IL-13 is an important stimulator of inflammation and tissue remodeling at sites of Th2 inflammation, which plays a key role in the pathogenesis of a variety of human disorders. We hypothesized that the ubiquitous transcription factor, early growth response-1 (Egr-1), plays a key role in IL-13-induced tissue responses. To test this hypothesis we compared the expression of Egr-1 and related moieties in lungs from wild type mice and transgenic mice in which IL-13 was overexpressed in a lung-specific fashion. We simultaneously characterized the effects of a null mutation of Egr-1 on the tissue effects of transgenic IL-13. These studies demonstrate that IL-13 stimulates Egr-1 via an Erk1/2-independent Stat6-dependent pathway(s). They also demonstrate that IL-13 is a potent stimulator of eosinophil- and mononuclear cell-rich inflammation, alveolar remodeling, and tissue fibrosis in mice with wild type Egr-1 loci and that these alterations are ameliorated in the absence of Egr-1.

Lastly, they provide insights into the mechanisms of these processes by demonstrating that IL-13 stimulates select CC and CXC chemokines (MIP-1α/CCL-3, MIP-1β/CCL-4, MCP-2/CCL-8, MCP-3/CCL-7, MCP-5/CCL-12, KC/CXCL-1, and Lix/CXCL-5), matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1, and apoptosis regulators (caspase-3, -6, -8, and -9 and Bax) and activates transforming growth factor-β1 and pulmonary caspasas via Egr-1-dependent pathways. These studies demonstrate that Egr-1 plays a key role in the pathogenesis of IL-13-induced inflammatory and remodeling responses.

Interleukin (IL)-13 is a 12-kDa product of a gene on chromosome 5q31 that is produced in large quantities by stimulated Th2 cells. It was originally described as an IL-4-like molecule based on shared effector properties including the ability to stimulate IgE production. Subsequent studies demonstrated that IL-13 stimulates select CC and CXC chemokines (MIP-1α/CCL-3, MIP-1β/CCL-4, MCP-2/CCL-8, MCP-3/CCL-7, MCP-5/CCL-12, KC/CXCL-1, and Lix/CXCL-5), matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1, and apoptosis regulators (caspase-3, -6, -8, and -9 and Bax) and activates transforming growth factor-β1 and pulmonary caspasas via Egr-1-dependent pathways. These studies demonstrate that Egr-1 plays a key role in the pathogenesis of IL-13-induced inflammatory and remodeling responses.

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Egr-1 Modulates IL-13 Effector Pathways

MATERIALS AND METHODS

Overexpression Transgenic Mice—CC10-IL-13 transgenic mice were generated in our laboratory, bred onto a C57BL/6 background, and used in these studies. These mice utilize the Clara cell 10-kDa protein (CC10) promoter to target IL-13 to the lung. The methods that were used to generate and characterize these mice were described previously (5). In this modeling system, IL-13 caused a mononuclear cell- and eosinophil-rich tissue inflammatory response, alveolar enlargement, subepithelial and parenchymal fibrosis, mucus metaplasia, and respiratory failure and death as previously described (5, 6, 14).

Breeding to Egr-1 Null Mutant (~/~), Stat6 Null, and Dominant-negative MEK-1 Overexpressing Mice—CC10-IL-13 transgenic animals were bred with mice with wild type and null Egr-1 or Stat6 loci. Egr-1 (~/~) mice were a generous gift from Dr. Jeffrey Milbrandt, Washington University, St. Louis, MO. Stat6 (~/~) mice were purchased from Jackson laboratory (Bar Harbor MA) (25). In all cases these mice had been bred for >10 generations onto a C57BL/6 genetic background. As a result of these crosses, CC10-IL-13 animals with (+/+ and (~/~) Egr-1 or Stat6 loci and CC10-IL-13 mice with (+) and without (~) the dominant-negative MEK-1 transgene were generated. Genotyping was accomplished as previously described (5, 26). The phenotypes of these mice were compared as described below.

In Vivo Administration of PD98059—Wild type and CC10-IL-13 animals were exposed to the MEK/Erk1/2 inhibitor PD98059 (Calbiochem) (5 mg/kg/day, via an intraperitoneal route) or its vehicle control for 14 days.

Bronchoalveolar Lavage (BAL)—Lung inflammation was assessed by BAL as described previously (6, 27). The BAL samples from each animal were pooled and centrifuged. The number and types of cells in the cell pellet were determined with light microscopy. The supernatants were stored at −20 °C until used.

