Ectopic expression of IL-5 identifies an additional CD4⁺ T cell mechanism of airway eosinophil recruitment

JEFFREY R. CROSBY, 1 H. H. SHEN, 1, 2 M. T. BORCHERS, 3 J. P. JUSTICE, 1 T. ANSAY, 1 J. J. LEE, 3 AND N. A. LEE 1

Divisions of 1Hematology/Oncology and 2Pulmonary Medicine, Department of Biochemistry and Molecular Biology, Mayo Clinic Scottsdale, Scottsdale, Arizona 85259; and 2Department of Respiratory Medicine, Second Hospital, Zhejiang University College of Medicine, Hangzhou 310009, People’s Republic of China

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Crosby, Jeffrey R., H. H. Shen, M. T. Borchers, J. P. Justice, T. Ansay, J. J. Lee, and N. A. Lee. Ectopic expression of IL-5 identifies an additional CD4⁺ T cell mechanism of airway eosinophil recruitment. Am J Physiol Lung Cell Mol Physiol 282: L99–L108, 2002.—CD4⁺ T cells have a critical role in the development of allergic pulmonary inflammation, including the recruitment of eosinophils to the airway lumen and interstitium. The expression of interleukin (IL)-5 by CD4⁺ T cells mediates eosinophil recruitment was assessed with gene knockout mice deficient for T cells or T cell subtypes and a unique IL-5 transgenic mouse (line NJ.1726) that constitutively overexpresses this cytokine in the lung epithelium. Pulmonary IL-5 expression is significantly attenuated in T cell- and CD4⁺-deficient animals, suggesting an obvious explanation for the lack of eosinophils in the lungs of T cell-deficient and CD4⁺(-/-) mice. However, although the constitutive expression of IL-5 in the lung epithelium of NJ.1726 mice elicited an eosinophilia in the airway lumen of both naive and ovalbumin-treated mice, in the absence of CD4⁺ cells, allergen-mediated eosinophil recruitment to the bronchoalveolar lavage fluid was abolished. Moreover, intranasal instillation of ovalbumin-specific chemokine eotaxin-2 was incapable of eliciting eosinophil recruitment in naive and ovalbumin-treated NJ.1726 CD4⁺(-/-) mice, suggesting that eosinophil trafficking during allergic inflammatory responses is a consequence of a CD4⁺ cell-mediated event(s) in addition to IL-5 expression and the establishment of a pulmonary chemokine gradient.

STUDIES OF ALLERGIC RESPIRATORY inflammation have centered on the relative roles of both resident pulmonary leukocytes (e.g., mast cells, natural killer cells, alveolar macrophages, dendritic cells) and leukocytes actively recruited to the lung as a consequence of antigen challenge (e.g., T lymphocytes and eosinophils). The recruitment of CD4⁺ T cells, and eosinophils in particular, correlates with the onset and progression of pulmonary dysfunction even in nominal cases of asthma, and the magnitude of the influx of these infiltrates is generally proportional to disease severity (5). Remarkably, this significant airway eosinophil recruitment has been a recognized marker of asthma even in the earliest clinical studies of this disease (see, for example, Ref. 25). Although debate exists regarding eosinophil effector activities occurring in the lung, at least five independent mechanisms potentially allow the eosinophil to modulate the intensity of pulmonary inflammation. 1) Eosinophils are immunoregulatory cells capable of secreting both Th helper (Th) type 1 and Th2 cytokines (54) as well as several chemokines (see, for example, Ref. 34). 2) Activated eosinophils generate and release copious amounts of reactive oxygen intermediates (10). 3) Recruited eosinophils are a source of small-molecule lipid mediators of inflammation [e.g., cysteinyl leukotrienes (24, 52)] and platelet-activating factor (PAF) (33). 4) Eosinophils secrete a series of degradative enzymes [e.g., lysophospholipase (39)] that may have significant effects on lung structure, including pulmonary surfactant activity. 5) The toxic granule proteins called eosinophil secondary granule proteins found in eosinophils recruited to the lung are toxic to epithelial cells at the concentrations present in respiratory secretions from asthma patients (15) and may increase vascular permeability (36). In addition, eosinophil secondary granule proteins potentially induce airway smooth muscle contraction (20) and activate several cell types, including epithelial goblet cells [see review by Gleich and Adolphson (19)].

