4+/CD8+ ratio, and no changes in T-cell activation in BAL at 10 minutes and 6 hours after allergen challenge. Frew et al^{13,14} demonstrated no increase in CD3+, CD4+, or CD8+ cells in bronchial biopsy specimens 24 hours after endobronchial challenge but reported an increase in T-cell activation. In all studies that have used antigen challenge protocols, there has been a consistency in reporting an increase in the number of eosinophils and the level of T-cell activation in BAL and biopsy specimens. However, it is not yet

clear whether there is an actual increase in the number of T cells in response to antigen challenge in the airways.

Increased cytokine gene expression after specific antigen challenge

Although the presence of T lymphocytes in allergic inflammation has been well demonstrated, the major reason for their importance relates to the cytokine profile that they express. We and others have previously characterized allergic responses by the predominant production of T_H2-cell cytokines, particularly IL-4, IL-5, and IL-13. These cytokines are increased in patients with asthma,¹⁵⁻¹⁹ rhinitis,²⁰⁻²² and atopic dermatitis^{23,24} and are upregulated after specific antigen challenge.^{6,17,24-26} Bentley et al⁶ reported an increase in IL-5 and granulocyte-macrophage colony-stimulating factor messenger RNA but no change in T-cell numbers in bronchial biopsy specimens

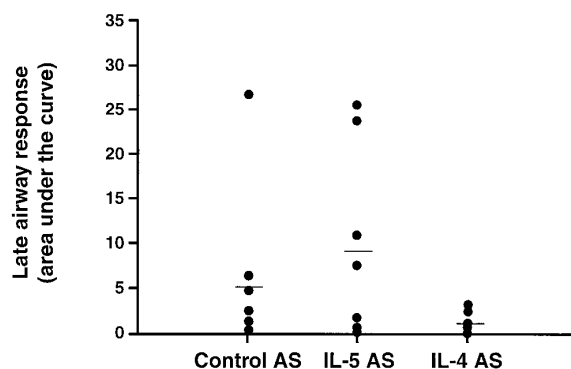


FIG 2. LARs to ovalbumin in Brown Norway rats. Unsensitized animals were injected intraperitoneally with CD4⁺ T cells that had been treated with control antisense oligonucleotides (*Control AS*), IL-5 antisense oligonucleotides (*AS*), or IL-4 antisense oligonucleotides. Two days later, the animals were challenged with ovalbumin. Lung resistance was measured at 3 to 8 hours for the LAR. IL-4 antisense oligonucleotides, but not IL-5 antisense oligonucleotides, inhibited the development of the LAR.