Lung Volume and Morphometric Assessments—Animals were anesthetized, the trachea was cannulated, and the lungs were removed and inflated with phosphate-buffered saline at 25 cm. The size of each lung was evaluated via volume displacement, and alveolar size was estimated from the mean chord length of the airspace as previously described by our laboratory (6). Chord length increases with alveolar enlargement.

Histologic Evaluation—Animals were sacrificed, a median sternotomy was performed, and right heart perfusion was accomplished with calcium and magnesium-free phosphate-buffered saline. The heart and lungs were then removed en bloc inflated at 25 cm pressure with neutral buffered 10% formalin, fixed in 10% formalin, embedded in paraffin, sectioned, and stained. Hematoxylin and eosin, Mallory’s trichrome, and periodic acid-Schiff with diastase stains were performed in the Research Histology Laboratory of the Department of Pathology at Yale University School of Medicine.

mRNA Analysis—mRNA levels were evaluated by conventional reverse transcription PCR analysis as described previously (6, 28). The primers that were employed have been described (6, 14, 16, 17). For each cytokine, the optimal numbers of cycles that will produce a quantity of cytokine product that is directly proportional to the quantity of input mRNA was determined experimentally. β-Actin was used as an internal standard. Amplified PCR products were detected using ethidium bromide gel electrophoresis, quantitated electronically, and confirmed by nucleotide sequencing. In selected experiments, real time reverse transcription PCR was used as previously described (28), and the data are presented in the supplemental materials.

Quantification of IL-13, TGF-β, and Chemokines—BAL IL-13, TGF-β, and chemokine levels were quantitated using commercial enzyme-linked immunosorbent assay kits (R&D Systems, Inc., Minneapolis, MN) per the manufacturer’s instructions.

Quantification of Lung Collagen—Collagen content was determined biochemically by quantifying total soluble collagen using the Sircol Collagen Assay kit (Biocolor, Northern Ireland) according to the manufacturer’s instructions (16). The data are expressed as the collagen content of the entire right lung.

TUNEL Evaluations—End labeling of exposure 3'-OH ends of DNA fragments was undertaken with the TUNEL in situ cell death detection kit AP (Roche Diagnostics) as described by the manufacturer. After staining, 20 fields of alveoli were randomly chosen for examination. The labeled cells were expressed as a percentage of total nuclei.

Immunoblott Analysis—Lung lysates were prepared, and Western analysis was undertaken with antibodies that reacted selectively with Egr-1, caspase-3, caspase-7, caspase-8, poly(ADP-ribose) polymerase (PARP), β-tubulin (Santa Cruz Biotechnology, Santa Cruz, CA), and inhibitor of caspase-activated DNase (ICAD) (Chemicon, Temecula, CA) as previously described (30).

Statistics—Normally distributed data are expressed as means ± S.E. and assessed for significance by Student’s t test or analysis of variance as appropriate. Data that were not normally distributed were assessed for significance using the Wilcoxon rank sum test.

RESULTS

IL-13 Regulation of Egr-1—To begin to understand the importance of Egr-1 in the pathogenesis of IL-13-induced tissue alterations, studies were undertaken to determine whether IL-13 regulated the expression and/or production of Egr-1 and related moieties. These experiments demonstrate that transgenic IL-13 is a potent stimulator of Egr-1 mRNA (Fig. 1 A) and Egr-1 protein (Fig. 1 B). These effects were not specific for Egr-1 because Egr-2 and Egr-3 are similarly regulated. However, the Egr-1-binding proteins NAB-1 and NAB-2 were not similarly altered (Fig. 1 A).

Role of Stat6 and Erk1/2 in IL-13 Stimulation of Egr-1—The signaling pathways that mediate the stimulatory effects of IL-13 were also evaluated. In these experiments we initially evaluated the role of Stat6, the canonical signaling pathway for IL-13. This was done by comparing the effects of transgenic IL-13 in mice with wild type and null Stat6 loci. Because Egr-1 expression can also be regulated via Erk1/2-dependent pathways (31–33), we also evaluated the role of Erk1/2 signaling in the stimulation of Egr-1 in IL-13 transgenic mice (Tg). This was done by crossing IL-13 Tg mice with mice in which a dominant-negative MEK-1 construct was overexpressed in a lung-specific fashion. As previously reported by our laboratory (34) these mice have a defect in Erk1/2 activation and significant alterations in IL-13 effector pathway activation. These results were compared with the results that were obtained with the specific pharmacologic Erk inhibitor (PD98059). IL-13 induction of Egr-1 was not significantly altered in IL-13 Tg mice in which dominant-negative MEK-1 was expressed (Fig. 1 C). PD98059 treatment also did not alter IL-13 stimulation of Egr-1 when compared with vehicle-treated Tg (+) animals (Fig. 1 C). In contrast, IL-13 stimulation of EGR-1 was completely abrogated by the null mutation of Stat6 (Fig. 1 C). Collectively, these results demonstrate that IL-13 induces Egr-1 via an Erk1/2-independent and Stat6-dependent pathway(s).