Despite the commonality of CD4⁺ T cell and eosinophil pulmonary recruitment, the hypothesized activities of CD4⁺ T cells are substantively different and are thought to orchestrate the underlying allergic inflammation primarily through the release of cytokine inflammatory signals. Particular focus has centered on the elaboration of a series of cytokines collectively characteristic of Th2-type inflammation. Th2-associ-
ated cytokines have pleiotropic inflammatory effects, eliciting mucus overproduction and goblet cell metaplasia [e.g., interleukin (IL)-4, -9, -13 (7, 8, 50)], eosinophil proliferation [e.g., IL-5 (48)], airway expression of chemokines with eosinophil agonist activities (57), immunoglobulin class switching [e.g., IL-4, -13 (27)], Th2 T cell differentiation [e.g., IL-4, -13 (2)], upregulation or activation of specific cell adhesion molecules [e.g., IL-4, -13 (31, 56)], and, in mice, B cell proliferation [e.g., IL-5 (45)]. The specific contributory activities of CD4+ cells during allergen sensitization or challenge that lead to pulmonary pathology have not been resolved because several of these CD4+ cell-dependent changes directly contribute to pulmonary pathology (e.g., mucus overproduction), whereas their effects on B cells [i.e., antibody production (29)] and mast cells (46) do not appear to be necessary.

The pleiotropic nature of CD4+ T cell function has been a significant obstacle to defining specific mechanisms by which these cells elicit eosinophil recruitment to the lung. That is, potential CD4+ cell-mediated effects range from attenuation of eosinophil proliferation in the marrow and inhibition of eosinophil recruitment from the circulation to survival of mature eosinophils in the airway lumen. For example, if CD4+ T cells are the prominent cell type expressing IL-5 and, in turn, are therefore necessary for eosinophil proliferation, the loss of pulmonary eosinophilia is a likely outcome of the depletion of CD4+ cells. Indeed, antibody depletion of CD4+ cells eliminated allergen-induced pulmonary eosinophilia to an extent similar to that seen in depletion studies of IL-5 itself (see, for example, Ref. 18 vs. Ref. 49). CD4+ T cell-mediated activities also appear necessary for the establishment of some chemokine gradients within the lung (57) that are presumably necessary, in part, for airway eosinophil recruitment. Alternatively, CD4+ T cell expression of IL-4 and/or IL-13 mediates changes in endothelial expression of cell surface adhesion molecules during inflammatory responses. Many of these changes appear to be necessary for the recruitment of eosinophils to the airways, including the upregulation of E-selectin (13) and the α4β1 integrin receptor ligand vascular cell adhesion molecule-1 (see, for example, Ref. 26). In addition, a series of elegant studies published by Cohn and colleagues (6, 8) suggested that IL-4 expression may have effects on eosinophil recruitment and survival through the upregulation of another T cell-derived cytokine, interferon (IFN)-γ. These studies suggest that increased levels of IFN-γ may elicit eosinophil apoptosis and thus abolish accumulation of airway eosinophils. Moreover, CD4+ T cell-mediated increases in granulocyte-macrophage colony-stimulating factor are also likely to have significant effects on eosinophil survival and, therefore, airway accumulation of these leukocytes (44).

The constitutive ectopic expression of IL-5 in the airway epithelium associated with the transgenic mouse line Nf1.1726 (32) was used, together with gene knockout mice deficient for specific T cell subtypes, to better define the specific CD4+ T cell-dependent mechanisms necessary for eosinophil recruitment to the lung. In particular, the issues of allergen-induced CD4+ T cell expression of IL-5 and the establishment of a chemokine gradient were assessed. The data presented show that although reconstitution of IL-5 expression in CD4+ T cell-deficient mice induced an eosinophil in the pulmonary interstitium of allergen-challenged mice, eosinophils were nonetheless incapable of migrating to the airway lumen. Furthermore, eosinophil recruitment to the lumen was not recoverable through the instillation of the potent eosinophil chemokine eotaxin-2, demonstrating that CD4+ T cell-mediated accumulation of eosinophils in the lungs is the result of a concurrent mechanism(s) that acts in addition to cell proliferation and vectorial movement in response to a chemoattractant.

