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IL-11 Selectively Inhibits Aeroallergen-Induced Pulmonary Eosinophilia and Th2 Cytokine Production

Jingming Wang,* Robert J. Homer, † Lielie Hong,* Lauren Cohn,* Chun Guen Lee,* Sungsoo Jung,‡ and Jack A. Elias*‡

IL-11 is a pleiotropic cytokine that induces tissue remodeling with subepithelial fibrosis when expressed in the airway. Its effects on the Th2-dominated airway inflammation that is characteristic of asthma, however, are poorly understood. To characterize the effects of IL-11 on Th2 tissue inflammation, we compared the inflammatory responses elicited by OVA in sensitized mice in which IL-11 is overexpressed in a lung-specific fashion (CC10-IL-11) with that in transgene− wild-type littermate controls. Transgene− and CC10-IL-11 transgene2 mice had comparable levels of circulating Ag-specific IgE after sensitization. OVA challenge of sensitized transgene− mice caused airway and parenchymal eosinophilic inflammation, Th2 cell accumulation, and mucus hypersecretion with mucus metaplasia. Exaggerated levels of immunoreactive endothelial cell VCAM-1, mucin (Muc) 5ac gene expression and bronchoalveolar lavage and lung IL-4, IL-5, and IL-13 protein and mRNA were also noted. In contrast, OVA challenge in CC10-IL-11 animals elicited impressively lower levels of tissue and bronchoalveolar lavage inflammation, eosinophilia, and Th2 cell accumulation, and significantly lower levels of VCAM-1 and IL-4, IL-5, and IL-13 mRNA and protein. IL-11 did not cause a comparable decrease in mucus hypersecretion, Muc 5ac gene expression, or the level of expression of RANTES, monocyte chemoattractant protein-2, or monocyte chemoattractant protein-3. In addition, IL-11 did not augment IFN-γ production demonstrating that the inhibitory effects of IL-11 were not due to a shift toward Th1 inflammation. These studies demonstrate that IL-11 selectively inhibits Ag-induced eosinophilia, Th2 inflammation, and VCAM-1 gene expression in pulmonary tissues.

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littermate controls. These studies demonstrate that the targeted expression of IL-11 in the murine lung diminishes aeroallergen-induced tissue and bronchoalveolar lavage (BAL)\(^3\) inflammation and eosinophilia. They also demonstrate that this inhibition is associated with a marked diminution of Th2 cytokine elaboration and endothelial cell VCAM-1 expression without comparable decreases in airway mucus metaplasia or Muc 5ac or RANTES gene expression or a significant increase in the elaboration of IFN-\(\gamma\).

Materials and Methods

Generation of CC10-IL-11 transgenic mice

Transgenic mice in which IL-11 is constitutively overexpressed in a lung-specific fashion using the Clara cell 10-kDa protein promoter (CC10; CC10-IL-11 mice) and transgenic littermate controls were employed in these studies. The methods that were used to generate these mice in our laboratory, the organ specificity of transgene expression, and the pathologic alterations induced by IL-11 have been described previously (22, 23). These mice were initially generated using CBA/C57BL/6 animals. Unless otherwise stated, progeny mice were used that were bred onto a C57BL/6 background. In selected experiments, CC10-IL-11 transgene mice and littermate controls were used that had been bred for \(\geq 10\) generations onto a BALB/c background.

OVA sensitization and challenge

OVA sensitization and challenge were accomplished using modifications of the protocols previously described by Yang et al. (30). In brief, 6- to 8-wk-old transgene and littermate control transgene mice received i.p. injections containing 20 \(\mu\)g of turkey OVA (Sigma, St. Louis, MO) complexed to alum (Resorptar, Indergen, New York, NY) or alum alone. This process was repeated 5 days later. After an additional 7 days, some animals were sacrificed (time zero), and others received aerosol challenge with OVA (1%, \(v/v\) in endotoxin-free PBS or endotoxin-free PBS alone. This was accomplished in a closed 27-\(\times\)20-\(\times\)10-cm plastic aerosol chamber in which the mouse was placed for 40 min. The aerosol was generated via a Omron NE-U07 ultrasonic nebulizer (Omron Healthcare, Vernon Hills, IL). Mice were sacrificed 4, 24, 48, or 72 h after aerosol exposure.

Treatment with recombinant human IL-11

Wild-type C57BL/6 mice were sensitized, challenged, and sacrificed as described above. Four hours before the aerosol OVA exposure, the mice were randomized to receive rIL-11 (1 \(\mu\)g) or PBS. These treatments were given in 50-\(\mu\)l volumes of PBS, which were applied to the noses of the mice and aspirated into their lungs under light anesthesia as previously described by our laboratory (16). BAL was performed as described below.

