Airway Epithelial Cell Expression of Interleukin-6 in Transgenic Mice
Uncoupling of Airway Inflammation and Bronchial Hyperreactivity

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Abstract

We produced transgenic mice which overexpress human IL-6 in the airway epithelial cells. Transgenic mice develop a mononuclear cell infiltrate adjacent to large and mid-sized airways. Immunohistochemistry reveals these cells to be predominantly CD4$^+$ cells, MHC class II$^+$ cells, and B220$^+$ cells. Transgenic mice and nontransgenic mice had similar baseline respiratory system resistance (0.47 ± 0.06 vs 0.43 ± 0.04 cmH$_2$O/ml per s at 9 wk of age, P = NS and 0.45 ± 0.07 vs 0.43 ± 0.09 cmH$_2$O/ml per s at 17 wk of age, P = NS). Transgenic mice, however, required a significantly higher log dose of methacholine to produce a 100% increase in respiratory system resistance as compared with nontransgenic littermates (1.34 ± 0.24 vs 0.34 ± 0.05 mg/ml, $P \le 0.01$). We conclude that the expression of human IL-6 in the airways of transgenic mice results in a CD4$^+$, MHC class II$^+$, B220$^+$ lymphocytic infiltrate surrounding large and mid-sized airways that does not alter basal respiratory resistance, but does diminish airway reactivity to methacholine. These findings demonstrate an uncoupling of IL-6–induced airway lymphocytic inflammation and airway hyperresponsiveness and suggest that some forms of airway inflammation may serve to restore altered airway physiology. (*J. Clin. Invest.* 1994. 94:2028–2035.) Key words: cytokines • airway physiology • lung biology • methacholine • airway hyperresponsiveness

Introduction

Asthma is a chronic disease characterized clinically by recurring episodes of bronchospasm, physiologically by airway hyperresponsiveness to a variety of stimuli, and pathologically by airway inflammation. Indirect evidence supports the hypothesis that the inflammatory response is responsible for the airway obstruction and airway hyperresponsiveness. For example, the number of eosinophils and activated T cells in asthmatic bronchial submucosa correlates with disease severity and degree of hyperresponsiveness (1–5). Although the mechanism by which airway inflammation causes reversible airway obstruction and hyperresponsiveness is unknown, it has been suggested that

they are a consequence of the elaboration of various mediators and cytokines in the asthmatic airway (6–8).

In contrast to normals, the presence of various cytokines has been demonstrated in the bronchoalveolar lavage fluid (BALF)[1] and/or biopsies of asthmatic airways. These include GM-CSF, TNF, interleukin (IL)-1, IL-2, IL-3, IL-4, IL-5, and IL-6 (9–12). In most reports, it is assumed that the dysregulated production of the cytokine being evaluated plays a role in the development of asthmatic manifestations. It is equally reasonable, however, to believe that the production of these cytokines may also represent a normal healing response in the damaged airway which is designed to normalize airway physiology. This quandary is well illustrated via an analysis of the in vitro effects of IL-6.

IL-6 has a variety of well documented proinflammatory effects that are potentially relevant to asthmatic inflammation, including its ability to stimulate the proliferation of thymocytes and mature T cells (13, 14), stimulate cytotoxic T lymphocyte differentiation (15), upregulate IL-4–dependent IgE production (16), and mediate the terminal differentiation and immunoglobulin production of B cells (13). In contrast, IL-6 has also been demonstrated to diminish tissue inflammation in animal models of hypersensitivity pneumonitis (17), oxygen toxicity (18), and endotoxin-induced lung injury (19), and to inhibit macrophage production of IL-1 and TNF in vitro (20).

To define the basic mechanisms by which inflammation alters airway hyperresponsiveness, we undertook studies designed to determine whether the inappropriate expression of one inflammatory cytokine in the airway would be sufficient to induce an inflammatory infiltrate and alter airway physiology. Since asthma is a chronic inflammatory disease, we took advantage of the distribution of the Clara cells in the airway (mouse conducting airways are 50–60% Clara cells) (21) and used the Clara cell CC10 promoter to express the human IL-6 gene chronically in airway epithelial cells. In this study we address three questions with respect to the biologic activity of IL-6 in vivo: (*a*) whether the upregulated expression of IL-6 in the airway is sufficient to produce an inflammatory response; (*b*) whether this leads to increased airway resistance; and (*c*) whether upregulated expression of IL-6 in the airway alters bronchial reactivity. These studies demonstrate that IL-6 can be expressed in high quantities in mouse lung using the CC10 promoter and that CC10–IL-6 transgenic mice develop a chronic peribronchial inflammatory response. Of importance, they also demonstrate that despite this inflammation CC10–IL-6 transgenic mice are hyporesponsive to methacholine, illustrat-

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1. *Abbreviations used in this paper:* BALF, bronchoalveolar lavage fluid; MCh, methacholine; PC100, provocative challenge 100.
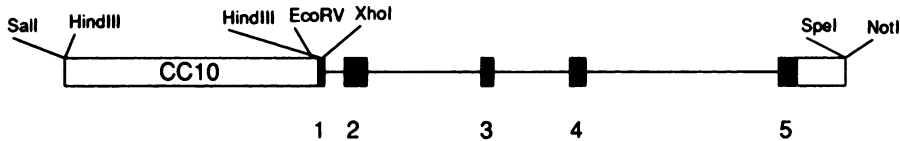
ing a remarkable dissociation of airway inflammation and airway reactivity.