MATERIALS AND METHODS

Mice. Transgenic mice constitutively expressing murine IL-5 from the lung epithelium (line NJ.1726) were generated as previously described (32) and were maintained by continual backcross to C57BL/6J. NJ.1726 animals were bred with gene knockout mice (Jackson Laboratory, Bar Harbor, ME) lacking either T cells [[(lpr/s); C57BL/6J TCR-βtm1Mom/TCR-γtm1Mom (37)], CD4+ cells [C57BL/6J CD4tm1Knw (35)], or CD8+ cells [C57BL/6 CD8αtm1Jax (16)] to generate compound IL-5 transgenic gene knockout mice deficient in T, CD4+, and CD8+ cells, respectively. The genotypes of mice derived from these crosses were determined by the presence of the IL-5 transgene (PCR of tail DNA) and loss of T cells or T cell subtypes as assayed by flow cytometry on peripheral blood with conjugated antibodies against mouse T cell receptor (TCR)-β, TCR-δ, CD4+, and CD8α as previously described (4). Control C57BL/6J mice were obtained from the Jackson Laboratory, and all procedures were conducted on mice 8–12 wk of age maintained in microisolator cages housed in a specific pathogen-free animal facility. The sentinel cages within this animal colony were negative for viral antibodies and the presence of known mouse pathogens. Protocols and studies involving animals were conducted in accordance with National Institutes of Health and Mayo Clinic Foundation guidelines.

Ovalbumin sensitization and challenge. Wild-type mice were sensitized by an intraperitoneal injection (100 μl) of 20 μg of chicken ovalbumin (Ova; Sigma, St. Louis, MO) emulsified in 2 mg of Imject Alum [Al(OH)3/Mg(OH)2; Pierce, Rockford, IL] on days 0 and 14. Mice were subsequently challenged with an aerosol generated from 1% Ova in saline or saline alone for 20 min by ultrasonic nebulization (DeVilbiss, Somerset, PA) on days 24, 25, and 26. Assessments of bronchoalveolar lavage (BAL) fluid IL-5 levels and airway or alveolar tissue eosinophils were performed on day 28.

BAL, eosinophil counts, and cytokine level determinations. Lungs were lavaged three times with 0.5 ml of PBS containing 2% FCS. The recovered BAL fluids were pooled and centrifuged, generating a BAL cell pellet and a cell-free supernatant. Total cell counts were determined with a hemacytometer, and eosinophil counts were performed on Wright-stained cytospin slides (Cytospin 3, Shandon Scientific, Pittsburgh, PA) by counting ≥300 cells. Cell-free lavage fluid was frozen on dry ice and stored at −80°C until used. Murine IL-5 levels were measured with a sandwich ELISA assay as described by the supplier of the anti-IL-5 antibodies (PharMin- gen, San Diego, CA).
CD4⁺ T cell-induced eosinophil recruitment

Immunochemical identification of lung eosinophils. Immunohistochemistry was performed with a rabbit polyclonal antibody against murine major basic protein (MBP). MBP–antigen-antibody complexes were detemined in 4-μm sections of formalin-fixed, paraffin-embedded sections of mouse lungs as previously described (11). Briefly, endogenous peroxidase was quenched in the lung sections with 3% hydrogen peroxide in methanol for 20 min at 25°C. Subsequently, the sections were pepsin digested (Zymed, San Francisco, CA) for 30 min at 25°C, washed in PBS (GIBCO BRL) for 15 min, and blocked with PBS containing 1.0% normal goat serum and 1% BSA for 30 min at 25°C before incubation with primary antibody (1:3,000 dilution, 60 min at 25°C). Antibody-bound slides were washed in PBS-1.0% BSA and incubated (30 min, 25°C) with biotinylated goat anti-rabbit antibody (1:500 dilution). Sections were incubated first with avidin-biotin-peroxidase complex (Vector Laboratories, Burlingame, CA) for 30 min at 25°C and then with diaminobenzidine (3 min, 25°C) for the development of a colored reaction product before being counterstained with methyl green. Tissue eosinophils were quantified by counting the total number of MBP-positive cells in five randomly selected high-power fields per section and expressed as eosinophils per square millimeter of lung tissue (n = 6 mice/group).