Bronchoalveolar lavage

BAL was performed as previously described (22, 23, 29). In brief, after anesthesia a median sternotomy was performed, the trachea was dissected free from the underlying soft tissues, and a 0.6-mm tube was inserted through a small incision in the trachea. BAL was performed by perfusing the lungs in situ with 0.6 ml of PBS and gently aspirating the fluid back. This was repeated three times. The samples were then pooled and centrifuged, and cell numbers and differentials were assessed. The cell-free BAL fluid was stored at \(-70^\circ\)C until used.

Peripheral blood leukocyte assessments

Retro-orbital bleeds were used to obtain peripheral blood from sensitized transgene and transgene mice before and after OVA challenge. Total leukocyte counts were obtained after erythrolysis with RBC lysis buffer (PharMingen, San Diego, CA), and cell differentials were assessed after Diff-Quick staining (Baxter, McGraw Park, IL).

Histologic analysis

Mice were anesthetized, a median sternotomy was performed, and the trachea was dissected free and cannulated as described above. The pulmonary vascular tree was then perfused with calcium- and magnesium-free PBS (pH 7.40) with a catheter in the right heart, and the lungs were inflated to 25 cm of water pressure with 10% formalin in PBS (pH 7.40). The lungs were then removed and postfixed in 10% formalin for 24 h. The tissues were processed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin, diastase-periodic acid-Schiff (D-PAS), or modified Congo Red stains. The stains were performed at the Department of Pathology of Yale University School of Medicine (New Haven, CT).

Quantification of tissue inflammation

Six to eight images of each lung section were captured at \(\times 25\) final magnification on an Olympus BH-2 microscope (Tokyo, Japan) using a Sony DXC-760 MD camera (Tokyo, Japan) attached to a Macintosh PPC100/80 (Apple Computer, Cupertino, CA) with a RasterOps 24 MxTv frame grabber board (RasterOps, Mountain View, CA). Images were collected at 16-bit color depth, 640 \(\times\) 480 pixels (final magnification, 0.75 pixels/\(\mu\)m), using the software that came with the framegrabber board. They were then analyzed with National Institutes of Health Image 1.62 using a computer-generated 18-line \(\times\)13-line overlay grid. Total intersections overlaying inflamed lung parenchyma, excluding vessels and airways, were counted and compared with total intersections overlaying parenchyma. The percentage of total points overlaying inflamed lung vessels per animal was then determined, and an average was made over each group.

Quantification of tissue eosinophils

On each Congo Red-stained slide, lungs were divided into six to eight rectangular areas using a 4-mm grid. From each area, the best defined, nonantagonistically cut bronchovascular areas were selected at \(\times 40\) final magnification using an Olympus BH-2 microscope. Eosinophils are not well visualized at that power. Eosinophils were then visualized at \(\times 200\) final magnification and counted. Area was quantitated using a rectangular 10-mm square reticule grid (American Optical, Buffalo, NY) inserted in one eyepiece. The number of lower left corner grid intersections overlapping the bronchovascular bundle was used as an approximation of area (31, 32). The ratio of total number of eosinophils to total area for each mouse lung was calculated, and the mean of these results was calculated for each experimental group. In our hands this method correlates well with eosinophils quantitated using tissue digestion.

Quantification of airway mucus

Mucus was quantitated by calculating the histologic mucus index (HMI) as previously described by our laboratories (32, 33). In brief, formalin-fixed, paraffin-embedded lungs were sectioned in a coronal plane at 5 \(\mu\)m until sections were obtained in which the central airways were visible. After D-PAS staining, marker dots in a grid with 2-mm spacing were placed over the entire lung section. The slide was examined at \(\times 100\) final magnification on an Olympus BH-2 microscope with a rectangular 10-mm square reticule grid (American Optical) inserted in one eye piece. Each marker dot was placed in the lower left corner of the field, and all intersections of airway epithelium with the reticule grid were counted in that field; mucus-containing and nonmucus-containing epithelia were separately quantitated. Approximately 25% of the total lung section was scored. The ratio of the total number of mucus intersections and the total of all intersections is referred to as the HMI and is equivalent to the linear percentage of epithelium positive for mucus.