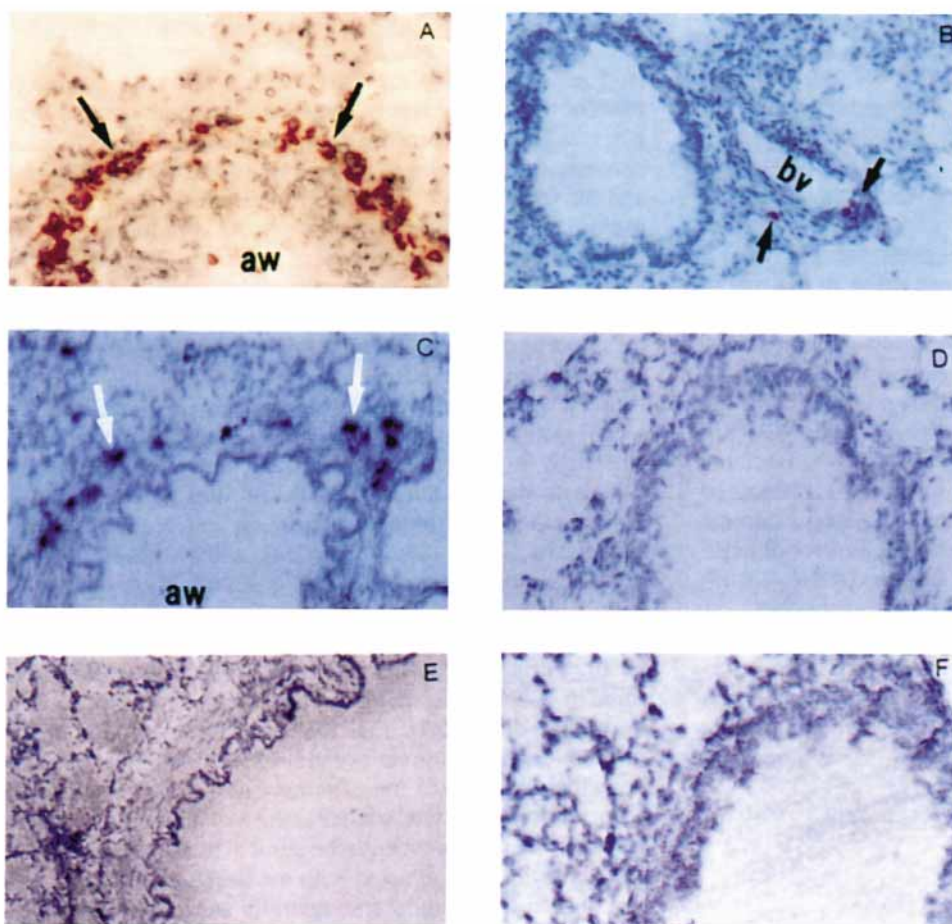


FIG 3. **A** and **B**, Immunocytochemical localization of major basic protein in lung explants of ovalbumin-sensitized Brown Norway rats. A large number of major basic protein-positive cells were demonstrated infiltrating the airway (aw) wall after antigen challenge (**A**). Fewer major basic protein-positive cells were demonstrated in and around blood vessels (bv) in explants obtained before challenge. **C** and **D**, Autoradiographs of cryostat sections obtained from lung explants of ovalbumin-sensitized rats, hybridized with radiolabeled IL-5 probe. Note the strong hybridization signal after challenge (arrows) in **C** compared with a section obtained before challenge where no significant hybridization signal was detected (**D**). **E**, An autoradiograph of an explant from a ovalbumin-sensitized animal after challenge; hybridization was with sense IL-5 probes as a control. **F**, An autoradiograph of an explant from a sham-sensitized animal that was hybridized with IL-5 probe. (Reproduced with permission from Eidelman DH, Minshall E, Dandurand RJ, Schotman E, Song YL, Yasrueel Z, Mogbel R, Hamid Q. Evidence for major basic protein immunoreactivity and interleukin 5 gene activation during the late phase response in expanded airways. *Am J Respir Cell Mol Biol* 1996;15:582-9. Official Journal of the American Thoracic Society. © American Lung Association.)

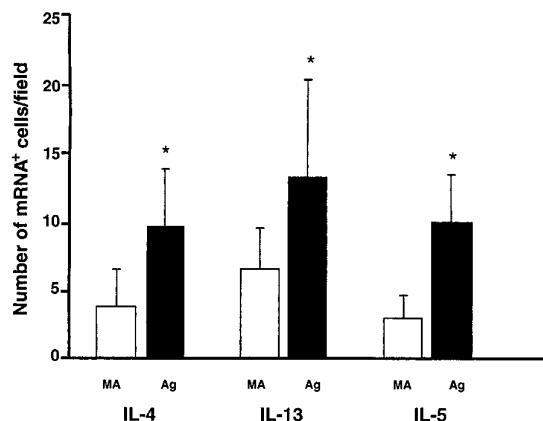


FIG 4. The number of cells that express mRNA coding for IL-4, IL-13, and IL-5 mRNA in sections of nasal mucosal tissue cultured with 500 PNU/mL ragweed allergen (Ag) or medium alone (MA; $P < .05$). Exposure to ragweed allergen resulted in increased numbers of cells that expressed mRNA for IL-4, IL-13, and IL-5. * $P < .05$.

after inhalation challenge. This was confirmed in a report from Robinson et al,¹⁷ which showed increases in IL-4, IL-5 (produced mainly by lymphocytes) but not IFN- γ and IL-2 mRNA⁺ cells in BAL after inhalation challenge. In addition to reporting an increase in the number of T_H2-cytokine-producing cells, studies have also shown an increase in IL-10, TNF- α , and IL-13 mRNA⁺ in BAL from asthmatic subjects after inhalation challenge.²⁷⁻²⁹ Similar results were reported in the nasal mucosa and skin after antigen challenge.

T-cell recruitment kinetics

The kinetics of T-cell recruitment and cytokine production in the skin after subcutaneous antigen injection has been investigated by Tsicopoulos et al.³⁰ In that study, increased numbers of T cells and T_H2 cytokine-producing cells were observed within 1 hour after allergen injection, with peak expression at 6 hours. They also showed that, in the same patients, injection of tuberculin increased T cells and the expression of T_H1 cell cytokines, between 48 and 96 hours after injection. Analysis of mRNA expression demonstrated that T_H2 cytokines in the lung, nose, and skin were primarily produced by CD3⁺ T cells^{20,31,32} and to a lesser extent by mast cells and eosinophils.³³⁻³⁵

Steroids have been shown to improve the symptoms of allergic individuals; the mechanisms of action of steroids illustrate their ability to reduce the recruitment of T cells and the expression of T_H2 cytokines.^{21,36,37} Indeed, treatment with topical corticosteroids before antigen challenge is associated with a reduction in T-cell recruitment and IL-4, IL-5, and IL-13 mRNA expression compared with placebo-treated patients.^{25,26}

