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Nonhematopoietic Expression of Janus Kinase 3 Is Required for Efficient Recruitment of Th2 Lymphocytes and Eosinophils in OVA-Induced Airway Inflammation

James W. Verbsky,* David A. Randolph,* Laurie P. Shornick,* and David D. Chaplin2*†

Tyrosine kinases of the Janus kinase (Jak) family transduce signals from the type I and type II cytokine receptors. Jak3 is unique in this family because its expression must be induced and is predominantly limited to cells of the lymphoid and myeloid lineages. Deficient expression of Jak3 interferes with normal development and function of T, B, and NK cells. Using irradiated Jak3-deficient (Jak3$^{-/-}$) mice reconstituted with normal bone marrow (Jak3$^{-/-}$ chimeric mice), we have investigated possible actions of Jak3 outside of the hematopoietic system. We show that efficient recruitment of inflammatory cells to the airways of OVA-sensitized mice challenged with aerosolized OVA requires the expression of Jak3 in radiosensitive nonhematopoietic cells. Failure to develop eosinophil-predominant airway inflammation in Jak3$^{-/-}$ chimeric mice is not due to failure of T cell sensitization, because Jak3$^{-/-}$ chimeric mice showed delayed-type hypersensitivity responses indistinguishable from wild-type chimeric mice. Jak3$^{-/-}$ chimeric mice, however, express less endothelial-associated VCAM-1 after airway Ag challenge. Given the key role of VCAM-1 in recruitment of Th2 cells and eosinophils, our data suggest that Jak3 in airway-associated endothelial cells is required for the expression of eosinophilic airway inflammation. This requirement for nonhematopoietic expression of Jak3 represents the first demonstration of a physiological function of Jak3 outside of the lymphoid lineages. The Journal of Immunology, 2002, 168: 2475–2482.

Cytokine receptors have been grouped into distinct superfamilies based on shared structural features. The type I receptor (cytokine/hematopoietic growth factor receptor) family and the type II receptor (IFNR) family use Janus kinase (Jak)$^1$ protein tyrosine kinases and STAT transcription factors to transduce signals from the receptors to the nucleus (1–6). There are four recognized members of the Jak family, Jak1, Jak2, Jak3, and tyrosine kinase 2. Jak3, the most recently discovered member of the family (7, 8), is distinct from the other three family members in two important ways. First, the expression of Jak3 is inducible, detected only after stimulation of the expressing cell with cytokines or polyclonal activators (7–10). Second, Jak3 is widely believed to be expressed only in cells of the lymphoid and myeloid lineages, while Jak1, Jak2, and tyrosine kinase 2 are ubiquitously expressed (2, 4, 5). Jak3 interacts with the common γ-chain ($\gamma_c$) of the IL-2R (IL-2R$\gamma_c$). It appears to be essential for responsiveness of receptors that use this subunit, including the receptors for IL-2, IL-4, IL-7, IL-9, and IL-15. Jak3$^{-/-}$ mice generated by gene targeting displayed a SCID phenotype, with developmental and proliferative defects of T, B, and NK cells (11, 12). However, Jak3$^{-/-}$ mice showed no detectable defects in the development of the myeloid lineages or other tissues (11, 12). Based on this mutant strain, it appears that Jak3 is essential for normal development and function of the lymphoid system but is not required for the development of other tissues.

The apparent restriction of Jak3 expression to cells of the hematopoietic lineage is striking because many types of nonhematopoietic cells do respond to cytokines that may use Jak3 for signal transduction. For example, IL-4 is known to alter the function of a variety of nonhematopoietic cell types. In particular, vascular endothelial cells respond to IL-4. In endothelial cells, IL-4 induces or modulates the expression of urokinase-type plasminogen activator (13), monocyte chemotactic protein-1 (14), IL-6 (15), VCAM-1 (16), eotaxin (17), thrombomodulin (18), and RANTES (19). These actions of IL-4 on endothelium are likely to contribute to the pathophysiology of asthma and atopic inflammation (20). By upregulating the repertoire of endothelial adhesion molecules and chemokines, especially VCAM-1 and eotaxin, IL-4 may contribute to the recruitment of lymphocytes and eosinophils to areas of inflammation (21–27).