Analysis of mucin gene expression

The levels of expression of mucin 5ac (Muc 5ac) and Muc 1 were analyzed using RT-PCR analysis. Total RNA from whole lung tissue was extracted using TRIzol reagent (Life Technologies, Grand Island, NY) as recommended by the manufacturer. RT-PCR was performed with the RT-PCR kit purchased from Promega (Madison, WI). In brief, 1 \(\mu\)g of whole lung RNA was reverse transcribed to cDNA and then amplified by PCR. The whole reaction was performed in 50 \(\mu\)l of reaction mixture containing 1 mM MgSO\(_4\); avian myeloblastosis virus/T\(\beta\) virus; 0.2 \(\mu\)M each of dATP, dCTP, dGTP, and dTTP; 100 \(\mu\)M avian myeloblastosis virus reverse transcriptase; 100 \(\mu\)M T\(\beta\) virus DNA polymerase; and 1 \(\mu\)M each of 5' and 3' primers (48°C for 45 min; 94°C for 2 min; 94°C for 45 s; annealing temperature, 1 min, 68°C at 2 min; 68°C at 7 min). The primers and conditions used in RT-PCR analysis for the cDNAs designed according to the published sequences (34, 35). For Muc 1, the primers were: 5', 5'-GCCAAGTTCTGTTCTGTTGTCTGTTGTTGTC; and 3', 5'-GATTTCACCCCACCAAGGCGCAAC. Annealing took place at 62°C, and a 250-bp fragment was produced. RT-PCR of \(\beta\)-actin was performed under the same conditions to confirm equal loading of RNA. The primers were: 5', 5'-GTTGGGCCCGTCTTGAAGCCCA; and 3', 5'-GTGGCTTTAC CCTGCAAGGGG. Annealing took place at 62°C, and a 241-bp fragment

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\(^3\) Abbreviations used in this paper: BAL, bronchoalveolar lavage; D-PAS, diastase-periodic acid-Schiff; HMI, histologic mucus index; RPA, RNase protection assay; IHIC, immunohistochemistry; MCP-2, monocyte chemoattractant protein-2.
was obtained (36). The RT-PCR products and m.w. markers were electrophoresed in 1% agarose gels containing ethidium bromide and visualized by UV illumination.

Cytokine mRNA quantification

**RNAse protection assays (RPA).** The levels of mRNA encoding the Th2 cytokines and selected chemokines were quantitated using RPA. Briefly, mice were sensitized and challenged, and the lungs were removed as described above. They were then digested in TRIzol reagent, and total RNA was obtained by processing the tissues according to the manufacturer’s specifications. The levels of specific mRNA transcripts were then evaluated by RPA using the RibonQuant kit (PharMingen) according to the instructions provided by the manufacturer.

**RT-PCR.** The levels of mRNA encoding MCP-2 and MCP-3 were quantitated by RT-PCR as described above. For MCP-2 the primers were: 5′-AGTGTCTTTTGGCTGCTGCTCATAG; and 3′, 5′-AGAGAAAAACCGCAGCCAGGACC. Annealing took place at 60°C, and a 389-bp product was appreciated. For MCP-3 the primers were: 5′, 5′-ACGCTCTGTGCCCCTGCTGCTCATAG; and 3′, 5′-GTAAAATAGGGAAAAAGGGGAGAAT. Amplification took place at 62°C, and a 241-bp product was appreciated. RT-PCR of β-actin was also performed to confirm equal RNA loading.

**Cytokine quantitation**

The levels of IL-4, IL-5, IL-13, and IFN-γ protein were quantitated by ELISA using commercial kits according to the instructions provided by the manufacturers (R&D Systems, Minneapolis, MN; Endogen, Cambridge, MA).

**Intracellular cytokine staining**

Transgene + and transgene − mice were sensitized and challenged with OVA as described above. Twenty-four hours after Ag challenge the animals were sacrificed, the pulmonary vascular tree was perfused with calcium- and magnesium-free PBS (pH 7.40) via a right heart catheter, and lung lymphocytes were prepared using collagenase III digestion, mechanical tissue disruption, and Ficoll density centrifugation. The resulting cells were randomized to receive no additional stimulation or anti-CD3 and soluble anti-CD28 anti-mouse Abs (PharMingen) for 6 h. Brefeldin A (1 μM; PharMingen) was added, and the cells were washed, fixed with 2% formaldehyde, permeabilized with 0.5% (w/v) saponin, and stained with specific Abs. For three-color staining, the cells were labeled with biotin-alkaline phosphatase (Zymed, San Francisco, CA) for 30 min, re-sensitized with PE-conjugated anti-IL-4 and FITC-labeled anti-IFN-γ, and then incubated with strepavidin CyChrome. After washing, specific Abs. For three-color staining, the cells were labeled with biotin-avidin-alkaline phosphatase (Zymed, San Francisco, CA) for 1.5 h at 25°C. The slides were then washed, incubated with strepavidin-alkaline phosphatase (Zymed, San Francisco, CA) for 30 min, re-sensitized with PE-conjugated anti-IL-4 and FITC-labeled anti-IFN-γ, and then incubated with strepavidin CyChrome. After washing, developed with Fast Red, and counterstained with hematoxylin.