Cytokines mediate T-cell recruitment

Recruitment of T cells to the site of allergic inflammation depends on a number of mechanisms that, although extensively studied, are not yet completely understood. However, the importance of cytokines in

this infiltration is becoming increasingly clear. IL-16 (initially called *lymphocyte chemoattractant factor*) is a cell-surface protein expressed predominantly by T cells and eosinophils that selectively induce the migration of CD4⁺ cells.^{38,39} We have recently demonstrated an increase in IL-16 mRNA expression and IL-16 in the epithelium and submucosa of the lung,⁴⁰ nose,⁴¹ and skin⁴² of allergic individuals. As shown in Fig 1, A,^{40,41} specific allergen challenge of the nasal mucosa has been associated with the increased expression of IL-16 mRNA and immunoreactivity relative to control.⁴⁰ Furthermore, the expression of IL-16 has been shown to correlate with the number of CD4⁺ T cells in the mucosa tissues (Fig 1, B). Further, IL-16 mRNA expression is inhibited by the use of topical steroids, both in the lung and nose (Fig 1, A). Additionally, the T_H2 cytokines IL-4 and IL-13 have been shown to enhance the endothelial expression of vascular cell adhesion molecule, an adhesion molecule that facilitates the infiltration of T cells and eosinophils into the inflamed tissue from the peripheral blood.^{43,44}

T lymphocytes and animal models

Although increased T-cell numbers in the tissue of allergic individuals has been well demonstrated, direct evidence for their importance in the pathogenesis of lung disorders and for their recruitment to the site of inflammation can only be accumulated indirectly with the use of animal models. A number of animal models have been used to study these phenomena, including mice and rats. In the Brown-Norway rat, which is characterized by high levels of IgE and late airway responses (LARs) after antigen challenge, administration of exogenous IL-2 (a T-cell growth factor) has been associated with increased LAR after antigen challenge.⁴⁵ Depletion of CD8⁺ T cells in these animals has been shown to increase the LAR,⁴⁶ whereas blocking CD4⁺ T cells decreases these responses.⁴⁷ Using this model, we demonstrated that adoptive transfer of antigen-primed CD4⁺, but not CD8⁺, T cells is directly involved in inducing the LAR.^{48,49} Intravenous injection of CD4⁺ cells (obtained from the lymph nodes of ovalbumin-sensitized animals) into naive animals leads to a high expression of T_H2 cytokines and an increase in the number of eosinophils in lung tissue and BAL.^{48,49} When the cells were labeled with bromodeoxyuridine, we observed a considerable number of these cells in the airways, which indicates that they were recruited from the peripheral blood to the site of inflammation. However, we were also able to demonstrate bromodeoxyuridine-positive cells in other organs including the spleen, liver, and lymph nodes. Interestingly, when T cells were administered to sensitized animals, we were not able to see a large number of bromodeoxyuridine-positive cells in the lung after antigen challenge. This observation supports the role of resident cells and their ability to elucidate LAR and inflammatory response. Furthermore, we have demonstrated that the LAR in the adoptive transfer model can be attenuated by the administration of antisense oligonucleotides for IL-4, but not

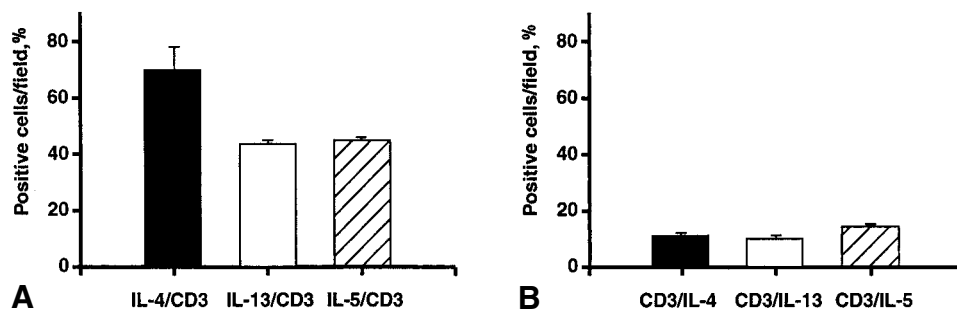


FIG 5. Localization of T_H2 cytokine expression to $CD3^+$ T cells. After ex vivo allergen exposure, a large proportion of T_H2 cytokine expression was colocalized to $CD3^+$ cells. As shown in **A**, 70% of IL-4, 43% of IL-13, and 44% of IL-5 mRNA⁺ cells were coexpressing $CD3$ immunoreactivity. However, as shown in **B**, only 11% of the total $CD3$ population were coexpressing IL-4 and IL-13 and 13% was coexpressing IL-5 mRNA.

IL-5, before T-cell transfer (Fig 2). These findings highlight the critical role of IL-4 in inducing T_H2 cytokine expression and the subsequent LAR. However, animals that were treated with IL-4 antisense oligonucleotides also exhibited an increase in IFN- γ mRNA⁺ cells, which indicates the importance of IL-4 in maintaining the T_H2 cytokine milieu.⁵⁰

Is T-cell recruitment essential for development of the allergic response?