We have previously shown that treatment of vascular endothelial and smooth muscle cells with TNF-α or IL-1β for 12–24 h induces Jak3 expression, and that Jak3 is phosphorylated in response to IL-4 (28). However, two other reports failed to detect expression of IL-2R$\gamma_c$ in HUVECs and concluded that these cells do not use this receptor subunit for their responses to IL-4 (29, 30). These two studies analyzed resting, unstimulated endothelial cells that express little Jak3. Our own data had shown that endothelial cells must be activated before Jak3 can be readily detected (28), indicating that the functional repertoire of this signaling molecule cannot be inferred from its expression pattern in resting cells.

To investigate possible roles of Jak3 in nonhematopoietic tissues, we have prepared chimeric mice. We reconstituted irradiated wild-type (Jak3$^{+/+}$) mice and Jak3$^{-/-}$ mice with normal bone
morrow or splenocytes to test whether absence of Jak3 in endothelial and other radioresistant nonhematopoietic cells might result in altered inflammatory responses. If Jak3 plays no obligatory role in nonhematopoietic tissues, then Jak3−/− mice reconstituted with wild-type bone marrow should manifest equivalent responses to similarly reconstituted Jak3+/+ chimera. We have evaluated two types of inflammatory responses in the chimeric mice: first, a delayed-type hypersensitivity (DTH) response; and second, an eosinophilic inflammatory response in the airway. Both of these inflammatory models are characterized by late responses, with peak inflammation at 48–72 h after Ag challenge. These time courses are consistent with potential roles for Jak3 in these responses, with the induced expression of Jak3 in endothelial cells peaking at 18–24 h after stimulation with inflammatory cytokines (J. W. Verbsky, D. A. Randolph, and D. D. Chaplin, unpublished observations). Late-phase inflammatory responses are orchestrated by T lymphocytes, with the character of the response largely determined by the phenotype of the Th cell. DTH responses are thought to depend on IFN-γ-producing Th1 cells and to be independent of IL-4. In contrast, allergic airway inflammation is believed to depend importantly on IL-4- and IL-5-producing Th2 cells. Consistent with the participation of Jak3 in signaling by the IL-4R, but not by the IFN-γR, our studies show that Jak3−/− chimeric mice (with absence of nonhematopoietic Jak3 expression) manifest impaired recruitment of Th2 cells and eosinophils to the airways in OVA-induced airway inflammation but no detectable impairment of DTH responses.

**Materials and Methods**

**Mice**

The Jak3−/− mice (12) were provided by Dr. L. J. Berg (University of Massachusetts Medical School, Worcester, MA) and were maintained on a mixed C57BL/6 × 129/Sv background (Thyl.1). Wild-type littermates were used as controls in all experiments. All other mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and were bred and housed in microisolator cages under specific pathogen-free conditions according to institutional animal care guidelines. Mice used in this study were regularly tested to assure absence of common mouse pathogens.

**Preparation of chimeric mice**

For generation of splenocyte chimeras, Jak3−/− and Jak3−/− littermates were sublethally irradiated (700 rad). Donor cell suspensions were prepared from spleens of Jak3−/− mice and filtered through 70-μm nylon mesh to remove debris. Splenocytes from one-half of a spleen were infused with a 1/2 dilution of supernatant from the anti-VCAM-1 hybridoma MK 2.7 (American Type Culture Collection, Manassas, VA). Sections were washed three times in PBS, then incubated for 45 min with a biotinylated anti-rat secondary Ab (1/200; Sigma-Aldrich), washed in PBS, then incubated for 45 min with the alkaline phosphatase-based ABC reagent (Vector Laboratories) according to the manufacturer’s instructions. Color was developed using the Alkaline Phosphatase Substrate Substrate kit 1 with added levamisole (Vector Laboratories) to block endogenous alkaline phosphatase activity. Slides from an individual experiment were stained together, with developing solution added simultaneously to all slides. When color development had reached an appropriate level, further development was stopped by rinsing all slides simultaneously in PBS.