**VCAM-1 immunohistochemistry (IHC)**

Immuno-reactive VCAM-1 was assayed by IHC as described by Yang et al. (30). Mice were sensitized, challenged with OVA, and sacrificed, and their lungs were inflated to pressure as described above. Fixation was under low calcium- and magnesium-free PBS (pH 7.40) via a right heart catheter, and lung lymphocytes were prepared using collagenase III digestion, mechanical tissue disruption, and Ficoll density centrifugation. The resulting cells were randomized to receive no additional stimulation or anti-CD3 and soluble anti-CD28 anti-mouse Abs (PharMingen) for 6 h. Brefeldin A (1 μM; PharMingen) was added, and the cells were washed, fixed with 2% formaldehyde, permeabilized with 0.5% (w/v) saponin, and stained with specific Abs. For three-color staining, the cells were labeled with biotinylated anti-mouse CD4 followed by strepavidin CyChrome. After washing, labeled cells were fixed and permeabilized as described above. They were then incubated with PE-conjugated anti-IL-4 and FITC-labeled anti-IFN-γ (Santa Cruz Biotechnology, Santa Cruz, CA) in saponin buffer for 1 h at room temperature. Analysis was performed on a FACScaliber flow cytometer (Becton Dickinson, San Jose, CA). Data are displayed as dot plots of FITC (x-axis) vs PE (y-axis) fluorescence (log scales). Quadrant markers were positioned to include ≥99% of control Ig-stained cells in the left lower quadrant.

**Statistical analysis**

Data are expressed as the mean ± SEM unless otherwise indicated. Data were assessed for significance using Student’s t test or ANOVA as appropriate.

**Results**

**Effect of IL-11 on OVA sensitization**

Studies were first undertaken to determine whether systemic Ag sensitization could be accomplished in IL-11-overexpressing mice. This was done by immunizing CC10-IL-11 transgene + mice and transgene − littermate controls with OVA plus alum or with alum alone on day 0 and again 5 days later. After an additional week, serum was obtained from transgene + and transgene − mice that have been handled in an identical fashion and the levels of IgE specific for turkey OVA were assessed. In the absence of Ag exposure, OVA-specific IgE could not be detected. In contrast, turkey OVA-specific IgE was readily detected in sensitized mice. Interestingly, comparable levels of turkey OVA-specific IgE were noted in the serum of transgene + and transgene − animals (data not shown), demonstrating that CC10-IL-11 mice can be systematically sensitized via the route that was employed.

**Effects of IL-11 on OVA-induced BAL and tissue eosinophilia**

OVA sensitization and challenge of transgene − mice resulted in a 2- to 4-fold increase in BAL cellularity and significant pulmonary inflammation. Eosinophils dominated this response, becoming the predominant inflammatory cell in BAL fluid (Fig. 1) and tissues (Figs. 2 and 3) 24–48 h after Ag exposure. Comparable degrees of BAL and tissue inflammation were not seen in animals that were not sensitized before aeroallergen challenge or that received systemic sensitization and nebulized saline (Fig. 2 and data not shown).

When similar experiments were undertaken in the CC10-IL-11 transgene + mice, impressive differences were noted. At the early time points after aerosol OVA exposure (4–24 h) modest to moderate decreases in BAL cellularity were seen (data not shown). IL-11 also markedly inhibited the recovery of eosinophils in BAL fluid.
fluid at all time points after aerosol Ag exposure (Fig. 1). This decrease in BAL eosinophils was associated with a significant increase in the percentages of BAL cells that were alveolar macrophages and insignificant alterations in the percentage of BAL lymphocytes and granulocytes (Fig. 1 and data not shown). The effects of IL-11 on BAL eosinophilia were particularly impressive 24 h after aerosol OVA exposure. At this time point, total eosinophil recovery in BAL fluids from transgene\(^1\) mice were 18.1% of the levels in fluids from transgene\(^2\) littermate controls (\(p < 0.01\)). This inhibitory effect of IL-11 was at least partially lung restricted, since circulating levels of lymphocytes and eosinophils in transgene\(^1\) mice were equal to or greater than those in transgene\(^2\) mice before and at all time points after OVA aerosol exposure (data not shown).

In accord with the BAL findings, IL-11 also caused a significant decrease in tissue inflammation (Fig. 2) and an impressive decrease in tissue eosinophil accumulation (Fig. 3). Overall, tissues from transgene\(^1\) mice contained ~25% as much inflammatory infiltrate (Fig. 2) and 5–20% as many eosinophils as those from transgene\(^2\) animals at all time points assessed (Fig. 3 and data not shown; \(p < 0.05\) at all time points). When viewed in combination, these studies demonstrate that IL-11 decreases BAL and tissue inflammation and markedly inhibits BAL and tissue eosinophilic responses after aeroallergen challenge.

**Effects of rIL-11**

As previously described by our laboratory (22, 23), the chronic expression of IL-11 causes structural alterations in the lungs of transgenic mice. To determine whether the IL-11 or the structural alterations were responsible for the diminished OVA-induced responses in the CC10-IL-11 transgene\(^1\) mice, studies were undertaken to determine whether rIL-11 manifest similar effects in sensitized, structurally normal, wild-type animals. As shown in Table I,
a single pulmonary dose of rIL-11 significantly inhibited OVA-induced total cell and eosinophil recovery in these animals. These studies demonstrate that the anti-inflammatory effector functions of IL-11 can be reproduced with recombinant protein. They also suggest that the inhibition of OVA-induced inflammation and eosinophilia in our transgene+ mice is due in great extent to the IL-11 that is expressed in these animals.