There are significantly more $CD4^+$ T cells in the bronchial biopsy specimens and BAL of asthmatic patients compared with control subjects. Despite this and the fact that T cells are the major source of T_H2 cytokines (including IL-4, IL-5, and IL-13), no strong correlates have been drawn between the number of these cells and disease severity (although markers of T-cell activation are increased in severe asthmatic patients⁵¹). One possible explanation for this is that the increase in T-cell number (ie, T-cell infiltration) may not be imperative for the allergic response in sensitized individuals. To delineate the contribution of resident versus recruited T cells, tissue explants are an ideal system, incorporating the advantages of both in vitro and in vivo experiments.

One of the first reports that challenged the importance of T-cell recruitment in the development of allergic airway responses was performed with the use of rat lung explants.^{52,53} In that study, the lungs of ovalbumin-sensitized Brown-Norway rats were sliced into thin sections and cultured in the presence or absence of ovalbumin for 6 hours. With image analysis, late-phase responses were monitored as assessed by the change in luminal area. Not only did most of these tissue sections demonstrate a physiologic late-phase response, there were also increased numbers of cells that expressed IL-4 and IL-5 mRNA and major basic protein, a marker of eosinophils. Furthermore, the late-phase response was correlated with the number of IL-4 and IL-5 mRNA⁺ cells, but not IL-2 and IFN- γ (Fig 3).^{52,53} These findings indicate that inflammatory cell recruitment to the target organ may not actually be a requirement for the generation of allergic responses.

In similar human experiments, we and others⁵⁴⁻⁵⁶ have demonstrated that, after ex vivo allergen exposure, nasal

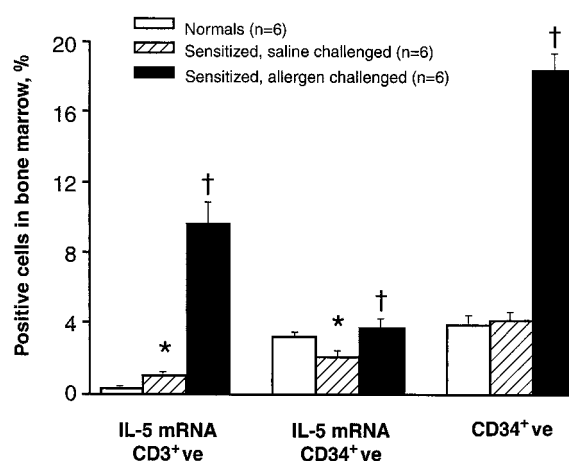


FIG 6. The presence of T cells within murine bone marrow preparations after intratracheal allergen challenge. IL-5 mRNA expression colocalized to $CD3^+$ progenitors and to $CD34^+$ T cells. * $P < .01$; † $P < .001$.

mucosal tissue from patients with allergic rhinitis express mRNA that encodes the T_H2 cytokines IL-4, IL-5, and IL-13 (Fig 4). IL-4 ushers activated naive T cells toward the T_H2 phenotype,⁵⁷⁻⁶¹ and in conjunction with IL-13, IL-4 induces a B-cell isotype switch to IgE.^{58,62} IL-5 is a required factor for eosinophil differentiation and also prolongs their survival.⁶³⁻⁶⁵ In addition to cytokine expression in the absence of cell recruitment, we have recently shown that this local production is sufficient to mediate a number of functions. Indeed, in nasal mucosal tissue from patients with allergic rhinitis, we have identified B cells undergoing epsilon germline transcription,⁵⁶ the initial step in isotype switching to IgE. These data indicate that the local production of IL-4 and IL-13 is sufficient to induce B-cell commitment to and de novo expression of IgE. Furthermore, we have demonstrated the presence of eosinophil precursor ($CD34^+/IL-5Ra^+$) in the respiratory mucosa of patients with asthma and allergic rhinitis and that these cells appear to differentiate locally in response to allergen exposure.^{55,66} In both cases, more than 40% of IL-4 and IL-5⁺ expression was attributed to T cells (Fig 5).^{55,56} We have also demon-

strated that the expression of GATA-3, a transcription factor that mediates IL-5 gene transcription and is selectively produced by T_H2 clones, is also increased in sensitized nasal mucosa that is cultured with specific allergen.⁶⁷ Collectively, these findings suggest that, in sensitized individuals, local activation of resident T cells is sufficient to mediate a number of events previously considered to depend on recruited T cells. The CD4⁺ T cells only recognize antigen that is associated with major histocompatibility complex class II. There are many cells capable of acting as antigen-presenting cells in respiratory mucosa (such as macrophages, dendritic cells, B cells, and even nasal epithelial cells).⁶⁸ As such, it seems likely that resident T cells may be activated at the site of allergen deposition and mediate the local response to allergen. Whether these cells go on to play a role in initiating the systemic response and recruiting more inflammatory cells to the target organ is not known.