Methods

Production of transgenic mice. The 5′-flanking region of the rat CC10 promoter was isolated and characterized as described previously (22). The CC10 promoter was isolated as a HindIII fragment and subcloned into the HindIII site in pKS-Bluescript. The locus encoding human IL-6 was the generous gift of Dr. S. Akira and Dr. T. Kishimoto. The IL-6 gene was isolated as a 4.8-kb XhoI (blunted with the Klenow fragment of DNA polymerase), SpeI fragment and subcloned into the EcoRV/SpeI site in pKS-CC10. The CC10–IL-6 plasmid was extracted from bacterial suspension and purified through two sequential CsCl gradients (23). The purified DNA was digested with NotI and SalI to generate the CC10–IL-6 fragment (Fig. 1), separated by electrophoresis through 1% agarose gel (SeaKem GTG; FMC Corp. BioProducts, Rockland, ME), and isolated by electroelution into dialysis tubing (23). The DNA fragment was purified through Elutip D columns by following the manufacturer's instructions (Schleicher and Schuell, Inc., Keene, NH) and dialyzed on filters against injection buffer (0.01 M Tris•HCl/0.1 mM EDTA, pH 7.5). Transgenic mice were made in (CBA × C57BL/6) F_2 animals as described (24), and positive animals were identified by Southern blot analysis of tail DNA using a ^{32}P-labeled 516-bp XhoI, XbaI IL-6 gene fragment as a probe (25).

IL-6 levels in BALF. BALF was obtained by inserting PE 50 tubing (Clay Adams, Parsippany, NJ) via a tracheotomy in pentobarbital (90 mg/kg) anesthetized mice and lavaging with three successive washes of 0.5 ml of phosphate-buffered saline (PBS) with 0.1% bovine serum albumin (BSA). Each BALF aliquot was centrifuged, and the supernatants were harvested and stored individually at −70°C until ready to use. All measurements were made on the first aliquot of BALF.

Measurements of IL-6 protein were made using an IL-6 ELISA kit following the manufacturer's instructions (Quantikine; R&D Systems, Inc., Minneapolis, MN). This ELISA has been shown to have no significant cross-reactivity with other cytokines and no cross-reactivity with mouse IL-6 at levels of 100 ng/ml (R&D Systems, Inc., product insert).

Measurements of IL-6 activity were made using B9.11 cells. B9.11 cells in log phase of growth were incubated with 100 μl of BALF in triplicate for 72 h in 96-well microtiter plates in a humidified 37°C, 5% CO_2 incubator. [^{3}H]Thymidine was added, and plates were incubated at 37°C for an additional 4 h. Cells were harvested, and [^{3}H]thymidine incorporation was counted by liquid scintillation.

Histologic sections. Lungs were inflated with 10% formalin, removed en block, placed in Tissue Tek III cassettes (Miles Inc., West Haven, CT) and placed overnight in 10% formalin. Paraffin embedding, tissue sectioning, slide mounting, hematoxylin and eosin staining, elastin staining, and trichrome staining were performed as a service by the Department of Pathology, Yale University School of Medicine.

Immunohistochemistry on frozen sections. Lungs were inflated with 1 × PBS/33% (vol/vol) OCT Tissue-Tek compound (Miles Inc.) and snap frozen in OCT by submersion into 2-methylbutane (Aldrich Chemical Co., Milwaukee, WI) cooled with dry ice. Tissue sections were cut, transferred onto silane-treated glass slides, fixed in 4% acetone for 15 min, and stained with various antibody reagents as described previously (26). Sections were blocked with avidin-biotin blocking kit (Vector Labs, Inc., Burlingame, CA) and BSA before reaction with biotinylated primary antibody. The slides were then washed three times in 0.1 M Tris buffer (pH 7.5), and the tissue sections were incubated with a prediluted streptavidin-alkaline phosphatase solution (Kirkegaard & Perry Laboratory, Gaithersburg, MD) for 1 h. The sections were washed and developed using Fast Red staining system (Sigma Immunochemicals, St. Louis, MO) in accordance with manufacturer's instructions. The slides were counterstained in Meyer's hematoxylin and then mounted with Aquamount histologic mounting medium (Lerner Laboratories, Pittsburgh, PA).