**Effect of IL-11 on airway mucus and mucin gene expression**

Mucus hypersecretion is a characteristic feature of the asthmatic airway that is nicely modeled in the OVA system (37). To determine whether the inhibitory effects of IL-11 on airway eosinophilia were associated with a comparable decrease in airway mucus, D-PAS staining was used to highlight the mucus-secreting cells in the airways of appropriately sensitized transgene− and transgene+ animals 24–72 h after aeroallergen inhalation. Morphometric approaches were also employed to calculate the HMI of these animals, and the levels of mRNA encoding Muc 5ac and Muc 1 were quantitated. PAS+ cells were not found or were extremely rare in the airways of sensitized transgene− animals that received a saline aerosol challenge and mice that were not sensitized before aerosol Ag exposure. In contrast, PAS+ cells were readily detected after aeroallergen exposure of appropriately sensitized transgene− animals (Fig. 4). OVA challenge of appropriately sensitized and challenged transgene+ mice was also associated with the induction of mRNA encoding Muc 5ac, but not Muc 1 (Fig. 5). IL-11 expression did not increase the frequency of D-PAS+ cells or levels of Muc 5ac or Muc 1 mRNA in the airways of saline-challenged mice or naive mice that received aerosol OVA alone. In addition, IL-11 did not significantly inhibit the mucus hypersecretion and mucus metaplasia induced by OVA in sensitized animals. This was easily seen in the histologic sections and confirmed by the HMI calculations (Fig. 4 and data not shown). Similarly, IL-11 did not inhibit, in a statistically significant fashion, the levels of mRNA encoding Muc 5ac or Muc 1 after OVA challenge (Fig. 5). Thus, IL-11 appears to selectively alter OVA-induced responses in the murine lung, inhibiting airway and tissue eosinophilia without comparably altering OVA-induced mucus hyperproduction, goblet cell hyperplasia, or Muc 5ac gene expression.

**Effect of IL-11 on Th2 and inflammation-regulating cytokines**

Studies were next undertaken to determine whether Th2 and other inflammation-regulating cytokines were induced during the course of the OVA-stimulated responses in transgene− and transgene+ mice. Twenty-four hours after OVA challenge, mRNA species encoding IL-4, IL-5, and IL-13 were easily detected in total RNA from sensitized transgene− animals (Fig. 6). The expression of all these Th2 cytokines was markedly decreased in the transgene+ animals (Fig. 6). In accord with these observations, IL-4, IL-5, and IL-13 protein were readily detected in the BAL fluids of sensitized and challenged transgene− animals and were present in significantly lower quantities in the BAL fluids from IL-11 transgene+ mice (Fig. 7). This inhibition was also most prominent 24 h after

![FIGURE 4](http://www.jimmunol.org) Comparison of mucus in transgene− animals and littermate controls. CC10-IL-11 transgene+ mice and transgene− littermate controls were sensitized, challenged with OVA, sacrificed, and processed for histologic evaluation 48 h later. D-PAS stains were performed to highlight mucus containing epithelial cells in transgene− animals (A) and transgene− littermate controls (B). Original magnification, ×250.
OVA exposure. At this time point, BAL fluids from IL-11 transgene mice contained 5.4, 7.6, and 1.6% as much IL-4, IL-5 and IL-13, respectively as BAL fluids from littermate controls (\(p < 0.05\) for all; Fig. 7). Intracellular cytokine staining also demonstrated that this inhibition was due in part to a decrease in the number of Th2 lymphocytes accumulating in the lungs of transgene mice after OVA challenge (Fig. 8). It was not, however, associated with a shift toward Th1 inflammation, since IFN-\(\gamma\) mRNA and protein and IFN-\(\gamma\)-producing cells were not increased in the lungs of transgene mice before or after Ag challenge (Figs.

**FIGURE 5.** Representative experiment (of five) demonstrating the mucin gene expression in transgene\(^+\) and transgene\(^-\) mice. CC10-IL-11 transgene\(^+\) mice and transgene\(^-\) littermate controls were sensitized and challenged with OVA. Animals were sacrificed before the OVA challenge (time zero) and 24 h later. Total lung RNA was extracted, and the levels of Muc 5ac, Muc 1, and \(\beta\)-actin gene expression were evaluated by RT-PCR as described in Materials and Methods.

**FIGURE 6.** Th2 cytokine mRNA in transgene\(^+\) and transgene\(^-\) animals. CC10-IL-11 transgene\(^+\) mice and transgene\(^-\) littermate controls were sensitized and challenged with OVA. Before (0 h) and 24 h after OVA challenge the levels of mRNA encoding Th2 cytokines were evaluated by RPA as described in Materials and Methods. Representative transgene\(^+\) and transgene\(^-\) animals (of five) are compared. The levels of expression of IL-4, IL-5, and IL-13 and the housekeeping gene L32 are compared.