EVIDENCE THAT THERE IS T-CELL RECIRCULATION

Eosinophilia has been referred to as the "hallmark of the allergic response." Although eosinophils are found constitutively within the bone marrow, their numbers are acutely elevated in response to allergen exposure.⁶⁹ For many years, *in vitro* studies have indicated the particular importance of IL-5 for eosinophil production.^{63,64} We have demonstrated the dependence of local eosinophil differentiation on IL-5, which is produced largely by T cells (Fig 5), but the bulk of eosinophil differentiation is considered to take place in the bone marrow. It is surprising, however, that until recently no real attempt had been made to determine the mechanism of signaling between antigen-induced inflammatory events in the tissue and the bone marrow response. The existence of a "serum factor" that is released by the lung after allergen challenge and its movement to the bone marrow has been suggested (see Inman, p. S235).⁷⁰ Although the nature of this factor remains to be established, it seems unlikely that it could be IL-5 because cytokines act primarily in paracrine fashion.

Recently, we demonstrated that when ovalbumin-sensitized mice are challenged intratracheally, they exhibit an increased number of bone marrow cells that express IL-5, compared with saline-challenged and naive mice. Although a considerable number of hematopoietic progenitor cells (CD34⁺) expressed IL-5, we observed an increase in the number of T cells in the bone marrow of sensitized and challenged mice and that these T cells expressed IL-5 (Fig 6).⁷¹ These findings indicated that the changes taking place in the bone marrow in response to allergen exposure may actually be a result of retrograde T-cell recruitment. That is, activated, cytokine-producing T cells may recirculate from the site of exposure back into the bone marrow.

To investigate the prospect of this event, we are currently pursuing experiments whereby sensitized T cells are tagged with radiolabel and injected into sensitized

mice, and their movement tracked after allergen challenge. By examining the lungs, lymph nodes, peripheral blood, and bone marrow compartments with a comprehensive kinetic protocol, we will characterize the movement of T cells in response to allergen exposure. Although there has been much work demonstrating that T cells are recruited to the lungs in response to allergen exposure, whether these cells then move to the bone marrow to provide the T_H2 cytokines required to drive the characteristic increased production of granulocytes remains elusive.

REFERENCES

- O'Brien RM, Xu H, Rolland JM, Byron KA, Thomas WR. Allergen-specific production of interferon-gamma by peripheral blood mononuclear cells and CD8 T cells in allergic disease and following immunotherapy. *Clin Exp Allergy* 2000;30:333-40.
- Dunnill MS. The pathology of asthma, with special reference to changes in the bronchial mucosa. *J Clin Pathol* 1960;13:37-3.
- Bentley AM, Menz G, Storz C, Robinson DS, Bradley B, Jeffery PK, et al. Identification of T lymphocytes, macrophages, and activated eosinophils in the bronchial mucosa in intrinsic asthma: relationship to symptoms and bronchial responsiveness. *Am Rev Respir Dis* 1992;146:500-6.
- 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.
- Azzawi M, Bradley B, Jeffery PK, Frew AJ, Wardlaw AJ, Knowles G, et al. Identification of activated T lymphocytes and eosinophils in bronchial biopsies in stable atopic asthma. *Am Rev Respir Dis* 1990;142:1407-13.
- Bentley AM, Meng Q, Robinson DS, Hamid Q, Kay AB, Durham SR. Increases in activated T lymphocytes, eosinophils, and cytokine mRNA expression for interleukin-5 and granulocyte/macrophage colony-stimulating factor in bronchial biopsies after allergen inhalation challenge in atopic asthmatics. *Am J Respir Cell Mol Biol* 1993;8:35-42.
- Gerblich AA, Campbell AE, Schuyler MR. Changes in T-lymphocyte subpopulations after antigenic bronchial provocation in asthmatics. *N Engl J Med* 1984;310:1349-52.
- Metzger WJ, Zavala D, Richerson HB, Moseley P, Iwamoto P, Monick M, et al. Local allergen challenge and bronchoalveolar lavage of allergic asthmatic lungs: description of the model and local airway inflammation. *Am Rev Respir Dis* 1987;135:433-40.
- Gerblich AA, Salik H, Schuyler MR. Dynamic T-cell changes in peripheral blood and bronchoalveolar lavage after antigen bronchoprovocation in asthmatics. *Am Rev Respir Dis* 1991;143:533-7.
- Montefort S, Gratziau C, Goulding D, Polosa R, Haskard DO, Howarth PH, et al. Bronchial biopsy evidence for leukocyte infiltration and upregulation of leukocyte-endothelial cell adhesion molecules 6 hours after local allergen challenge of sensitized asthmatic airways. *J Clin Invest* 1994;93:1411-21.
- Gratziau C, Carroll M, Walls A, Howarth PH, Holgate ST. Early changes in T lymphocytes recovered by bronchoalveolar lavage after local allergen challenge of asthmatic airways. *Am Rev Respir Dis* 1992;145:1259-64.
- Gratziau C, Carroll M, Montefort S, Teran L, Howarth PH, Holgate ST. Inflammatory and T-cell profile of asthmatic airways 6 hours after local allergen provocation. *Am J Respir Crit Care Med* 1996;153:515-20.
- Frew AJ, St-Pierre J, Teran LM, Trefiliet A, Madden J, Peroni D, et al. Cellular and mediator responses twenty-four hours after local endobronchial allergen challenge of asthmatic airways. *J Allergy Clin Immunol* 1996;98:133-43.
- Frew AJ. Cloning T cells from sites of allergic inflammation [editorial; comment]. *Clin Exp Allergy* 1999;29:1155-7.
- Hamid Q, Azzawi M, Ying S, Moqbel R, Wardlaw AJ, Corrigan CJ, et al. Expression of mRNA for interleukin-5 in mucosal bronchial biopsies from asthma. *J Clin Invest* 1991;87:1541-6.
- Robinson DS, Hamid Q, Ying S, Tsicopoulos A, Barkans J, Bentley AM, et al. Predominant T_H2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992;326:298-304.