Eosinophil transmigration assay. Polycarbonate membrane (5 μm) Transwell inserts in 24-well tissue culture polystyrene plates (Costar, Corning, NY) were preincubated with medium (RPMI 1640 containing 5% FCS) for 1 h. The medium was removed, and the eosinophils (1 × 10⁶ cells) and recombinant mouse IL-5 (30 ng/ml) in a total volume of 200 μl of medium were placed in the inserts, and, in turn, the inserts were subsequently seated in the wells of the plate, each of which contained 500 μl of medium alone or medium containing mouse eotaxin-2. The plates were incubated at 37°C in 5% CO₂ for 90 min. Eosinophil movement is expressed as a migration index reflecting the ratio of the number of cells migrating in response to the chemokine relative to the number of cells migrating in response to the medium alone.

Intranasal instillation of eotaxin-2. Lyophilized recombinant murine eotaxin-2 (PeproTech, Rocky Hill, NJ) was reconstituted with medium (RPMI 1640 containing 5% FCS) for 1 h. The medium was removed, and the eosinophils (1 × 10⁶ cells) and recombinant mouse IL-5 (30 ng/ml) in a total volume of 200 μl of medium were placed in the inserts, and, in turn, the inserts were subsequently seated in the wells of the plate, each of which contained 500 μl of medium alone or medium containing mouse eotaxin-2. The plates were incubated at 37°C in 5% CO₂ for 90 min. Eosinophil movement is expressed as a migration index reflecting the ratio of the number of cells migrating in response to the chemokine relative to the number of cells migrating in response to the medium alone.

Antibody-mediated depletion of CD4⁺ T cells. CD4⁺ T cells were depleted in some groups of mice with a rat anti-mouse CD4⁺ monoclonal antibody (GK1.5). The protocol used was first described by Gavett and colleagues (18) and includes intraperitoneal administration of 0.5 mg of GK1.5 (or control rat IgG; Sigma) to naïve NJ.1726 mice 1 wk before eotaxin-2 instillation. BAL fluid leukocytes were enumerated 6 h after chemokine instillation.

Statistical analysis. Data presented are means ± SE. Statistical analysis was performed on parametric data with Student’s t-tests, with differences between means considered significant when P < 0.05.

RESULTS

CD4⁺ T cells are necessary for the elaboration of airway IL-5 levels in allergen-sensitized and -challenged mice. The pulmonary recruitment of eosinophils that occurs as a consequence of an allergic inflammatory response depends on the elaboration of eosinophil proliferative signals such as IL-5 (14). This is exemplified in Ova-sensitized and aerosol-challenged mice (see MATERIALS AND METHODS for details of allergen protocol) because IL-5 levels increased dramatically over the undetectable levels in control saline-challenged animals (Fig. 1). This allergen-induced IL-5 increase was a T cell-dependent phenomenon and was abolished in Ova-treated T cell-deficient [αβ(−/−)γδ(−/−)] mice. Moreover, assessment of knockout mice with deficiencies of specific T cell subtypes demonstrated that the presence of CD4⁺ and not CD8⁺ cells is required for IL-5 expression with this sensitization and challenge protocol.

Allergen-induced pulmonary eosinophilia does not occur in CD4⁺ mice. A direct and predictable consequence of eliminating IL-5 expression was the abolition of allergen-induced pulmonary eosinophilia. Immunohistochemical staining with a rabbit antimouse eosinophil MBP polyclonal antiserum demonstrated that eosinophil infiltration of the lung did not occur in Ova-treated T cell-deficient and CD4⁺(-/-) mice but did occur in CD8⁺(-/-) mice (Fig. 2). Moreover, recruitment of eosinophils to the airways was similarly affected because BAL fluid eosinophil numbers after Ova challenge were abolished only in T cell-deficient and CD4⁺(-/-) mice (Fig. 3). It is noteworthy that this effect was restricted to eosinophils be-
cause the numbers of other cell types (i.e., macrophages, lymphocytes, and neutrophils) in the BAL fluid were similar among the different knockout mice; the percentage of each of these cells reflected only the presence or absence of an allergen-induced pulmonary eosinophilia (data not shown).