**FIGURE 7.** Cytokine levels in BAL fluid from transgene\(^+\) and transgene\(^-\) mice. CC10-IL-11 transgene\(^+\) mice and transgene\(^-\) littermate controls were sensitized and challenged with OVA. The levels of IL-4 and IL-13 (A) and IL-5 and IFN-\(\gamma\) (B) in the BAL fluids from these animals were quantitated by ELISA 24 h after OVA challenge. ■, Transgene\(^+\) animals; □, transgene\(^-\) animals. *, \(p < 0.05\).

7 and 8 and data not shown). In addition, RANTES, MCP-2, and MCP-3 mRNA were not comparably inhibited 4 and 24 h after OVA challenge in the IL-11 transgene mice (Figs. 9 and 10 and data not shown). These studies demonstrate that IL-11 selectively inhibits aeroallergen-induced Th2 cytokine gene expression.
and protein elaboration and Th2 cell accumulation without inducing IFN-γ in respiratory structures.

Effect of IL-11 on VCAM-1 expression

Eosinophils are selectively recruited into sites of atopic tissue inflammation, in part via the ability of Th2 immune responses to augment endothelial cell VCAM-1 expression and the interaction of endothelial VCAM-1 and eosinophil VLA-4 (38). Studies were thus undertaken to determine whether the diminished tissue eosinophilia noted in OVA-sensitized and challenged transgene 1 mice was associated with an alteration in the ability of these animals to express endothelial VCAM-1. Endothelial VCAM-1 was not detected in significant quantities by IHC in wild-type animals that received aerosol saline challenges or Ag in the absence of sensitization (data not shown). VCAM-1 was readily detected in wild-type animals that were appropriately sensitized and challenged with OVA (Fig. 11). IL-11 production did not alter the expression of VCAM-1 in the airways of saline-challenged mice or naive mice that received aerosol OVA alone. IL-11 did, however, cause a significant decrease in the expression of VCAM-1 on endothelial cells of OVA-sensitized and challenged animals (Fig. 11). Thus, IL-11 decreases endothelial cell VCAM-1 expression in this Th2-dominated modeling system.

Discussion

To further understand the in vivo effector functions of IL-11 in the lung, we compared the response to aeroallergen of appropriately sensitized and challenged mice in which IL-11 was overexpressed in a lung-specific fashion and of transgene 1 littermate controls. These studies demonstrate for the first time that IL-11 has an inhibitory effect in this setting. Although transgene 1 mice and transgene 2 mice manifest comparable systemic IgE responses to OVA, their pulmonary responses differed markedly. Transgene 1 animals contained 21.1% as many cells, 2.9% as many eosinophils, 10.4% as many lymphocytes, 27.3% as much IL-4, 12.5% as much IL-5, and 3.3% as much IL-13 as BAL fluids from identically treated littermate controls (Figs. 12 and 13). These studies demonstrate that IL-11 is a potent inhibitor of OVA-induced responses in BALB/c as well as C57BL/6 mice.

Effect of IL-11 on BALB/c mice

C57BL/6 and BALB/c mice differ in their propensity to mount specific types of T cell-mediated responses, with the former having a propensity toward Th1-dominated inflammation, and the latter a propensity toward Th2-dominated inflammation. The studies noted above demonstrate that IL-11 inhibits aeroallergen-induced pulmonary eosinophilia in C57BL/6 mice. Studies were next undertaken to determine whether IL-11 had similar inhibitory effects in the setting of a genetic background that is predisposed to Th2 immunity. In these experiments CC10-IL-11 transgene 1 mice on a BALB/c background and appropriate littermate controls were sensitized and challenged as described above. In the transgene 1 littermate controls a brisk response was elicited that was associated with a 4- to 5-fold increase in total BAL cellularity and an impressive increase in BAL eosinophils and lymphocytes. By 24–48 h after OVA challenge eosinophils made up >65% of the BAL cell population (Fig. 12). IL-4, IL-5, and IL-13 were also readily detected in the BAL fluid from these animals (Fig. 13). In accord with our observations in the C57BL/6 mice, IL-11 potently inhibited this response in BALB/c background animals. Twenty-four hours after OVA exposure, the BAL fluid from IL-11 transgene 1 animals contained 21.1% as many cells, 2.9% as many eosinophils, 10.4% as many lymphocytes, 27.3% as much IL-4, 12.5% as much IL-5, and 3.3% as much IL-13 as BAL fluids from identically treated littermate controls (Figs. 12 and 13). These studies demonstrate that IL-11 is a potent inhibitor of OVA-induced responses in BALB/c mice.
responses in the lung, with transgene" animals manifesting markedly diminished expression of IL-4, IL-5, and IL-13, but not MCP-2, MCP-3, or RANTES. Lastly, they demonstrate that this inhibition of Th2-dominated inflammation is not the result of a shift toward a Th1 immune response, since IFN-γ was not induced in the IL-11-overexpressing animals.