17. Robinson DS, Ying S, Bentley AM, Meng Q, North J, Durham SR, et al. Relationships among numbers of bronchoalveolar lavage cells expressing messenger ribonucleic acid for cytokines, asthma symptoms, and airway methacholine responsiveness in atopic asthma. *J Allergy Clin Immunol* 1993;92:397-403.
18. Naseer T, Minshall EM, Leung DY, Laberge S, Ernst P, Martin RJ, et al. Expression of IL-12 and IL-13 mRNA in asthma and their modulation in response to steroid therapy. *Am J Respir Crit Care Med* 1997;155:845-51.
19. Minshall EM, Hogg JC, Hamid QA. Cytokine mRNA expression in asthma is not restricted to the large airways. *J Allergy Clin Immunol* 1998;101:386-90.
20. Kay AB, Ying S, Durham SR. Phenotype of cells positive for interleukin-4 and interleukin-5 mRNA in allergic tissue reactions. *Int Arch Allergy Immunol* 1995;107:208-10.
21. Cameron LA, Durham SR, Jacobson MR, Masuyama K, Juliusson S, Gould HJ, et al. Expression of IL-4, Cepsilon RNA, and Iepsilon RNA in the nasal mucosa of patients with seasonal rhinitis: effect of topical corticosteroids. *J Allergy Clin Immunol* 1998;101:330-6.
22. Pawankar RU, Okuda M, Hasegawa S, Suzuki K, Yssel H, Okubo K, et al. Interleukin-13 expression in the nasal mucosa of perennial allergic rhinitis. *Am J Respir Crit Care Med* 1995;152:2059-67.
23. Hamid Q, Boguniewicz M, Leung DY. Differential in situ cytokine gene expression in acute versus chronic atopic dermatitis. *J Clin Invest* 1994;94:870-6.
24. Durham SR, Ying S, Varney VA, Jacobson MR, Sudderick RM, Mackay IS, et al. Cytokine messenger RNA expression for IL-3, IL-4, IL-5, and granulocyte/macrophage-colony-stimulating factor in the nasal mucosa after local allergen provocation: relationship to tissue eosinophilia. *J Immunol* 1992;148:2390-4.
25. Durham SR, Gould HJ, Thienes CP, Jacobson MR, Masuyama K, Rak S, et al. Expression of epsilon germ-line gene transcripts and mRNA for the epsilon heavy chain of IgE in nasal B cells and the effects of topical corticosteroid. *Eur J Immunol* 1997;27:2899-906.
26. Ghaffar O, Laberge S, Jacobson MR, Lowhagen O, Rak S, Durham SR, et al. IL-13 mRNA and immunoreactivity in allergen-induced rhinitis: comparison with IL-4 expression and modulation by topical glucocorticoid therapy. *Am J Respir Cell Mol Biol* 1997;17:17-24.
27. Robinson DS, Tsiocopoulos A, Meng Q, Durham S, Kay AB, Hamid Q. Increased interleukin-10 messenger RNA expression in atopic allergy and asthma. *Am J Respir Cell Mol Biol* 1996;14:113-7.
28. Huang SK, Xiao HQ, Kleine-Tebbe J, Paciotti G, Marsh DG, Lichtenstein LM, et al. IL-13 expression at the sites of allergen challenge in patients with asthma. *J Immunol* 1995;155:2688-94.
29. Keatings VM, O'Connor BJ, Wright LG, Huston DP, Corrigan CJ, Barnes PJ. Late response to allergen is associated with increased concentrations of tumor necrosis factor-alpha and IL-5 in induced sputum [see comments]. *J Allergy Clin Immunol* 1997;99:693-8.
30. Tsiocopoulos A, Hamid Q, Haczku A, Jacobson MR, Durham SR, North J, et al. Kinetics of cell infiltration and cytokine messenger RNA expression after intradermal challenge with allergen and tuberculin in the same atopic individuals. *J Allergy Clin Immunol* 1994;94:764-72.
31. Ying S, Durham SR, Barkans J, Masuyama K, Jacobson M, Rak S, et al. T cells are the principal source of interleukin-5 mRNA in allergen-induced rhinitis. *Am J Respir Cell Mol Biol* 1993;9:356-60.
32. Ying S, Durham SR, Jacobson MR, Rak S, Masuyama K, Lowhagen O, et al. T lymphocytes and mast cells express messenger RNA for interleukin-4 in the nasal mucosa in allergen-induced rhinitis. *Immunology* 1994;82:200-6.
33. Ying S, Humbert M, Barkans J, Corrigan CJ, Pfister R, Menz G, et al. Expression of IL-4 and IL-5 mRNA and protein product by CD4+ and CD8+ T cells, eosinophils, and mast cells in bronchial biopsies obtained from atopic and nonatopic (intrinsic) asthmatics. *J Immunol* 1997;158:3539-44.
34. Burd PR, Thompson WC, Max EE, Mills FC. Activated mast cells produce interleukin 13. *J Exp Med* 1995;181:1373-80.
35. Moqbel R, Ying S, Barkans J, Newman TM, Kimmitt P, Wakelin M, et al. Identification of messenger RNA for IL-4 in human eosinophils with granule localization and release of the translated product. *J Immunol* 1995;155:4939-47.