**Results**

To test whether expression of Jak3 in nonhematopoietic tissues is required for the development and expression of a DTH response, chimeric mice were prepared in which wild-type splenocytes were adoptively transferred to sublethally irradiated Jak3−/− and Jak3−/− mice. Twenty-four hours later, all reconstituted mice were immunized s.c. with 0.1 mg of β-galactosidase in CFA. Seven days later the mice were challenged in the footpad with 25 μg of β-galactosidase in PBS or with PBS alone. Ag-induced footpad

**Induction of OVA-induced airway inflammation**

In preliminary experiments, we found that sublethally irradiated wild-type mice that had been reconstituted with wild-type splenocytes showed inconsistent eosinophilic inflammatory responses in the lungs and airways following OVA sensitization and aerosol challenge. Consequently, we used transfer of wild-type bone marrow to lethally irradiated Jak3−/− and Jak3−/− mice to test the role of nonhematopoietic Jak3 in this form of inflammation. Eight or more weeks after bone marrow transplantation, these mice were sensitized by i.p. injection with 8 μg of OVA (Sigma-Aldrich) absorbed to 2 mg of OVA and rechalld with a second dose 7 days later. Control mice received alum alone. Seven to 10 days after the booster immunization, mice were challenged with an aerosol of 1% (w/v) OVA in sterile PBS for 20 min using an Ultra Neb 99 nebulizer (De Vilbiss Healthcare/Sunrise Medical, Somerset, PA). Treatment with OVA aerosol was repeated 4–6 h later. At the indicated times after challenge, bronchoalveolar lavage (BAL) fluid was collected, and total and differential cell counts were obtained as previously described (31). Blood was allowed to clot at room temperature for 15 min, and serum samples were stored at −70°C until tested by ELISA for OVA-specific IgG1 and IgE as previously described (32).

**Immunohistology**

After recovery of BAL fluid, 0.8 ml of OCT compound (Miles, Elkhart, IN) in PBS (1/1) was infused into the lungs to expand the tissue, and then the lungs and trachea were embedded in OCT at −80°C. Eight-micrometer sections were cut from frozen tissues and fixed in acetone for 5 min, then blocked in PBS with 5% goat serum for 15 min at room temperature using a commercial biotin-blocking kit (Vector Laboratories, Burlingame, CA). Sections were then incubated for 1 h at room temperature with a 1/2 dilution of supernatant from the rat anti-mouse VCAM-1 antibody MK 2.7 (American Type Culture Collection, Manassas, VA). Sections were washed three times in PBS, then incubated for 45 min with a biotinylated anti-rat secondary Ab (1/200; Sigma-Aldrich), washed in PBS, then incubated for 45 min with the alkaline phosphatase-based ABC reagent (Vector Laboratories) according to the manufacturer’s instructions. Color was developed using the Alkaline Phosphatase Substrate Substrate kit 1 with added levamisole (Vector Laboratories) to block endogenous alkaline phosphatase activity. Slides from an individual experiment were stained together, with developing solution added simultaneously to all slides. When color development had reached an appropriate level, further development was stopped by rinsing all slides simultaneously in PBS.

For two-color immunofluorescence analysis, 8-μm frozen sections were fixed in acetone and blocked for 15 min at room temperature with PBS containing 5% goat serum. Sections were then incubated for 1 h at room temperature with a 1/2 dilution of supernatant from the anti-VCAM-1 antibody and with a 1/1000 dilution of a rabbit anti-human von Willebrand Factor (vWF) serum (provided by D. Dean, Washington University). This serum also detects murine vWF (D. Dean, unpublished observation). Sections were washed in PBS, then incubated for 45 min with a biotinylated anti-rat secondary Ab (1/200; Sigma-Aldrich), washed in PBS, then incubated for 45 min with the alkaline phosphatase-based ABC reagent (Vector Laboratories) according to the manufacturer’s instructions. Color was developed using the Alkaline Phosphatase Substrate Substrate kit 1 with added levamisole (Vector Laboratories) to block endogenous alkaline phosphatase activity. Slides from an individual experiment were stained together, with developing solution added simultaneously to all slides. When color development had reached an appropriate level, further development was stopped by rinsing all slides simultaneously in PBS.