Airway biopsies and autopsy samples from patients with asthma have demonstrated an inflammatory response of the mucosa and submucosa with lymphocytes, eosinophils, mast cells, and hyperplasia of goblet cells. Many of these features have been reproduced in acute aeroallergen models of airway inflammation such as the one used in this report. Studies of these models have defined the critical importance of CD4" Th2 cells in the development of these inflammatory responses (39–41). In keeping with the importance of Th2 cells in this model, we noted that the ability of IL-11 to inhibit tissue inflammation and eosinophilia was associated with a significant decrease in Th2 cytokine gene expression and protein elaboration. This finding stands in contrast to the demonstrated ability of IL-11 to augment Th2 responses in other modeling systems (24) and the ability of IL-11 to inhibit the elaboration of Th1

FIGURE 11. Vascular VCAM-1 expression in transgene" and transgene" mice. CC10-IL-11 transgene" mice (B and D) and transgene" littermate controls (A and C) were sensitized and challenged with OVA. The levels of endothelial VCAM-1 were evaluated by IHC as described in Materials and Methods. In A and B, anti-VCAM-1 was employed; in C and D, an isotype control Ab was employed. In A, the endothelial staining is highlighted with arrows. In the representative bronchovascular bundles in all panels, airways contain triangles, and blood vessels contain asterisks. Original magnification, ×100.

FIGURE 12. Total cell, eosinophil, and lymphocyte recovery in BAL fluids from transgene" and transgene" BALB/c mice. CC10-IL-11 transgene" mice and transgene" littermate controls on a BALB/c background were sensitized and challenged with OVA, and BAL was performed 24 h later. Total cell, lymphocyte, and eosinophil recovery and the percentage of BAL cells that were lymphocytes and eosinophils are illustrated. *, p < 0.05; **, p < 0.01.

FIGURE 13. Cytokine levels in BAL fluids from transgene" and transgene" BALB/c mice. CC10-IL-11 transgene" mice and transgene" controls on a BALB/c background were sensitized and challenged with OVA, and BAL was performed 24 h later. The levels of IL-5, IL-4, and IL-13 were quantitated by ELISA as described in Materials and Methods (p < 0.05 for all comparisons).
cytokines (such as IL-12) in vitro (25, 42). The mechanism by which IL-11 inhibits Th2-dominated inflammation in this model is not clear. It is possible that the ability of IL-11 to inhibit macrophage production of primary cytokines such as IL-1 and/or TNF (25, 27) or inhibit macrophage NF-kB activation (26, 28) may play a role(s) in this response. Our studies also demonstrate that IL-11 inhibits Th2 cell accumulation while decreasing endothelial cell VCAM-1 expression. In this setting, IL-11 could primarily inhibit Th2 inflammation, with the failure to up-regulate VCAM-1 being a consequence of this inhibition. Alternatively, the failure to up-regulate VCAM-1 expression may be the primary regulatory event, and diminished Th2 cell accumulation may be the result of faulty Th2 cell recruitment due to the lack of VCAM-1 expression (43). Our studies do not allow these possibilities to be differentiated. They do, however, demonstrate that the inhibition of Th2-dominated inflammation is not due to an augmented Th1 immune response with Th1 counter-regulation, since the levels of IFN-g were not augmented in aeroallergen-challenged transgenic animals vs those in appropriate littermate controls.

An interesting feature of our studies was the demonstration that IL-11 inhibits tissue and BAL eosinophilia and Th2 cytokine elaboration without causing comparable decreases in airway mucus induction or Muc 5ac gene expression. These observations demonstrate that IL-11 selectively alters facets of the airway response in our model. This observation is in accord with that made by Anguita et al., who demonstrated that IL-11 inhibits only specific manifestations of Lyme disease in a murine model (44). These studies also suggest that eosinophils and eosinophil-derived mediators are not major inducers of the mucus metaplasia, mucus hyperproduction, or Muc 5ac gene expression in this modeling system. This contention is supported by the recent demonstration by Cohn et al. that airway eosinophils and mucus induction can be readily dissociated in the murine airway (32, 39). The demonstration that IL-11 inhibits Th2 cytokine production without causing a comparable decrease in mucus induction, however, is more problematic, since a variety of evidence suggests that Th2 cytokines, in particular IL-13, may play an important role in this inductive response (4, 33). There are a number of possible explanations for our findings. First, mucus induction in our modeling system may be mediated by a Th2 cytokine-independent process. It is well known that a variety of mediators, including leukotrienes, PGs, platelet-activating factor, and histamine, also induce mucus secretion (37, 45, 46). Alternatively, the relationship between Th2 cytokine elaboration and airway mucus induction may not be linear, and the combined effects of the low levels of IL-4, IL-5, IL-13, and other Th2 cytokines that are produced in our IL-11-overexpressing transgenic mice, individually or via synergistic interactions, may be adequate to induce significant mucus induction. Support for this latter possibility comes from studies from our laboratory using inducible overexpression transgenic mice that generate IL-13 in a lung-specific fashion. In these studies only picogram per milliliter quantities of BAL IL-13 were required to cause significant mucus metaplasia in the murine airway (Z. Zhu and J. A. Elias, unpublished observation).