Areas of cellular infiltration were photographed. Infiltrates were divided into quadrants, and cells were counted. Positively staining cells with each antibody were counted and expressed as a percentage of the total number of cells in the infiltrate.

Physiologic assessment. Mice were anesthetized with pentobarbital (90 mg/kg) and tracheostomized with an 18-gauge angiocatheter (Baxter Scientific, McGraw Park, IL). Airway resistance in mice was measured using a modification of the techniques described by Martin et al. (27). With these techniques, changes in the lung volume of anesthetized and tracheostomized mice were measured plethysmographically by determining pressure in a Plexiglas chamber using an in line Microswitch pressure transducer. Flow was measured by differentiation of the volume signal, and transpulmonary pressure was determined by a second Microswitch pressure transducer placed in line with the plethysmograph and an animal ventilator. Resistance was then calculated using the method of Amdur and Mead (28). Resistance of the tracheostomy catheter was eliminated, and baseline measurements of pulmonary resistance were obtained by ventilating the mouse in the plethysmograph at volumes of 0.4 ml at a rate of 150 breaths/min previously shown to produce normal arterial blood gases (27). Each mouse was studied at baseline. Bronchial hyperreactivity was then determined by methacholine (MCh) challenge as described previously (29). Increasing concentrations of MCh in PBS were administered by nebulization (20 1-ml breaths), and the pulmonary resistance was calculated precisely 1 min later. Stepwise increases in MCh dose were then given until the pulmonary resistance, in comparison with baseline level, had at least doubled. The data were expressed as the provocative challenge 100 (PC100), the dose at which pulmonary resistance was 100% above baseline level.

Statistical analysis. Values were expressed as means±SE. The data were normally distributed, and group means were compared with Student's two-tailed, unpaired t test with StatView software for Macintosh.

Results

Production of transgenic mice. We wished to test the hypothesis that IL-6 directly mediates local inflammation in vivo and that an inflammatory response localized to the airway is sufficient to result in altered susceptibility to MCh-induced bronchoconstriction. We constructed several lines of transgenic mice in which expression of the human IL-6 gene was regulated by the CC10 promoter. This promoter has been successfully used previously to direct the expression of chloramphenicol acetyltransferase in the conducting airways of the lung (22). Of 35 progeny screened by Southern blot analysis of tail DNA, 6 were positive for the transgene with copy numbers ranging from 5 to 50 per genome (data not shown). Founders 9 (10 copies), 11 (20 copies), and 17 (50 copies) were bred with C57BL/6 mice.

Table I. IL-6 Levels in BALF and Serum

Sample	Transgene +	Transgene −	P value
	ng/ml	*ng/ml*	
BALF	10.1±3.6	< 0.003	≤ 0.025
Serum	0.243±0.010	< 0.003	≤ 0.0005

The levels of human IL-6 in BALF and serum of transgene-positive (+) and -negative (−) mice were assessed by ELISA as described. IL-6 values represent mean±SEM, n = 2–3 samples per group.

Figure 2. IL-6 activity. B9.11 cells were incubated with BALF from transgene-positive (*Tg Pos*) and transgene-negative (*Tg Neg*) mice. Cell proliferation was measured as [³H]thymidine incorporation in counts per minute. Cells incubated with BALF from Tg Pos mice had significantly more proliferation than cells incubated with BALF from Tg Neg mice (30 × 10³ vs 1 × 10³ cpm) (P ≤ 0.0005). Each assay was done in triplicate on BALF of individual mice. BALF was obtained from four Tg Pos and four Tg Neg mice. The bars represent the means±SEM.

The CC10–IL-6 transgene is translated into IL-6 in the airway. To determine if the CC10–IL-6 transgene is appropriately expressed in these animals, BAL was performed on transgene-positive and transgene-negative littermates of progeny of founder 9 and 17, and human IL-6 protein levels were assayed by ELISA. Human IL-6 was found only in the transgene-positive animals of each line, at similar levels (Table I, data from line 9 only). Transgenic animals had a mean of 10.1±3.6 ng/ml of BALF IL-6 while nontransgenic animals had no (< 0.003 ng/ml) detectable human IL-6 (P ≤ 0.025). The levels of serum IL-6 were also assayed. Significant IL-6 could be detected in the serum of the transgenic but not in the serum of the nontransgenic animals (Table I), 243±10 pg/ml versus nondetectable (< 3.13 pg/ml) (P ≤ 0.0005). In all cases, the levels of serum IL-6 were significantly lower than the levels of BALF IL-6.