**Analysis of intracellular cytokines**

BAL cells from three mice in each experimental group were pooled and prepared for analysis of intracellular IL-4 and IFN-γ as previously described (31).
Jak3/fl mice in each group and are representative of two separate experiments. Data shown are the average differences in footpad swelling of three littermates. On day 8, footpad swelling was measured with calipers, and the difference between right and left footpad thickness is reported in micrometers. Data shown are the average differences in footpad swelling of three mice in each group and are representative of two separate experiments.

swelling 24 h later was indistinguishable between Jak3+/+ and Jak3−/− chimeric mice (Fig. 1). Similar results were obtained using Jak3+/+ and Jak3−/− mice that had been lethally irradiated and reconstituted with wild-type bone marrow (data not shown). Thus, there was no evidence of an essential requirement for Jak3 in non-hematopoietic cells during the sensitization or effector phases of the DTH response.

**Impaired recruitment of inflammatory cells to the airways in Jak3−/− chimeric mice**

An essential action of Jak3 in the development of eosinophil predominant airway inflammation has been shown by Malaviya et al. (33). These investigators compared the inflammatory responses in lungs of wild-type and Jak3−/− mice that had been sensitized systemically with OVA and challenged using aerosolized OVA. Although a robust eosinophil predominant inflammatory response was observed in wild-type mice, no eosinophil inflammation was detected in the Jak3-deficient strain. To dissect a specific nonhematopoietic role of Jak3 for the formation of allergic airway inflammation, we used bone marrow chimeric mice because preliminary experiments had shown poor responses to i.p. OVA sensitization in wild-type mice reconstituted with Jak3+/+ splenocytes. Jak3−/− and wild-type chimeric mice were sensitized to OVA by immunization two times separated by 1 wk. Seven to 10 days after the second sensitizing dose, all of the mice were challenged simultaneously with aerosolized OVA. Three days after challenge, BAL cells were collected and differential cell counts were obtained. Unsensitized Jak3+/+ chimeric mice showed primarily mononuclear phagocytes in the BAL, with small numbers of lymphocytes following OVA challenge (Fig. 2). BAL from unsensitized Jak3−/− chimeric mice showed increases in both lymphocytes and mononuclear phagocytes, with the lymphocytes representing a higher fraction than in wild-type chimeras. When Jak3+/+ chimeric mice were sensitized and challenged with OVA, a dramatic influx of lymphocytes and eosinophils was observed (Fig. 2). In contrast, in sensitized Jak3−/− chimeras, OVA challenge induced no significant increase in BAL cells, and unexpectedly there was a near complete absence of BAL eosinophils. Histological examination of the lungs confirmed the results of BAL. Jak3+/+ chimeric mice sensitized and challenged with OVA...
showed perivascular infiltrates containing abundant eosinophils, as detected by two different eosinophil-specific stains (data not shown). In contrast, Jak3\(^{-/-}\) chimeric mice sensitized and challenged with OVA showed no detectable change in the amount or characteristics of infiltrating parenchymal leukocytes. As seen for BAL, there were similar perivascular infiltrates of T and B lymphocytes in both sensitized and unsensitized Jak3\(^{-/-}\) chimeric mice. However, most striking was the complete lack of eosinophils in the infiltrates of the sensitized and challenged Jak3\(^{-/-}\) chimeric mice.

**Ab responses are independent of nonhematopoietic Jak3**

Analysis of anti-OVA Abs in peripheral blood of immunized Jak3\(^{+/+}\) and Jak3\(^{-/-}\) chimeric mice showed similar induction of IgG1 and IgE anti-OVA Abs (Fig. 3A). This provides clear evidence of both T and B cell sensitization of the chimeric mice. Similarly, analysis of peripheral blood differential cell counts showed equal numbers of circulating eosinophils and lymphocytes in Jak3\(^{+/+}\) and Jak3\(^{-/-}\) chimeric mice (Fig. 3B). Thus, failure of eosinophil recruitment to the lungs and airways of Jak3\(^{-/-}\) chimeric mice is not due to an absolute deficiency of eosinophils or lymphocytes.

**Requirement for nonhematopoietic Jak3 for recruitment of Th2 lymphocytes**

Effective recruitment of eosinophils to sites of inflammation is known to depend on T cells, with the Th2 products IL-4 and IL-5 playing particularly prominent roles. IL-4 acts to induce high levels of VCAM-1, which is important for emigration of eosinophils from the circulation into sites of tissue inflammation, and IL-5 acts to up-regulate production, chemotaxis, and survival of eosinophils (34, 35). To test whether the failure of eosinophil recruitment might be associated with a failure of Th2 cell recruitment in the Jak3\(^{-/-}\) chimeric mice, we determined the content of Th1 and Th2 cells in BAL fluid from sensitized and unsensitized Jak3\(^{+/+}\) and Jak3\(^{-/-}\) chimeric mice 72 h after challenge with OVA.