Airway remodeling with subepithelial fibrosis has been described in patients with mild and severe asthma and, to a lesser extent, patients with allergic rhinitis (7). Although its significance is not clear, evidence has been presented suggesting that airway remodeling contributes to disease severity, the generation of airways hyper-responsiveness, and the acquisition of an airways phenotype with partial and/or irreversible obstruction (reviewed in Ref. 7). It is still not known, however, if this remodeling response represents a healing and repair response by the host and/or a manifestation of disease pathogenesis. This question is relevant to IL-11, which induces significant airway fibrosis when expressed in transgenic mice (22, 23) and is selectively expressed in tissues from patients with severe asthma and severe asthmatic airway remodeling (47). We reasoned that these not mutually exclusive options might be able to be differentiated by determining the effect of IL-11 on airway inflammation, since a mediator of healing and repair (such as TGF-b1) would be expected to induce fibrosis while inhibiting airway inflammation, whereas fibrogenic mediators that are not involved in healing might not alter or might actually augment local inflammatory responses. Our studies demonstrate that IL-11 is a potent inhibitor of aeroallergen-induced airway inflammation and tissue and BAL eosinophilia. Studies from our laboratory and others have also demonstrated that IL-11 has protective effects in the setting of radiation-induced thoracic injury (27), immune complex lung injury (28), and protective and cytoprotective effects in the setting of oxidant-induced lung injury (29). When viewed in combination, these studies suggest that IL-11 may be a healing cytokine with the ability to induce tissue fibrosis, minimize tissue injury, mediate cytoprotection, and inhibit tissue inflammation.

Eosinophils are recruited into tissues as a result of a coordinated multistep process. Key events in this process include the selective induction of endothelial VCAM-1 and the elaboration of eosinophil chemoattractants. This allows endothelial VCAM-1 to interact with VLA-4 on the eosinophil surface (38) and the eosinophil to subsequently transmigrate into tissues. Our studies demonstrate that the effects of IL-11 on tissue and BAL eosinophilia cannot be attributed solely to the effects of IL-11 on the expression of MCP-2, MCP-3, or RANTES. They do, however, demonstrate that IL-11 inhibits aeroallergen-induced VCAM-1 expression in appropriately sensitized animals. This observation is not without precedent, since IL-6, a closely related cytokine, inhibits VCAM-1 expression in the central nervous system, and IL-11 inhibits endothelial ICAM-1 expression without inhibiting CXC chemokine expression in a rat immune complex lung injury model (28, 48). Additional experimentation will be required to determine whether the diminished expression of VCAM-1 in the IL-11 transgene+ mice is the result of the ability of IL-11 to inhibit Th2 cytokine or IL-1 or TNF elaboration (25, 27, 29) or whether IL-11 also has direct effects on endothelial VCAM-1 expression as seen with IL-6 and astrocytes (46).

C57BL/6 and BALB/c mice differ in their propensity to develop Th2 vs Th1 inflammatory responses. Recent studies demonstrated that this difference may be the result of a difference in the number of lymphocytes in each that are susceptible to Th2/Th1 polarization and not a function of differing levels of cytokine production on a per cell basis (49). This difference has been referred to as probabilistic gene expression and has been attributed to a poorly defined genetic locus in these animals (49, 50). To shed light on the mechanism by which IL-11 regulates Th2-dominated inflammation, we compared the effects of IL-11 in mice with C57BL/6 and BALB/c backgrounds. IL-11 had qualitatively similar effects in both circumstances. This demonstrates that the inhibitory effects of IL-11 on Th2 inflammation are not overcome by the genetic differences that determine the types of inflammatory response in C57BL/6 and BALB/c animals.

In summary, these studies demonstrate, for the first time, that IL-11 selectively inhibits aeroallergen-induced Th2-dominated tissue inflammation and eosinophilia. This inhibition was associated with a marked decrease in the production of Th2 cytokines, diminished Th2 cell accumulation, and diminished endothelial VCAM-1 expression in the absence of comparable alterations in mucus metaplasia or the expression of MCP-2, MCP-3, or RANTES. They also demonstrate that this inhibition is not due to Th1 polarization, since IL-11-induced increases in IFN-g were not
noted. The anti-inflammatory, fibrotic, and cytoprotective properties of IL-11 suggest that it is a healing and/or protective cytokine in the lung. These properties also suggest that rIL-11 may be a useful therapeutic that can be used to regulate Th2-dominated inflammatory disorders.

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References