To determine if the IL-6 was biologically active, B9.11 cells were incubated with BALF from transgene-positive and -negative littermates. B9.11 cells had significantly more proliferation when incubated with BALF from transgene-positive animals than when incubated with BALF from transgene-negative littermates as demonstrated by [³H]thymidine incorporation, 30 × 10³ counts per minute (cpm) vs 1 × 10³ cpm, P ≤ 0.0005 (Fig. 2).

Expression of IL-6 in the airway results in airway inflammation. The expression of IL-6 in the airway was sufficient to induce an overwhelming infiltration of mononuclear cells that was consistently seen in areas adjacent to large and mid-sized airways in transgenic progeny from lines 9, 11, and 17 but not in transgene-negative littermates (Fig. 3). The epithelium in the transgenic animals appeared normal. No eosinophils were seen in the infiltrate, nor was there any subepithelial fibrosis by trichrome or elastin staining (data not shown). All other organs were examined histologically and appeared normal (data not shown).

The airway infiltrating cell population consists of CD3+, CD4+, CD8+, MHC class II+, and B220+ cells. The results obtained with these IL-6 transgenic mice suggested that expression of IL-6 in the airway was sufficient to initiate an inflammatory response. The phenotype of the infiltrating cells was therefore analyzed by immunohistochemistry of frozen lung sections. These data reveal that the predominant cell type is an MHC class II+, B220+ cell with the remainder being CD3+, CD4+ cells. Few CD8+ cells and no F4/80+ cells were noted (Fig. 4). Areas of infiltrate were divided into quadrants, and cells were counted. CD3+ cells make up 25% of the cells in the infiltrate, of which 90% are CD4+ and 10% are CD8+. B220+ cells make up 75% of the cells in the infiltrate. None of the antibodies reacted with sections of lung from transgene-negative
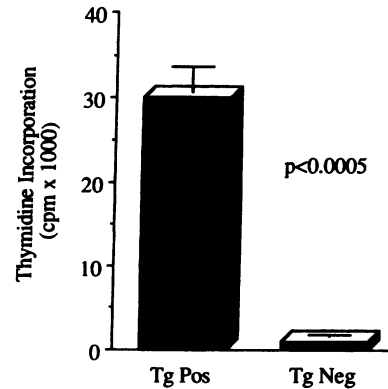
mice because there was no airway infiltration in these animals (data not shown).

Expression of IL-6 results in stunted growth. The expression of IL-6 in the airway caused transgenic mice to be stunted in growth compared with the transgene-negative animals. As seen in Fig. 5, transgenic mice are of equal weight to their littermate negatives when they are young, but as they age the littermate negatives have a steeper growth curve. At 16 wk of age, transgenic males weigh 26.2±0.78 g while nontransgenic males weigh 30.8±1.3 g (P ≤ 0.025). The difference in transgenic and nontransgenic females was even more marked and could be seen as early as 11 wk of age, 19.8±0.67 vs 21.7±0.6 g (P ≤ 0.05) and 21.38±0.78 vs 23.68±0.67 g (P ≤ 0.01), respectively. No difference was found in males at 9 and 11 wk or in females at 9 wk.

IL-6 expression and airway inflammation does not increase basal airway resistance. To determine whether the infiltrating lymphocytes affected airway resistance, we measured the resistance of the respiratory system in the transgene-positive mice and compared them with age-matched transgene-negative littermate mice. The results show that IL-6 transgenic mice have no difference in respiratory resistance despite having massive airway inflammation (Table II), 0.47±0.06 vs 0.43±0.04 cm H_2O/ml per s (P = NS) at 9 wk and 0.45±0.07 vs 0.43±0.09 cm H_2O/ml per s (P = NS) at 17 wk for transgenic and nontransgenic animals, respectively. We concluded that, despite having massive airway inflammation, there is no change in baseline pulmonary resistance.

IL-6–expressing transgenic mice are less reactive to methacholine. To assess the degree of hyperresponsiveness in IL-6 transgenic mice, MCh challenge was performed. The results show that IL-6 transgenic mice require a significantly higher log dose of MCh to achieve a 100% increase (PC100) in the respiratory resistance, 1.14±0.25 vs 0.63±0.12 (P ≤ 0.05) for females and 1.34±0.24 vs 0.34±0.05 (P ≤ 0.01) for males in two separate experiments (Fig. 6). We concluded that IL-6 overexpression decreases MCh-induced bronchoconstriction in these animals.