Role of Egr-1 on IL-13-induced Inflammation—Studies were next undertaken to define the role of Egr-1 plays in IL-13-induced inflammation. In these experiments we compared the BAL and tissue inflammatory responses in transgene (Tg) (~) and Tg (+) mice with wild type (+/+ and null mutant (~/) Egr-1 loci. As previously reported, transgenic IL-13 caused BAL and tissue inflammation with enhanced total...
cell, eosinophil, and lymphocyte responses (Fig. 2, A and B, data not shown). Egr-1 appeared to play an important role in these responses because tissue (Fig. 2A) and BAL inflammation were all significantly diminished in Tg (+/H11001) mice with null mutant Egr-1 loci (Fig. 2B). These studies demonstrate that Egr-1 plays a critical role in IL-13-induced pulmonary inflammation.

Role of Egr-1 in IL-13-induced Pulmonary Fibrosis—Because IL-13 is a major fibrogenic mediator at sites of Th2 inflammation (1, 3), studies were undertaken to define the role of Egr-1 in the IL-13-induced fibrotic response. In these studies we used trichrome evaluations and lung collagen assessments to characterize the fibrotic response in Tg (+/H11002) and Tg (+/H11001) mice with (+/H11001) and (+/H11002) Egr-1 loci. As previously reported by our laboratory (5), transgenic IL-13 caused peribronchial and interstitial fibrosis in Tg (+/H11001) mice with (+/H11001) Egr-1 loci (Fig. 3A). This induction was readily appreciated in trichrome evaluations (Fig. 3A) and biochemical assays (Fig. 3B). This fibrotic response was Egr-1-dependent because trichrome and biochemical assays demonstrated marked decreases in collagen accumulation in Tg (+/H11001) mice with null mutant versus (+/H11002) Egr-1 loci (Fig. 3, A and B). Thus, IL-13-induced tissue fibrosis is mediated via a mechanism that is, at least in part, Egr-1-dependent.

Role of Egr-1 in IL-13-induced Alveolar Remodeling—Previous studies from our laboratory highlighted the ability of IL-13 to induce an alveolar remodeling response (6). Thus, studies were undertaken to define the role of Egr-1 in these responses. In accord with our previous observations in Egr-1-sufficient mice (6), transgenic IL-13 caused impressive increases in pulmonary compliance and alveolar enlargement after pressure fixation (Fig. 4). Egr-1 played an important role in these responses because IL-13-induced alveolar remodeling (Fig. 4A) and alveolar enlargement (Fig. 4, A and B) were diminished in Tg (+/H11001) mice with null mutant Egr-1 loci. These studies demonstrate that IL-13 induces alveolar remodeling via a mechanism that is, at least in part, Egr-1-dependent.

Effect of Egr-1 Deficiency on IL-13 Elaboration—A deficiency of Egr-1 could modify IL-13-induced tissue responses by altering IL-13 production or modifying IL-13 effector functions. To differentiate among these options, we compared the levels of BAL IL-13 in Tg (+/H11002) and Tg (+/H11001) mice with wild type and null mutant Egr-1 loci. IL-13 was not readily apparent in BAL fluids from Tg (−/−) mice with wild type or null mutant Egr-1 loci. In contrast, significant levels of BAL of IL-13 were noted in doxycycline-treated Tg (+/+) animals. These levels, however, were similar in Tg (+/+) mice with wild type and null mutant Egr-1 loci (data not shown). This demonstrates that the ablation of Egr-1 alters the IL-13 phenotype by modifying IL-13-induced effector pathway activation.