36. Bentley AM, Hamid Q, Robinson DS, Schotman E, Meng Q, Assoufi B, et al. Prednisolone treatment in asthma: reduction in the numbers of eosinophils, T cells, tryptase-only positive mast cells, and modulation of IL-4, IL-5, and interferon-gamma cytokine gene expression within the bronchial mucosa. *Am J Respir Crit Care Med* 1996;153:551-6.
37. Masuyama K, Jacobson MR, Rak S, Meng Q, Sudderick RM, Kay AB, et al. Topical glucocorticosteroid (fluticasone propionate) inhibits cells expressing cytokine mRNA for interleukin-4 in the nasal mucosa in allergen-induced rhinitis. *Immunology* 1994;82:192-9.
38. Berman JS, Cruikshank WW, Center DM, Theodore AC, Beer DJ. Chemoattractant lymphokines specific for the helper/inducer T-lymphocyte subset. *Cell Immunol* 1985;95:105-12.
39. Rand TH, Cruikshank WW, Center DM, Weller PF. CD4-mediated stimulation of human eosinophils: lymphocyte chemoattractant factor and other CD4-binding ligands elicit eosinophil migration. *J Exp Med* 1991;173:1521-8.
40. Laberge S, Ernst P, Ghaffar O, Cruikshank WW, Kornfeld H, Center DM, et al. Increased expression of interleukin-16 in bronchial mucosa of subjects with atopic asthma. *Am J Respir Cell Mol Biol* 1997;17:193-202.
41. Laberge S, Durham SR, Ghaffar O, Rak S, Center DM, Jacobson M, et al. Expression of IL-16 in allergen-induced late-phase nasal responses and relation to topical glucocorticosteroid treatment. *J Allergy Clin Immunol* 1997;100:569-74.
42. Laberge S, Ghaffar O, Boguniewicz M, Center DM, Leung DY, Hamid Q. Association of increased CD4+ T-cell infiltration with increased IL-16 gene expression in atopic dermatitis. *J Allergy Clin Immunol* 1998;102:645-50.
43. Schleimer RP, Sterbinsky SA, Kaiser J, Bickel CA, Klunk DA, Tomioka K, et al. IL-4 induces adherence of human eosinophils and basophils but not neutrophils to endothelium: association with expression of VCAM-1. *J Immunol* 1992;148:1086-92.
44. Bochner BS, Klunk DA, Sterbinsky SA, Coffman RL, Schleimer RP. IL-13 selectively induces vascular cell adhesion molecule-1 expression in human endothelial cells. *J Immunol* 1995;154:799-803.
45. Renzi PM, Sapienza S, Wasserman S, Du T, Olivenstein R, Wang NS, et al. Effect of interleukin-2 on the airway response to antigen in the rat. *Am Rev Respir Dis* 1992;146:163-9.
46. Olivenstein R, Renzi PM, Yang JP, Rossi P, Laberge S, Wasserman S, et al. Depletion of OX-8 lymphocytes from the blood and airways using monoclonal antibodies enhances the late airway response in rats. *J Clin Invest* 1993;92:1477-82.
47. Olivenstein R, Martin JG, Xu LJ, Yang JP, Renzi PM. Effects of W3/25 monoclonal antibody on pulmonary inflammation and the late airway response (LR) in Brown-Norway (BN) rats [abstract]. *Clin Invest Med* 1994;17(suppl):B123.
48. 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.
49. Watanabe A, Mishima H, Kotsimbos TC, Hojo M, Renzi PM, Martin JG, et al. Adoptively transferred late allergic airway responses are associated with Th2-type cytokines in the rat. *Am J Respir Cell Mol Biol* 1997;16:69-74.
50. Molet S, Ramos-Barbon D, Martin JG, Hamid Q. Adoptively transferred late allergic response is inhibited by IL-4, but not IL-5, antisense oligonucleotide. *J Allergy Clin Immunol* 1999;104:205-14.
51. Corrigan CJ, Kay AB. CD4 T-lymphocyte activation in acute severe asthma: relationship to disease severity and atopic status. *Am Rev Respir Dis* 1990;141:970-7.
52. Eidelman DH, Minshall E, Dandurand RJ, Schotman E, Song YL, Yasruel Z, et al. Evidence for major basic protein immunoreactivity and interleukin 5 gene activation during the late phase response in explanted airways. *Am J Respir Cell Mol Biol* 1996;15:582-9.
53. Minshall EM, Dandurand RJ, Hamid Q, Eidelman DH. Local cytokine messenger ribonucleic acid expression and in vitro allergic late phase responses in Brown-Norway rats. *Eur Respir J* 1998;11:630-5.
54. Okamoto Y, Shirotori K, Kudo K, Ishikawa K, Ito E, Togawa K, et al. Cytokine expression after the topical administration of substance P to human nasal mucosa: the role of substance P in nasal allergy. *J Immunol* 1993;151:4391-8.
55. Cameron L, Christodoulouopoulos P, Lavigne F, Nakamura Y, Eidelman D, McEuen A, et al. Evidence for local eosinophil differentiation within allergic nasal mucosa: inhibition with soluble IL-5 receptor. *J Immunol* 2000;164:1538-45.
56. Cameron L, Hamid Q, Wright E, Nakamura Y, Christodoulouopoulos P, Muro S, et al. Local synthesis of epsilon germline gene transcripts, IL-4 and IL-13 in allergic nasal mucosa after ex vivo allergen exposure. *J Allergy Clin Immunol* 2000;106:46-52.