Ectopic overexpression of IL-5 in the lung epithelium results in a pulmonary eosinophilia that is independent of the presence or absence of T cell subtypes. Our laboratory (22) previously reported the creation of a transgenic mouse line (NJ.1726) that constitutively expresses IL-5 from lung epithelium with a rat CC10 promoter fragment. This earlier study showed that homeostatic baseline pulmonary eosinophil numbers are greatly elevated relative to wild-type control animals. The data presented in Fig. 4 demonstrate that these elevated levels of pulmonary eosinophils did not increase further in response to Ova sensitization and aerosol challenge of NJ.1726 mice. In addition, the pulmonary eosinophil levels in Ova-treated NJ.1726 mice were also independent of the presence of T cells or T cell subtypes. Eosinophil numbers per unit area of the lung were equivalent in Ova-treated NJ.1726 and CD4(−/−) mice (Fig. 4).

Eosinophils in lung alveolar tissue were identified with immunohistochemistry with a rabbit anti-mouse eosinophil major basic protein polyclonal antiserum. Photomicrographs represent sections from 5 mice/group. Arrows indicate eosinophil. A: C57BL/6J mice challenged with Sal aerosol. B: C57BL/6J mice challenged with Ova aerosol. C: T cell(-/-) mice challenged with Ova aerosol. D: CD4(-/-) mice challenged with Ova aerosol. E: CD8(-/-) mice challenged with Ova aerosol. F: quantitative assessment of eosinophil recruitment data as a function of tissue area. Values are means ± SE derived from multiple sections/mouse; n = 5 mice/group. *Significant differences, P < 0.05.
magnification views presented in Fig. 5 demonstrate that, compared with NJ.1726 mice, eosinophils appear less frequently in the alveolar spaces of NJ.1726 CD4(−/−) animals.

**CD4** T cells are required for allergen-mediated eosinophil recruitment to the airways of IL-5 transgenic mice. Although the pulmonary eosinophilia of NJ.1726 mice was not substantially affected by Ova sensitization and aerosol challenge, this was not true of the allergen-induced airway eosinophilia. Ova treatment of NJ.1726 mice resulted in a robust BAL fluid eosinophilia (Fig. 6), dwarfing by >10-fold the eosinophil numbers recruited to the BAL fluid of allergen-sensitized/aerosol-challenged wild-type mice (compare Figs. 3 and 6). This dramatic increase was obviously due in part to the large number of available eosinophils in the perivascular/peribronchial regions of NJ.1726 mice. However, allergen sensitization and aerosol challenge of compound NJ.1726 gene knockout mice deficient for specific T cells or T cell subtypes demonstrated that the simple availability of eosinophils is insufficient to elicit allergen-induced recruitment to the BAL fluid. Allergen sensitization and aerosol challenge of compound transgenic/gene knockout mice deficient for T cells or cells of the CD4+ subtype, but not the CD8+ subtype, failed to induce the recruitment of eosinophils to the BAL fluid despite the availability of exaggerated levels of pulmonary eosinophils (Fig. 6).

Eosinophils from NJ.1726 CD4(−/−) mice are able to respond to mouse eotaxin-2. The inability of eosinophils from NJ.1726 CD4(−/−) mice to migrate to the airway lumen in response to Ova treatment was potentially a consequence of a previously unknown yet significant perturbation(s) associated with eosinophils (i.e., a cell autonomous effect caused by the CD4+ deficiency). However, these effects appear unlikely. Migration of eosinophils from NJ.1726 CD4(−/−) mice (assessed by in vitro Transwell assay) in response to eotaxin-2, a CCR3-binding chemokine with eosinophil agonist activity (57), is equivalent to the migration exhibited by eosinophils derived from NJ.1726 mice (Fig. 7).