Role of Egr-1 and IL-13-induced Chemokine Elaboration—To investigate the mechanism(s) by which Egr-1 deficiency inhibited IL-13-induced inflammation, we compared the expression of selected chemokines in Tg (−/−) and Tg (+/+) mice with wild type and null mutant Egr-1 loci. In Tg (−/−) mice with wild type or null mutant Egr-1 loci, the levels of mRNA encoding MIP-1α/CCL-3, MIP-1β/CCL-4, MIP-2/CXCL2/3, MCP-1/CCL-2, MCP-2/CCL-8, MCP-3/CCL-7, MCP-5/CCL-12, KC/CXCL-1, and Lix/CXCL-5 were at or below the limits of detection.
in our assays (Fig. 5). As previously reported by our laboratory (35), IL-13 caused a marked increase in the levels of mRNA encoding these chemokine moieties in Tg (+/) mice with wild type Egr-1 loci. These alterations in mRNA were associated with comparable alterations in the levels of these chemokines in BAL fluids (data not shown). In the absence of Egr-1, however, the ability of IL-13 to stimulate MIP-1α/CCL-3, MIP-1β/CCL-4, MIP-2/CXCL2/3, MCP-1/CCL-2, MCP-2/CCL-8, MCP-3/CCL-7, MCP-5/CCL-12, KC/CXCL-1, and Lix/CXCL-5 mRNA and/or protein were markedly diminished (Fig. 5). Interestingly, Mig/CXCL-9, IP-10/CXCL-10, SDF-1/CXCL-12, and

FIGURE 2. Role of Egr-1 in IL-13-induced inflammation. 2-month-old Tg (+/) and TG (-/) littermate controls with (+/+ ) and (-/-) Egr-1 loci were generated. The histologic appearance of their lungs on hematoxylin and eosin evaluations (A, 10× original magnification) and BAL cell recovery (B) are compared. A is representative of a minimum of five similar evaluations, and in B the values represent the mean ± S.E. of evaluations in a minimum of five animals (*, p < 0.01).

FIGURE 3. Role of Egr-1 in IL-13-induced fibrosis. The collagen content of lungs from 3-month-old IL-13 Tg (-/) and (+/) mice with (+/+ ) and (-/-) Egr-1 loci were compared using Mallory's trichrome (A) and sircol (B) collagen evaluations. A is representative of a minimum of five similar evaluations. In B, each value represents the mean ± S.E. of evaluations in a minimum of four mice (*, p < 0.05).
lungkine/CXCL-15 were not altered by IL-13, and their levels of expression were not modified by the absence of Egr-1 in Tg (−) and Tg (+) animals (Fig. 5). IL-13 also induces potent eosinophil chemoattractant eotaxin/CCL11 and its receptor CCRL3. Their expression was markedly decreased in the absence of Egr-1, whereas the expression of the neutrophil chemoattractant RANTES/CCL-5 and its receptor CCR1 were not altered in the absence of Egr-1 (see Fig. 1 in supplemental materials). When viewed in combination, these studies demonstrate that IL-13 stimulates select CC and CXC chemokines and their receptors in the lung via Egr-1-dependent pathways.

Importance of Egr-1 on IL-13-induced Protease Alterations—We reasoned that a deficiency of Egr-1 could modulate IL-13-induced inflammatory and alveolar phenotypes by decreasing the production of respiratory proteases (6, 17). To test this hypothesis, we compared the levels of mRNA encoding lung-relevant MMPs and cathepsins in Tg (−) and Tg (+) mice with wild type and null mutant Egr-1 loci. Comparable levels of mRNA encoding MMP-2, MMP-9, MMP-12, MMP-14, TIMP-1, TIMP-2, cathepsin-B, -H, -K, -L, -S, and cystatin C were found in lungs from Tg (−) mice with wild type and null mutant Egr-1 loci (Fig. 6 and Fig. 1 in supplemental materials). In accord with previous studies from our laboratory (6) doxycycline induction of IL-13 increased the levels of expression of these MMPs and cathepsins (Fig. 6 and Fig. 1 in supplemental materials). Interestingly, Egr-1 deficiency decreased the ability of IL-13 to stimulate the accumulation of mRNA encoding MMP-9 and TIMP-1. Egr-1 deficiency did not alter the ability of IL-13 to regulate the accumulation of mRNA encoding MMP-2, MMP-12, MMP-14, TIMP-2, or cathepsin-B, -H, -K, -L, and -S. These studies demonstrate that IL-13 induces MMP-9 and TIMP-1 via a pathway that is partially Egr-1-dependent.