57. Abehsira-Amar O, Gibert M, Jolij M, Theze J, Jankovic DL. IL-4 plays a dominant role in the differential development of Th0 into Th1 and Th2 cells. *J Immunol* 1992;148:3820-9.
58. Del Prete G, Maggi E, Parronchi P, Chretien I, Tiri A, Macchia D, et al. IL-4 is an essential factor for the IgE synthesis induced in vitro by human T cell clones and their supernatants. *J Immunol* 1988;140:4193-8.
59. Rousset F, Robert J, Andary M, Bonnin JP, Souillet G, Chretien I, et al. Shifts in interleukin-4 and interferon-gamma production by T cells of patients with elevated serum IgE levels and the modulatory effects of these lymphokines on spontaneous IgE synthesis. *J Allergy Clin Immunol* 1991;87:58-69.
60. Romagnani S. The role of lymphocytes in allergic disease. *J Allergy Clin Immunol* 2000;105:399-408.
61. Gauchat JF, Lebman DA, Coffman RL, Gascan H, de Vries JE. Structure and expression of germ-line epsilon transcripts in human B cells induced by interleukin 4 to switch to IgE production. *J Exp Med* 1990;172:463-73.
62. Punnonen J, Aversa G, Cocks BG, McKenzie AN, Menon S, Zurawski G, et al. Interleukin 13 induces interleukin 4-independent IgG4 and IgE synthesis and CD23 expression by human B cells. *Proc Natl Acad Sci U S A* 1993;90:3730-4.
63. Clutterbuck EJ, Hirst EM, Sanderson CJ. Human interleukin-5 (IL-5) regulates the production of eosinophils in human bone marrow cultures: comparison and interaction with IL-1, IL-3, IL-6, and GM-CSF. *Blood* 1989;73:1504-12.
64. Shalit M, Sekhsaria S, Malech HL. Modulation of growth and differentiation of eosinophils from human peripheral blood CD34+ cells by IL5 and other growth factors. *Cell Immunol* 1995;160:50-7.
65. Ochiai K, Kagami M, Matsumura R, Tomioka H. IL-5 but not interferon-gamma (IFN-gamma) inhibits eosinophil apoptosis by up-regulation of bcl-2 expression. *Clin Exp Immunol* 1997;107:198-204.
66. Sehmi R, Wood LJ, Watson R, Foley R, Hamid Q, O'Byrne PM, 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.
67. Nakamura Y, Christodoulou P, Cameron L, Wright E, Lavigne F, Toda M, et al. Upregulation of the transcription factor GATA-3 in upper airway mucosa after in vivo and in vitro allergen challenge. *J Allergy Clin Immunol* 2000;105:1146-52.
68. Nonaka M, Nonaka R, Jordana M, Dolovich J. GM-CSF, IL-8, IL-1R, TNF-alpha R, and HLA-DR in nasal epithelial cells in allergic rhinitis. *Am J Respir Crit Care Med* 1996;153:1675-81.
69. 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;94:1217-24.
70. Inman MD, Denburg JA, Ellis R, Dahlback M, O'Byrne PM. Allergen-induced increase in bone marrow progenitors in airway hyperresponsive dogs: regulation by a serum hemopoietic factor. *Am J Respir Cell Mol Biol* 1996;15:305-11.
71. 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.