**FIGURE 3.** Jak3\(^{+/+}\) and Jak3\(^{-/-}\) chimeric animals exhibit similar peripheral blood differential cell counts and OVA-specific Ab responses. A, Jak3\(^{+/+}\) and Jak3\(^{-/-}\) animals were treated as described in Fig. 2. OVA-specific IgG1 and IgE levels were determined by ELISA. Serum samples were diluted 1/10 in PBS (IgE) or 1/100 in PBS (IgG1), and the absorbance value was read at 30 min and normalized to a standard serum sample used in all experiments. Each data point indicates the relative absorbance units (RU) of a serum sample from one animal from five different experiments. B, Peripheral blood smears were stained with Wright-Geimsa. Differential blood cell counts were obtained and reported as a percentage of total peripheral blood leukocytes.

**FIGURE 4.** Sensitized Jak3\(^{-/-}\) chimeric animals fail to recruit Th2 lymphocytes following OVA aerosol challenge. Jak3\(^{+/+}\) and Jak3\(^{-/-}\) chimeric animals were treated as described in Fig. 2. Seventy-two hours post-Ag challenge, BAL cells from three to five animals in each experimental group were collected, pooled, and stained for intracellular cytokines as described in Materials and Methods. All of the cells in the BAL were analyzed, and the staining profiles of CD4-gated cells are shown. Cells staining high for IFN-\(\gamma\) and low for IL-4 are Th1 lymphocytes, and cells staining high for IL-4 and low for IFN-\(\gamma\) are Th2 lymphocytes. The total number of CD4\(^{+}\) cells analyzed was 2,237 for unsensitized Jak3\(^{+/+}\) chimeric mice, 25,940 for sensitized Jak3\(^{+/+}\) chimeric mice, 13,280 for unsensitized Jak3\(^{-/-}\) chimeric mice, and 38,772 for sensitized Jak3\(^{-/-}\) chimeric mice. Mice in each experimental group were sensitized to Ag in a similar manner as shown by similar levels of serum OVA-specific IgG1 and IgE (data not shown).

BAL cells were collected 72 h after aerosol challenge, then stimulated for 6 h with PMA, ionomycin, and monensin (to prevent exocytosis of cytokines). The cells were then stained with Abs
Impaired induction of vascular VCAM-1 in lungs of Jak3\textsuperscript{−/−} chimeric mice

The failure to recruit Th2 cells to the airways can explain the failure to recruit eosinophils in this model. To investigate why Th2 lymphocytes were not being recruited, we analyzed the expression of VCAM-1 in the lungs 36 h after Ag challenge. This time point correlates with the peak recruitment of lymphocytes in this model of inflammation (J. W. Verbsky, D. A. Randolph, and D. D. Chaplin, unpublished observations). VCAM-1 was chosen for study because it has been shown to be critical for the recruitment of both lymphocytes and eosinophils in this model of inflammation (35). Unsensitized Jak3\textsuperscript{−/−} and Jak3\textsuperscript{+/+} chimeric mice express little VCAM-1 (Fig. 5, A and C). When sensitized Jak3\textsuperscript{+/+} mice were analyzed, significant increases in both the number of VCAM-1-positive vessels and the intensity of staining were apparent (Fig. 5B). The most prominent VCAM-1 staining was detected in small to medium-sized arteries in close proximity to a respiratory bronchiole. These vessels also supported efficient recruitment of eosinophils, as shown by histochemical and eosinophil-specific stains (data not shown). However, sensitized Jak3\textsuperscript{−/−} chimeric animals showed nearly undetectable induction of VCAM-1 following OVA challenge (Fig. 5D). The levels of VCAM-1 staining in OVA-challenged Jak3\textsuperscript{−/−} chimeric animals correlated generally with the degree of Ag sensitization as assessed by the levels of serum anti-OVA IgE (data not shown). Consequently, for these experiments, we compared Jak3\textsuperscript{+/+} and Jak3\textsuperscript{−/−} chimeric mice that showed similar levels of IgE anti-OVA.