Discussion

We studied the in vivo role of IL-6 in airway inflammation, airway obstruction, and reactivity to methacholine. Our goal in
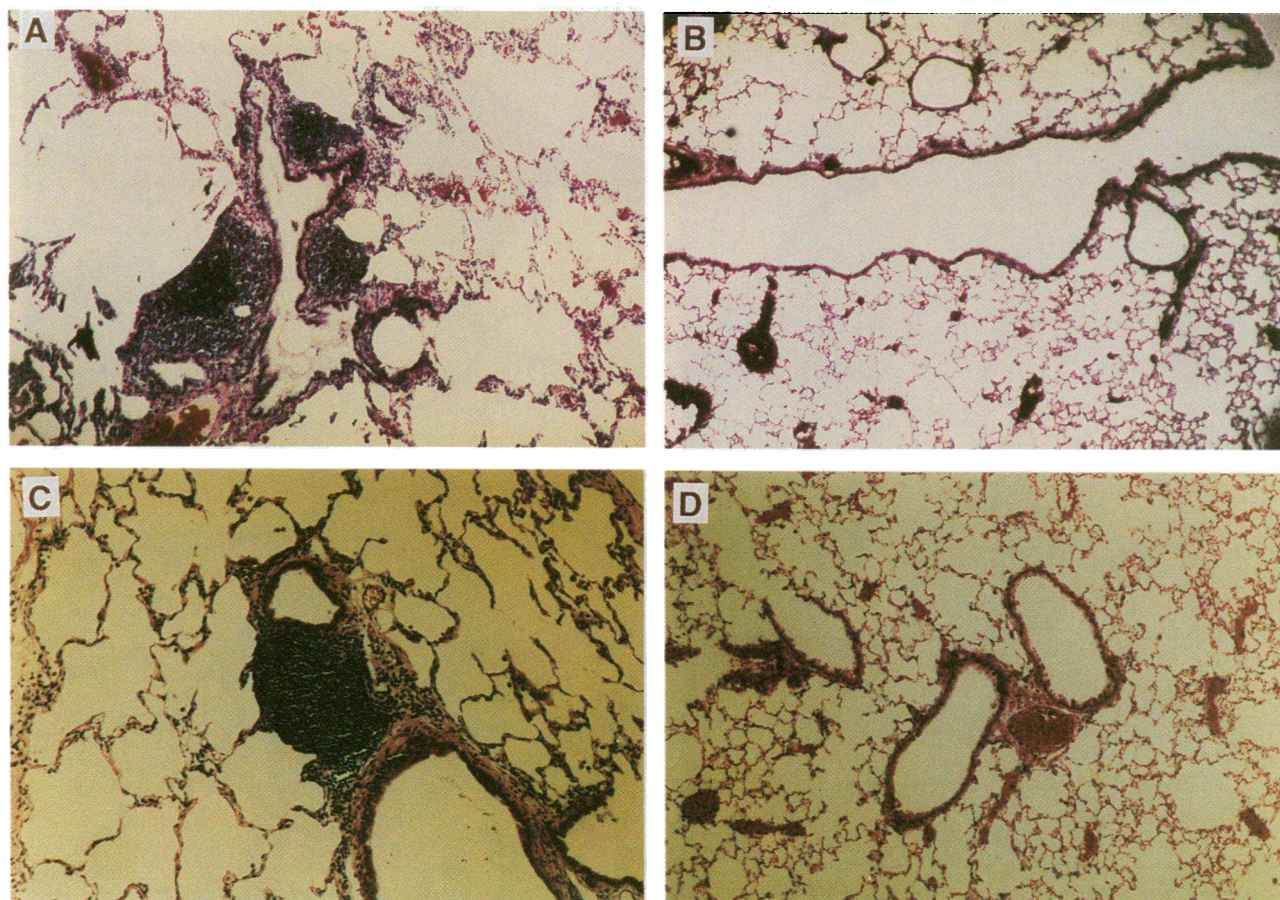
Figure 3. Lung histology. Lungs were inflated and fixed in 10% formalin overnight. Sections were embedded in paraffin, cut onto slides, and stained with hematoxylin and eosin. Large airways are shown in the top panels. (*A*) Transgene positive, ×4; (*B*) transgene negative, ×4. Mid-sized airways are shown in the bottom panels. (*C*) Transgene positive, ×10; (*D*) transgene negative, ×10.

these studies was twofold: first, to determine whether IL-6 causes an inflammatory response, and, second, to determine whether this inflammation is sufficient to produce airway obstruction or airway hyperresponsiveness. Our hypothesis was that the inappropriate expression of IL-6 in the airway would be a sufficient signal to initiate and sustain an inflammatory response and that this inflammation would cause airway hyperresponsiveness. To provide a constant supply of cytokine in the microenvironment of the airway while limiting the complex systemic effects of this cytokine, we produced transgenic mice in which the IL-6 gene was expressed in airway epithelial cells under the direction of the Clara cell CC10 promoter. Our results show that IL-6 expression in airway epithelial cells was sufficient to induce a lymphocytic inflammatory response around large and mid-sized airways (Fig. 3). Importantly, our results disproved the second half of our hypothesis since this inflammation did not alter basal airway resistance (Table II) and diminished the methacholine sensitivity of these animals (Fig. 6).