Intranasal instillation of eotaxin-2 elicits the recruitment of eosinophils to the airways of IL-5 transgenic but not IL-5 transgenic CD4(+/−) mice. CD4+ T cells have been hypothesized to elicit airway recruitment of eosinophils during allergic inflammatory responses through the induced expression of eosinophil agonist chemokines and the establishment of a vectorial gradient (41). In particular, recent data (57) have suggested that eotaxin-2 represents an archetype signal characterizing this potential mechanism. These studies demonstrated that eotaxin-2 is a powerful and specific eosinophil chemoattractant whose expression in the lung is induced as a consequence of allergen sensitization and aerosol challenge. The ability of eotaxin-2 alone to mediate vectorial movement of eosinophils to the airway lumen is demonstrated in Fig. 8A. Intranasal instillation of eotaxin-2 into the lungs of naive NJ.1726 mice was sufficient to elicit the recruitment of eosinophils to the BAL fluid relative to unmanipulated or vehicle-instilled animal groups. In contrast, the instillation of eotaxin-2 failed to elicit eosinophil recruitment in naive compound NJ.1726 CD4(−/−) mice (Fig. 8A). Moreover, eotaxin-2 instillation in Ova-sensitized and aerosol-challenged NJ.1726 CD4(−/−) mice also failed to elicit eosinophil recruitment, demonstrating that eotaxin-2-initiated movement does not require an allergen-mediated costimulatory signal. The unique necessity for the presence of CD4+ cells for eosinophil trafficking in response to eotaxin-2 instillation is demonstrated in the antibody depletion study presented in Fig. 8B. Treatment of naive NJ.1726 mice [i.e., CD4+(+/−) animals] with anti-mouse CD4+ monoclonal antibodies (GK1.5) before eotaxin-2 instillation abolished the ability of this chemokine to elicit eosinophil recruitment to the lumen. These data show that CD4+ cells are a critical component of eosinophil trafficking within the lung, independent of potential developmental defects associated with CD4-deficient knockout mice.

**DISCUSSION**

The hypothesis that CD4+ T cells mediate much of the pulmonary pathology associated with allergic inflammation is supported by extensive studies of both human asthma patients (28) and the characterization of several mouse models of respiratory inflammation (17). In particular, adoptive transfer of CD4+ cells to syngeneic hosts (53), the use of neutralizing antibodies (18), and, more recently, the availability of gene knockout mice have demonstrated that these cells are necessary (and possibly sufficient) to elicit the recruitment of eosinophils to the lung (21). The CD4+ cell-mediated activity commonly suggested to be primarily responsible for pulmonary eosinophil recruitment is the elaboration of the eosinophilopoietic Th2 cytokine IL-5. The underlying mechanism hypothesized is that after antigen provocation, CD4+ cells are required to generate IL-5, which, in turn, is necessary for the release of the
reservoir of eosinophils stored in the bone marrow (30), the proliferation of marrow-derived eosinophil lineage-committed progenitors (48), and the subsequent recruitment of these cells to the lung (38). Moreover, IL-5 has direct effects on eosinophil maturation, survival, and activation (1, 3, 55), suggesting that elaboration of IL-5 levels potentially elicits multiple mechanisms leading to the accumulation of pulmonary eosinophils. The increase in lung eosinophil levels as a consequence of CD4+ T cell-dependent elaboration of pulmonary IL-5 levels confirms the importance of this CD4+ T cell activity; i.e., the loss of Ova-induced eosinophil recruitment to the BAL fluid of T cell- and CD4+ cell-deficient mice, relative to wild type, parallels a similar loss of allergen-induced pulmonary IL-5 levels.

However, it is noteworthy that IL-5 levels were not extinguished in either T cell- or CD4+ cell-deficient mice. Previous studies (23) have demonstrated that under specific circumstances, CD8+ cells are a potential source of IL-5. In addition, studies (51) with an intraperitoneal sensitization model system have demonstrated that NK cells are a prodigious source of IL-5. Nonetheless, these data suggest that although alternative sources of IL-5 may exist, CD4+ cells are the predominant source of IL-5 during allergic inflammatory responses in the lung.