To identify the cell type expressing the high levels of VCAM-1 in sensitized Jak3\textsuperscript{+/+} chimeric animals, lung sections were stained with Abs against vWF (TRITC, shown in red in Fig. 6), an endothelial-specific marker, and VCAM-1 (FITC, shown in green in Fig. 6) using two-color immunofluorescence. Intense VCAM-1 staining was observed in small arterial vessels, which appeared to be luminal. When the same vessels were examined for vWF immunoreactivity, it was apparent that the most intense VCAM-1 staining colocalized with vWF, suggesting that endothelial cells are the main source of VCAM-1 in this model (Fig. 6). We anticipate that Jak3 is also expressed in endothelial cells; however, efforts to localize the expression of Jak3 by immunohistochemistry and immunofluorescence microscopy using several different anti-Jak3 Abs have been unsuccessful (data not shown).

**FIGURE 5.** Jak3\textsuperscript{−/−} chimeric animals fail to up-regulate VCAM-1 in the lung in response to aerosolized Ag. Unsensitized Jak3\textsuperscript{+/+} chimeras (A), sensitized Jak3\textsuperscript{+/+} chimeras (B), unsensitized Jak3\textsuperscript{−/−} chimeras (C), and sensitized Jak3\textsuperscript{−/−} chimeras (D) were prepared as described in Fig. 2. Thirty-six hours after aerosol Ag challenge, mice were sacrificed and the lungs were expanded and embedded with OCT, sectioned, and stained for VCAM-1 as described in Materials and Methods. Representative sections are shown from three mice in each experimental group. All mice showed similar levels of sensitization to Ag as shown by similar levels of serum IgG1 and IgE anti-OVA. Original magnification, ×100.
Discussion

The above studies using chimeric mice show that nonhematopoietic expression of Jak3 is required for efficient allergic airway inflammation but is dispensable for the DTH response. DTH is controlled by Th1 cells, which selectively produce IFN-γ, a key mediator in this type of inflammation. In contrast, allergic inflammation is dependent on Th2 lymphocytes, which produce IL-4, IL-5, IL-10, and IL-13. IL-4 has an important role in allergic inflammation, including this model of allergic airway inflammation. BAL fluid IL-4 levels correlate with the degree of eosinophilia in asthmatic patients (34). In murine experimental allergic airway inflammation, IL-4 is detected in the BAL fluid, and systemic treatment with Abs against IL-4 decreased leukocyte recruitment and abrogated eosinophil recruitment to the airways (36, 37). In addition, transgenic mice expressing IL-4 in the lung develop lymphocytic and eosinophilic inflammation (38). Our data of a selective block in allergic inflammation in Jak3−/− chimeric mice correlate well with the known function of Jak3 in cytokine receptor signaling. Jak3 is not involved in signaling through the IFN-γR and would not be expected to play a role in nonhematopoietic tissues during a DTH response (4, 39). However, Jak3 is involved in signaling through the receptor for IL-4, and nonhematopoietic expression of Jak3 could be expected to contribute to allergic inflammatory responses.

Cohn et al. (40) demonstrated that if CD4+ T lymphocytes from DO11.10 TCR-transgenic mice are differentiated in vitro to the Th2 phenotype in response to OVA, then transferred into mice and challenged with aerosolized OVA, inflammation characteristic of asthma is seen, with mucus production and lymphocyte and eosinophil recruitment to the lung. When CD4+ T lymphocytes from IL-4-deficient animals were used in the transfer experiments, no lymphocyte or eosinophil recruitment to the lung was apparent. Because the cells were differentiated in vitro with exogenous IL-4, the defect must lie in the recruitment of inflammatory cells, and the authors mentioned that this defect was likely due to the inability of the IL-4-deficient T cells to up-regulate VCAM-1. We propose that the inability of Jak3-deficient tissues to respond to IL-4 results in the failure to recruit lymphocytes in the Jak3−/− chimeric animals.

In the experiments reported in this study, the mice had been reconstituted with normal bone marrow cells and thus had similar peripheral blood leukocyte populations and similar Ab responses to OVA, as shown by serum OVA-specific IgG1 and IgE. The failure of Th2 lymphocyte recruitment was associated with an impairment of VCAM-1 induction (Fig. 5).