IL-6 is a pleotropic cytokine that is the product of a single gene on chromosome 7. It is produced by a wide variety of cells including fibroblasts, monocyte/macrophages, endothelial cells, T and B lymphocytes, and keratinocytes. Studies from this and other laboratories have shown that IL-6 is induced by a number of cytokines (IL-1, TNF, PDGF), viruses, and endotoxin via transcriptional and posttranscriptional mecha-

nisms (for review see reference 30). IL-6 possesses an impressively diverse spectrum of biologic activities, both proinflammatory and antiinflammatory. The proinflammatory effects include the ability to stimulate the proliferation of thymocytes and mature T cells (13, 14), stimulate cytotoxic T lymphocyte differentiation (15), upregulate IL-4–dependent IgE synthesis (16), mediate the terminal differentiation of B cells, and induce synthesis of IgM, IgG, and IgA by B cells (31). The antiinflammatory properties of IL-6 include the induction of the hepatic acute phase response (32), stimulation of the proliferation of keratinocytes (33), inhibition of macrophage and monocyte TNF production (20, 34), inhibition of macrophage proliferation (35), and reduction of monocyte cytotoxicity (34). Not surprisingly, IL-6 has been implicated in the pathogenesis of a variety of inflammatory, infectious, and malignant disorders including wound healing (36), acute transplant rejection (37), diabetes mellitus (38), rheumatoid arthritis (39), Castleman's disease (40), cardiac myxomas (41), mesangioproliferative glomerulonephritis (42), myeloblastic leukemia (43), Kaposi's sarcoma (44), and asthma (45–48). In the majority of these disorders, however, only an association between abnormal and/ or dysregulated IL-6 production and disease expression has been documented. In some, IL-6 might have directly contributed to disease pathogenesis. In others, it is felt that IL-6 elaboration is a nonspecific generalized alarm signal which limits tissue injury via the induction of the hepatic acute phase response and
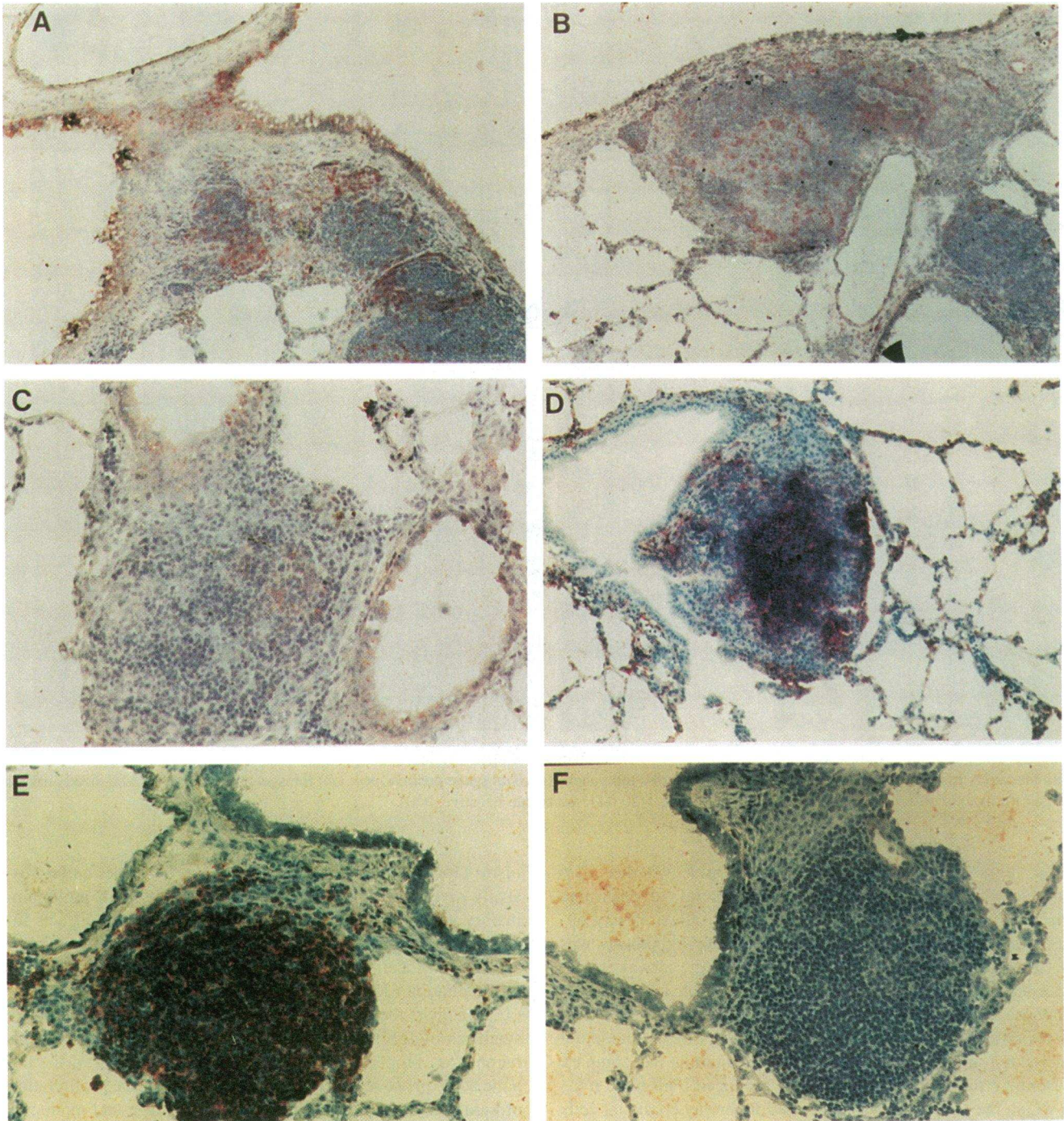
Figure 4. Immunohistochemistry. Lungs were fixed in acetone and frozen at −70°C, cut onto silane-coated slides, stained with appropriate 1° biotinylated antibodies, incubated with streptavidin-alkaline phosphatase, and developed using Fast Red, counterstained with hematoxylin. All sections shown are from transgene-positive animals. (*A*) Anti-CD3, ×10; (*B*) anti-CD4, ×10; (*C*) anti-CD8, ×20; (*D*) anti−MHC class II, ×10; (*E*) anti-B220, ×20; and (*F*) anti-F4/80, ×20.