Role of Egr-1 in IL-13-induced DNA Injury and Cell Death—Previous studies from our laboratory demonstrated that Egr-1 is an important regulator of DNA injury and cell death in the cytokine-treated murine lung (30). Thus, studies were undertaken to determine whether transgenic IL-13 caused DNA injury and cell death, and the role of Egr-1 in this response was evaluated. In these studies we compared the level of DNA injury and cell death by comparing TUNEL evaluations of lungs from Tg (−) and Tg (+) mice with wild type and null mutant Egr-1 loci. As noted in Fig. 7, TUNEL (+) cells were not readily apparent in lungs from Tg (−) mice with wild type or null mutant Egr-1 loci. In contrast, TUNEL staining was readily apparent in lungs from Tg (+) mice with wild type Egr-1 loci (Fig. 7A). This staining was readily apparent in alveolar macrophages and could also be appreciated in alveolar structures. In these structures, double labeling experiments demonstrated that many of the TUNEL (+) cells were alveolar type II cells that stained positively for surfactant apoprotein-C (data not shown). Egr-1 played an important role in this TUNEL response because TUNEL staining was markedly diminished in lungs from Tg (+) mice with null mutant Egr-1 loci (Fig. 7A and B). In accord with this finding, IL-13 increased the levels of mRNA encoding caspase-3, -6, -8, -9 and -Bax (Fig. 7C), enhanced the activation of caspase-3, -7, and -8, and enhanced cleavage of the caspase targets ICAD and PARP (Fig. 7D). These responses were also Egr-1-dependent with the levels of mRNA encoding caspase-3, -6,
-8, and -9, and Bax, the levels of activation of caspase-3, -7, and -8, and the levels of ICAD and PARP cleavage being diminished in Tg (+) mice with null Egr-1 loci (Fig. 7, C and D). These studies demonstrate that IL-13 is a potent inducer of DNA injury and cell death in the murine lung where it induces and activates the caspases and Bax. These studies also demonstrate that this IL-13-induced DNA injury and caspase induction and activation are mediated by a pathway that is, at least in part, Egr-1-dependent.

**Role of Egr-1 in TGF-β1 Induction and Activation**—To begin to understand the mechanism by which a deficiency in Egr-1 altered IL-13-induced tissue fibrosis, studies were undertaken to determine whether TGF-β1 is regulated in an Egr-1-dependent fashion. In these studies we compared the stimulation and activation of TGF-β1 in Tg (−) and Tg (+) mice with wild type and null Egr-1 loci. TGF-β1 was not readily detected in BAL fluids from Tg (−) mice with wild type or null mutant Egr-1 loci (Fig. 8). Interestingly, a significant amount of this TGF-β1 was spontaneously bioactive because significant levels of TGF-β1 could be detected in BAL fluids in Tg (+) mice with wild type Egr-1 loci in the absence of BAL fluid acidification (Fig. 8B). Egr-1 did not play a significant role in the production of total TGF-β1 because comparable levels of total TGF-β1 were appreciated in BAL fluids from Tg (+) mice with wild type and null mutant Egr-1 loci (Fig. 8A). Egr-1 did, however, play a critical role in the activation of TGF-β1 because the ability of IL-13 to activate TGF-β1 was markedly diminished in comparisons of BAL fluids from Tg (+) mice with wild type and null mutant Egr-1 loci (Fig. 8B). These studies demonstrate that IL-13 stimulation and activation of TGF-β1 in the murine lung are mediated by Egr-1-independent and -dependent mechanisms, respectively.

**Potential Mechanisms of Egr-1 Regulation of TGF-β1 Activation**—The studies noted above demonstrate that Egr-1 plays an important role in TGF-β1 activation in the murine lung. Previous studies from our laboratory demonstrated that MMP-9 and uPA contributed to this response (16). In addition, thrombospondin and CD44 have been demonstrated to contribute to TGF-β1 activation in other settings (36, 37). Thus, studies were undertaken to determine whether IL-13 regulated the expression of these and other TGF-β1-regulating moieties, and this
regulation was compared in Tg (+) mice with wild type and null Egr-1 loci. The levels of latent TGF-β1-binding protein, CD36, thrombospondin-1 (TSP-1), integrin-β6, uPA, plasminogen activator inhibitor-1, and CD44 were comparable in Tg (–) mice with wild type and null mutant Egr-1 loci. IL-13 increased the levels of mRNA encoding CD36, TSP-1, uPA and CD44 in lungs from mice with wild type Egr-1 loci (Fig. 8C). Interestingly, IL-13 induction of TSP-1, uPA, and CD44 in Tg (+) mice were all diminished in animals with null Egr-1 loci, whereas IL-13 regulation of CD 36 was not altered in the absence of Egr-1 (Fig. 8C). These studies demonstrate that the decreased ability of IL-13 to activate TGF-β1 in the setting of Egr-1 deficiency is associated with decreased MMP-9, TSP-1, uPA, and CD44 induction in this murine modeling system.