Several lines of evidence now suggest, however, that IL-5 expression by CD4+ cells, although an important activity leading to allergic pulmonary eosinophilia, is but one activity necessary for the recruitment of eosin-

Fig. 4. Constitutive ectopic expression of IL-5 from airway epithelial cells elicits a pulmonary eosinophilia independent of allergen challenge or the presence or absence of T cell subtypes. Pulmonary eosinophils in IL-5 transgenic (NJ.1726) mice and compound NJ.1726 gene knockout mice deficient for T cells or T cell subtypes were identified in tissue sections with a rabbit anti-mouse eosinophil major basic protein polyclonal antiserum. Photomicrographs represent sections from 5 mice/group. A: IL-5 transgenic mice challenged with Sal aerosol. B: IL-5 transgenic mice challenged with Ova aerosol. C: compound IL-5 transgenic αβ and γδ TCR(−/−) mice challenged with Ova aerosol. D: compound IL-5 transgenic CD4+ cell(−/−) mice challenged with Ova aerosol. E: compound IL-5 transgenic CD8+ cell(−/−) mice challenged with Ova aerosol. F: quantitative assessment of eosinophil recruitment data as a function of tissue area. Values are means ± SE derived from multiple sections/mouse; n = 5 mice/group. *Data not significantly different.
ophils to the airway lumen. Collectively, the implicit conclusion of this evidence is that CD4⁺ cell-mediated eosinophil production is not solely responsible for the dramatic increase in pulmonary eosinophils associated with allergic inflammation. I) Little difference is observed in the number of circulating peripheral blood eosinophils between Ova-sensitized and aerosol-challenged wild-type mice and gene knockout mice deficient in T cells or T cell subtypes (21) (data not shown). 2) Adoptive transfer of Ova-specific IL-4-deficient Th2 cells before an aerosol challenge failed to induce an airway eosinophilia despite increased levels of IL-5 and an induced pulmonary eosinophilia (6). 3) Constitutive ectopic expression of IL-5 from the lung epithelium induces a dramatic pulmonary eosinophilia independent of CD4⁺ T cells; however, in the absence of these cells, allergen-induced eosinophil trafficking to the airways is abolished. Interestingly, the loss of eosinophil recruitment occurring in mice deficient for this T cell was equivalent to the loss observed in mice devoid of all T cells (i.e., αβγδ TCR-deficient mice), suggesting a necessary and possibly sufficient role for CD4⁺ cells alone in this model system.

A concurrent CD4⁺ cell-mediated activity, apparently critical for the recruitment of eosinophils to the airways during allergen challenge, is the establish-

Fig. 5. Recruitment of eosinophils within the lung depends on CD4⁺ cell-mediated activity(ies). Relative to Ova-sensitized and -challenged NJ.1726 mice (A), eosinophil nos. in airway submucosa of Ova-treated transgenic mice were nominally reduced in the absence of CD4⁺ cells (B). A more significant difference is observed in the outlying alveolar spaces. In contrast to Ova-sensitized and -challenged NJ.1726 mice (C), eosinophils were reduced in Ova-treated compound NJ.1726/CD4(−/−) animals (D). Scale bar, 50 μm.

Fig. 6. Pulmonary expression of IL-5, and thus an induced eosinophilia of the alveolar tissue, is insufficient to elicit allergen-mediated trafficking of eosinophils to the airways in CD4⁺ T cell(−/−) mice. Total recovered eosinophils were enumerated in fluid from BAL performed 48 h after the last of 3 Sal or Ova aerosol challenges. Cohorts (n = 6/group) representing Sal- vs. Ova aerosol challenges. *P < 0.05.