other poorly clarified mechanisms. This is clearly the case in animal models of hypersensitivity pneumonitis where treatment with IL-6 decreases pulmonary inflammation and scarring while treatment with an anti−IL-6 antibody increases pulmonary inflammation and scarring (17), in animal models of oxygen toxicity where IL-6 enhances TNF and IL-1−induced protection against oxygen toxicity (18), and in animal models of pulmonary inflammation where intratracheal IL-6 inhibits the acute

neutrophilic lung infiltration that occurs after the administration of intratracheal endotoxin (19).

A variety of studies have demonstrated dysregulated IL-6 production in situations relevant to the asthmatic diathesis. Elevated levels of IL-6 have been found in the BALF of patients with asthma (45, 46). Immunocytochemical evaluation of BAL cells from these asthmatics demonstrates that IL-6 is predominantly produced by nonciliated epithelial cells (Clara cells) and
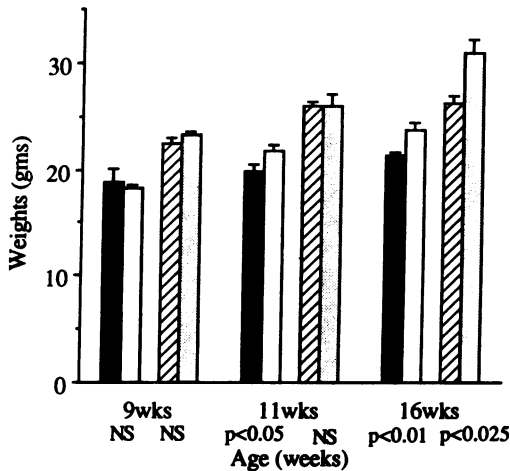
Figure 5. Body weights. Transgene-positive males (*Tg Pos M*, striped bars) weighed significantly less than transgene-negative males (*Tg Neg M*, dotted bars) at 16 wk of age, 26.2±0.78 vs 30.8±1.3 g, $P \leq 0.025$. Transgene-positive females (*Tg Pos F, filled bars*) also weighed significantly less than transgene-negative females (*Tg Neg F, open bars*) at 11 and 16 wk, 19.8±0.67 vs 21.7±0.6 g, $P \leq 0.05$ and 21.38±0.78 vs 23.68±0.67 g, $P \leq 0.01$, respectively. There was no difference in the males at 9 or 11 wk or in the females at 9 wk. Each data point represents the mean of two to six mice±SEM.
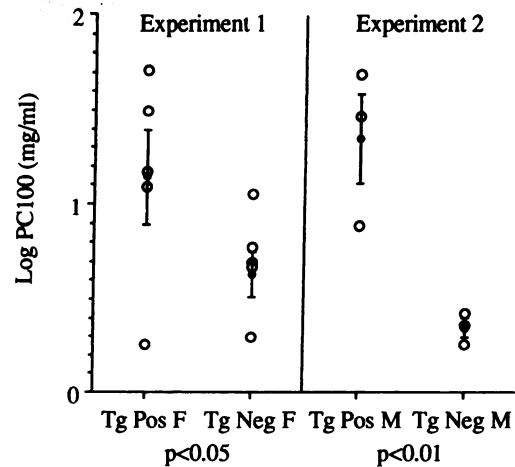
Figure 6. Airway hyperresponsiveness. Two separate experiments were done comparing age-matched transgene-positive and transgene-negative mice. Transgene-positive females (*Tg Pos F*) had a significantly higher log PC100 than transgene-negative females (*Tg Neg F*) (1.15 vs 0.6) ($P \leq 0.05$); transgene-positive males (*Tg Pos M*) had a significantly higher log PC100 than transgene-negative males (*Tg Neg M*) (1.35 vs 0.3) ($P \leq 0.01$), thus, transgene-positive mice are less responsive to methacholine than transgene-negative mice. Each open point represents one mouse, and the solid point represents the mean of the group±SEM.