It is likely that the failure to recruit Th2 lymphocytes underlies the lack of eosinophil recruitment to the BAL and lung parenchyma 72 h after Ag challenge. The IL-4 that is required for the induction of expression of VCAM-1 on endothelium is most probably derived from the recruited Th2 cells (16). Eosinophils, but not neutrophils, bind to IL-4-activated endothelium, and this has been shown to occur as a result of VCAM-1 expression (25, 41). Furthermore, the eosinophil-selective chemokine eotaxin was cloned by its ability to induce chemotaxis of eosinophils, and its level of expression has been shown to parallel the eosinophil influx in this model of allergic inflammation (42). Eotaxin is induced by IL-4 and IL-4-secreting tumors (17, 43). Dermal fibroblasts, an intestinal epithelial cell line, and a bronchial epithelial cell line express eotaxin in response to IL-4 (44–46). Thus Th2 lymphocytes are likely to induce the expression of this chemokine by the production...
of IL-4. Using an RNase protection assay we have detected re-duced levels of eotaxin mRNA in the lungs of Jak3−/− chimeric mice compared with Jak3+/+ chimeric mice 72 h after Ag chal-lenge (data not shown).

VCAM-1 expression has been shown to be critical in this model of allergic airway inflammation. Studies using Ab blocking of VCAM-1, ICAM-1, LFA-1, and very late activation Ag 4 (VLA-4) show that anti-VCAM-1 Abs decreased eosinophil recruitment by 75%, as did anti-VLA-4 (a leukocyte-specific ligand for VCAM-1) Abs (35). Anti-ICAM-1 and anti-LFA-1 Abs had no effect on eo-sinophil recruitment. Anti-VCAM-1 and anti-VLA-4 Abs blocked CD4+ T lymphocyte recruitment by 72%, while anti-ICAM-1 and anti-LFA-1 Abs blocked only 44% of the CD4+ T cell recruitment. These studies also examined the expression of ICAM-1 and VCAM-1 before and after Ag challenge. They showed that ICAM-1 is not significantly increased after Ag challenge, while VCAM-1 expression was increased ~8-fold. They also showed that induction of VCAM-1 could be blocked by treatment of mice with anti-IL-4 Abs. Thus, the failure to induce VCAM-1 in the Jak3−/− animals, likely due to an inability to respond normally to IL-4, would be expected to prevent eosinophil and lymphocyte recruitment in this model.

A variety of cell types have been reported to express VCAM-1, including smooth muscle cells, endothelial cells, fibroblasts, and macrophages (47–50). In these experiments, two-color immuno-fluorescence was used to colocalize the VCAM-1 staining with an endo-thelial-specific marker, vWF. Endothelial-associated VCAM-1 was indeed most severely affected by the lack of Jak3 (data not shown). Because VCAM-1 stains were analyzed on pairs of mice with equival-ent OVA-specific IgG1 and IgE to control for differences in sensi-tization levels, and because all mice were challenged simultaneously, these results suggest that the loss of Jak3 in endothelium results in a decreased responsiveness to IL-4. We believe that the failure to in-duce endothelial-associated VCAM-1 expression results in a failure to recruit Th2 lymphocytes, which in turn prevents eosinophils from entering the tissues.

A recent study by Uckun and colleagues (33) has used a Jak3-specific inhibitor in this model of allergic airway inflammation. These investigators showed that treating wild-type mice with the Jak3 inhibitor WHI-P97 inhibited eosinophil recruitment and air-way hyperresponsiveness following OVA challenge. This effect was thought to be due to this compound’s ability to inhibit leuko-triene synthesis and mast cell activation that then leads to the in-hibition of eosinophil recruitment. Our findings suggest rather that it is the inhibition of Jak3 in endothelium and other stromal ele-ments that accounts for this result, because mast cells are bone marrow derived and should express Jak3 in reconstituted Jak3−/− mice. Our data underscore the critical requirement for activation of the lung vasculature in Ag-driven recruitment of inflammatory cells to the airway. Jak3, most likely in the endothelial cells them-selves, is essential for the transduction of signals that support this Ag-driven inflammatory cell recruitment.

Acknowledgments

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References