**DISCUSSION**

To further understand the cellular and molecular events involved in IL-13-induced phenotype generation, we took advantage of transgenic systems developed in our laboratory in which IL-13 effector pathways can be selectively assessed *in vivo* and used these systems to characterize the role(s) of Egr-1 in the pathogenesis of IL-13-induced alterations in the lung. These studies demonstrate that IL-13 is a potent stimulator of Egr-1 and other Egr family proteins and IL-13 induces Egr-1 via Stat6-dependent pathway. They also demonstrate that Egr-1 plays a central role in the pathogenesis of the IL-13 phenotype because IL-13-induced TGF-β1 is a potent stimulator of Egr-1 (30). Egr-1 can also stimulate TGF-β1 production, be stimulated by TGF-β1, and inhibit TGF-β RII expression *in vitro* (21, 39–41). When viewed in combination, one can envision a scenario in which IL-13 stimulates Egr-1, which in turn contributes to the induction of MCP-1/CCL2, uPA, and MMP-9. This would augment the production of active TGF-β1, which would feed back to further stimulate Egr-1. This would result in an amplification loop that could contribute to the chronicity, progression, and/or severity of pulmonary and extrapulmonary fibrotic disorders.

Our studies demonstrate that IL-13 regulates downstream genes via Egr-1-dependent and -independent pathways. Studies of a number of genes including MMP-9, uPA, TSP-1, and CD44 have highlighted the
transcriptional nature of the effects of Egr-1 (42–46) and the functional Egr-1 and/or Sp-1 binding sites in their promoters (42–45). However, the mechanisms underlying this differential regulation have not been fully addressed. In addition, the relationship(s) between the Egr-1-dependent alterations in MMP-9, uPA, or TSP-1 and the Egr-1-dependent alterations TGF-β1 activation will need further evaluation.

In addition to its well documented ability to induce eosinophilic inflammation, mucus metaplasia, and airways hyperresponsiveness (2, 3, 5), studies from our laboratory and others have also highlighted the ability of IL-13 to induce alveolar remodeling while stimulating MMPs and cathepsins and inhibiting a-1 antitrypsin (6). The present studies demonstrated that Egr-1 plays an essential role(s) in IL-13-induced alveolar enlargement. Surprisingly, however, Egr-1 ablation only altered the expression of MMP-9 and TIMP-1 and did not alter the expression of the other MMPs, cathepsins, and antiproteases that were assessed. This suggests that Egr-1 may also regulate protease-independent aspects of the IL-13-induced alveolar remodeling response. Previous studies from our laboratory demonstrated that IL-13 induces and activates TGF-β1 (16). They also demonstrated that TGF-β1 induces alveolar enlargement and destruction and that this response is critically dependent on Egr-1-mediated epithelial apoptosis (30). Interestingly, the present studies demonstrate that IL-13 also induces Egr-1-dependent apoptosis. As a result, it is tempting to speculate that Egr-1 contributes to the IL-13-induced alveolar remodeling response, at least in part, via its ability to modulate TGF-β1 activation and cellular apoptosis. Additional experimentation will be required, however, to address this hypothesis.

In summary, our studies demonstrate that IL-13 is a potent stimulator of Egr-1 and that Egr-1 plays an essential role in the pathogenesis of IL-13-induced inflammation, fibrosis, alveolar remodeling, and apoptosis in vivo. They also demonstrate that Egr-1 plays an important role in the ability of IL-13 to stimulate chemokines, proteases, antiproteases, and apoptosis regulators and activate TGF-β1 and caspases. Exaggerated IL-13 production has been implicated in the pathogenesis of a variety of disorders including asthma, chronic obstructive pulmonary disease, pulmonary fibrosis, scleroderma, hepatic cirrhosis, and nodular sclerosing Hodgkin's disease(2,7–12,47). The present studies suggest that the effects of IL-13 in these disorders can be beneficially controlled by Egr-1 ablation only altered the expression of MMP-9 and TIMP-1 and did not alter the expression of the other MMPs, cathepsins, and antiproteases that were assessed.