Fig. 7. NJ.1726 CD4(−/−) eosinophil migration in response to eotaxin-2 was equivalent to the migration observed with eosinophils from NJ.1726 (i.e., CD4(+/+)) mice. Migration of eosinophils isolated from NJ.1726 and NJ.1726 CD4(−/−) mice were assessed in response to mouse eotaxin-2 with an in vitro Transwell assay. Values are means ± SE of duplicate determinations conducted on 3 separate occasions.
CD4+ T CELL-MEDIATED EOSINOPHIL RECRUITMENT

Fig. 8. Creation of a pulmonary chemokine gradient by intranasal instillation of eotaxin-2 did not induce eosinophil recruitment to airways of compound NJ.1726 CD4(-/-) mice (A) or NJ.1726 mice (B) transiently depleted of CD4+ cells with an anti-CD4+ antibody. Four experimental groups of animals were examined (from left to right): naive NJ.1726, naive compound NJ.1726 deficient in CD4+ cells, Ova-sensitized and aerosol-challenged NJ.1726 CD4(-/-), and naive NJ.1726 mice depleted of CD4+ cells by intraperitoneal administration of GK1.5 monoclonal antibody (B; control mice were administered nonspecific rat IgG). No differences were observed in BAL fluid eosinophil nos. recovered from control groups of naive NJ.1726 after either no instillation or instillation of 20 μl of vehicle (PBS). *P < 0.05.

The inability to elicit eosinophil recruitment in CD4+ cell-deficient mice (knockout or antibody-mediated depletion) by reconstituting the dominant eosinophil agonist activities associated with these cells (i.e., IL-5 expression and, therefore, eosinophil proliferation as well as the establishment of an eotaxin pulmonary gradient) suggests that an additional CD4+ cell-dependent mechanism(s) is required for eosinophil trafficking. Hypotheses of CD4+ cell-mediated eosinophil recruitment that require direct physical interactions between T cells and eosinophils are limited because of the logistical constraints of pulmonary eosinophil numbers relative to resident CD4+ cells as well as their relative location within the lung (i.e., there are too many eosinophils relative to T cells, and the two cell types are often not found together). However, the recent demonstration (42) that eosinophils act as antigen presentation cells in the lung suggests that at some levels, direct physical interactions between T cells and eosinophils are occurring and may contribute to the activation state of a subpopulation of either or both cell types. The identity of additional CD4+ cell-mediated mechanisms is likely associated with either direct T cell-eosinophil interactions through a soluble intermediate(s) or T cell-induced changes in the lung on which eosinophil trafficking is dependent. Studies in support of either potential mechanism have been described in the literature. Adoptive transfer experiments with Th2-differentiated lymphocytes derived from gene knockout mice have implicated IFN-γ as a potential signal with direct eosinophil agonist effects (6, 8). These studies delineate a T cell regulatory network in which IL-4/IL-13 expression downregulates the levels of T cell-derived IFN-γ. In turn, IFN-γ appears to...
directly modulate eosinophil accumulation in the lung through a mechanism restricted to activities on hematopoietic cells, with eosinophils being a likely target (6). In this paradigm, the loss of CD4+ cells leads to decreased levels of IL-4/IL-13 and concomitant increases in IFN-γ, resulting in either an inhibition of eosinophil recruitment to the airway or an increase in eosinophil turnover (e.g., apoptosis). In either case, the net effect is a substantive decrease in steady-state levels of eosinophils in the lumen that is not recoverable by reconstitution of IL-5 or an eotaxin gradient. Alternatively, several studies (reviewed in Ref. 43) have shown that inflammatory signals released by CD4+ cells lead to priming/activation of both effector leukocytes and capillary endothelial cells. In particular, this interaction leads to changes in cell surface adhesion molecule (and/or receptor) expression on eosinophils (9, 12, 47) and endothelial cells (40) and thus may have significant effects directly on adhesion molecule-mediated eosinophil recruitment or on the activation necessary for eosinophil survival. The loss of CD4+ cells in this model prevents the required cell adhesion molecule (and/or receptor) changes, thus preventing eosinophil airway accumulation. Similar to the paradigm described above, the CD4+ cell signals required for these cell surface changes are not recoverable by simply reconstituting IL-5 expression or the pulmonary chemokine gradient (i.e., eosinophils are produced and attracted to the lung but are unable to move from the interstitium). The commonality of either explanation is the description of potential CD4+ cell activities independent of IL-5-mediated eosinophil proliferation or chemokine-mediated recruitment, highlighting the importance of additional CD4+ T cell dependent mechanisms in the establishment of the pulmonary eosinophilia associated with allergic respiratory inflammation.

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REFERENCES