alveolar macrophages (45). Bronchial epithelial cells obtained by bronchial biopsy of asthmatic patients and placed in culture produce elevated levels of IL-6 (47). Alveolar macrophages obtained after bronchial allergen challenge of asthmatic patients secrete elevated levels of IL-6 (48). Similarly, an asthmagenic stimulus, ozone, induces elevated levels of IL-6 in the BALF of normal human subjects (49). The role that IL-6 plays in these asthma states, however, has not been determined. Our studies suggest that IL-6 in these disorders may actually serve to normalize airway physiology.

Studies of the inflammatory response in asthmatic airways have demonstrated that T lymphocytes, particularly Th₂-type cells (9) and eosinophils (1–7), appear to play an important role in this process. Using monoclonal antibodies, we have demonstrated that the peribronchial infiltrate in IL-6 transgenic mice consists predominantly of MHC class II^{+}, B220^{+} cells (B cells), and CD4^{+} cells (T cells) (Fig. 4). Despite this impressive inflammatory response, however, these animals do not have altered basal airway resistance (Table II) and have a significantly higher log PC100 (Fig. 6), thus are less responsive to methacholine than their transgene-negative littermates.

The difference in physiology produced by IL-6–induced

Table II. Airway Resistance

Age	Transgene +	Transgene −	P value
wk	*cmH₂O/ml per s*	*cmH₂O/ml per s*	
9	0.47±0.06	0.43±0.04	NS
17	0.45±0.07	0.43±0.09	NS

Airway resistance was measured in transgene-positive (+) and -negative (−) mice as described. Resistance values represent mean±SEM, and there were three to seven mice per group.

airway inflammation and that associated with airway inflammation in asthma is striking. This may be the result of differences in the subset, state of activation, or location of cells that are infiltrating the airways in these two processes. Alternatively, eosinophilic infiltration and/or subepithelial fibrosis may be crucial to the development of asthmatic airway hyperreactivity since these features are commonly seen in asthmatics (50, 51) and are lacking in our IL-6 transgene-positive animals.

Three transgenic models have been constructed previously with IL-6. We have used the rat insulin promoter to stimulate the selective expression of IL-6 in the pancreas of NOD (spontaneously diabetic) mice. These animals have delayed onset of diabetes compared with their littermate control NOD mice, thus suggesting protection (our unpublished data). Suematsu et al. (52) used the IgG heavy chain promoter to overexpress IL-6 systemically. These mice developed a polyclonal hyperglobulinemia and a diffuse plasmacytosis. In contrast, Turksen et al. (53) used the keratin promoter to express IL-6 in the epidermis. In this location, IL-6 did not produce psoriasis and did not cause inflammatory cell infiltration. Instead, there was thickening of the stratum corneum suggesting that, in this model, IL-6 production served as a protective mechanism against infection or injury (53). These results illustrate the importance of analyzing the effects of IL-6 in an organ-specific fashion and support our use of an airway-selective promoter to analyze the role of IL-6 in the asthmatic diathesis.

The relationship between airway inflammation and hyperresponsiveness remains poorly defined. In the model described in this manuscript, we can unambiguously implicate the product of a single well defined gene (IL-6) in airway inflammation. More importantly, we show for the first time in an animal model an uncoupling of airway inflammation and airway hyperresponsiveness. This finding leads us to hypothesize that some forms of airway inflammation may not necessarily lead to changes in basal airway muscle tone, airway edema, or hyperreactivity

typical of the asthmatic diathesis. Our findings suggest that IL-6 promotes a type of airway inflammation that decreases airway hyperresponsiveness to bronchoconstrictor agents. Thus, although IL-6 may contribute to airway inflammation, it is unlikely to be the only cytokine responsible for the establishment and maintenance of all aspects of the asthmatic diathesis. IL-6 production in asthmatic airways may in fact represent an attempt to mount a healing response designed to restore normal airway responsiveness in airways that are inflamed and hyperresponsive as a result of other insults and/or mediators. The demonstration that IL-6–induced airway inflammation can diminish airway responsiveness has important implications for the pathogenesis and treatment of the asthmatic diathesis.

Acknowledgments

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