Transgenic Modeling of Transforming Growth Factor-β_1

Role of Apoptosis in Fibrosis and Alveolar Remodeling

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Inflammation and tissue remodeling with pathologic fibrosis are common consequences of Th2 responses in the lung and other organs. Interleukin (IL)-13 and transforming growth factor-β1 (TGF-β_1) are frequently coexpressed in these responses and are believed to play important roles in the pathogenesis of Th2-induced pathologies. To shed light on the mechanisms of these responses, overexpression transgenic approaches were used to selectively target each of these cytokines to the murine lung. IL-13 proved to be a potent stimulator of eosinophilic inflammation, mucus metaplasia, tissue fibrosis, and alveolar remodeling. CC chemokines, specific chemokine receptors (CCR2, CCR1), adenosine metabolism, vascular endothelial growth factor, and IL-11 contributed to the genesis of these responses. IL-13 also induced tissue fibrosis, at least in part, via its ability to induce and activate TGF-β_1. In the TGF-β_1 transgenic mouse, epithelial apoptosis preceded the onset of tissue fibrosis and alveolar remodeling. In addition, chemical (Z-VAD-fmk) and genetic (null mutations of early growth response gene 1) interventions blocked apoptosis and ameliorated TGF-β_1–induced fibrosis and alveolar restructuring. These studies define an IL-13–TGF-β_1 pathway of tissue remodeling that regulates inflammation, mucus metaplasia, apoptosis, vascular responses, and fibrosis in the lung. They also highlight the intimate relationship between apoptosis and fibrosis induced by TGF-β_1. By defining the complexities of this pathway, these studies highlight sites at which therapies can be directed to control these important responses.

Keywords: asthma; fibrosis; interleukin-13; transforming growth factor-β1; transgenic

Fibrosis is an important cause of morbidity and mortality in the lung. This is illustrated in airways disorders such as bronchiolitis obliterans syndrome and asthma, which are characterized by chronic inflammation and subepithelial/airway fibrosis (1, 2). The consequences of these responses are significant because asthma affects approximately 18 million people in the United States, and asthmatic airway remodeling is believed to be an important determinant of disease severity and natural history (1). This is also illustrated in the interstitial lung diseases (ILD), including idiopathic pulmonary fibrosis, scleroderma, and bleomycin lung, where pulmonary fibrosis is a dreaded and sometimes fatal disease endpoint (3). The consequences of these diseases are also significant, with ILD manifesting a prevalence from 3 to 26 per 100,000 per year and undiagnosed ILD occurring at a rate that is 10 times that of recognized disease (3). The mechanisms of tissue fibrosis in these lung disorders are poorly understood.

A chronic Th2-dominated inflammatory response is believed to be the cornerstone of the asthmatic diathesis, and IL-13 is believed to be the key fibrogenic effector in this disorder (4–6). Studies from our laboratory recently demonstrated that IL-13 mediates its fibrogenic effects, at least in part, via its ability to induce and activate transforming growth factor (TGF)-β_1 (7). These findings are in accord with and support the "type 2 cytokine hypothesis of fibrosis," which suggests that fibrosis occurs in chronic inflammatory disorders when cytokine balance shifts in a Th2 (Type II) direction (8). In keeping with this hypothesis, IL-13 is also dysregulated in, and may play a critical role in, many interstitial lung disorders, including idiopathic pulmonary fibrosis, scleroderma, radiation-induced pulmonary fibrosis, and bleomycin lung (9–15). TGF-β_1 also plays a critical role in the pathogenesis of these and other disorders (7, 16–24).

TGF-β_1 EFFECTOR FUNCTIONS

TGF-β_1 family proteins are multifunctional cytokines that play pivotal roles in diverse biologic processes, including the regulation of cell growth and survival, cell and tissue differentiation, development, inflammation, immunity, hematopoiesis, and tissue remodeling and repair. On superficial analysis, TGF-β_1 can be accurately described as a healing molecule that manifests antiinflammatory and fibrotic effects while inducing wound healing. This perspective, however, is only partially correct, and the effector profile of TGF-β_1 can seem confusing and even contradictory. This is noted in the setting of inflammation where TGF-β_1 has important antiinflammatory and immunosuppressive effects in some settings (25, 26) and proinflammatory effects in others (26, 27). This is also seen in oncogenesis, where TGF-β_1 can exert potent growth inhibitory effects on tumor cells while enhancing tumor cell migration and invasion. Particularly relevant to the present study are the studies that demonstrate that, in the proper setting, TGF-β_1 is essential for wound healing, stimulates matrix molecule deposition and angiogenesis, and is an essential mediator of the pathologic scarring in fibrotic disorders (7, 17, 28–31). On the other hand, TGF-β_1 can induce tissue injury (32) and cellular apoptosis, decrease epithelialization, and inhibit wound healing (33–36).

The complexity of TGF-β_1 effector function can be attributed to a number of items. First, the effects of TGF-β_1 proteins vary with the state of activation and differentiation of the target cell and the presence of other stimuli in the local microenvironment. In addition, it has been demonstrated that TGF-β_1 can exert its seemingly antagonistic effects via different effector pathways. This is illustrated in Smad-3 null mutant mice, which manifest enhanced wound healing and epithelialization (33–36). The fact that these responses are perceived to be contradictory may also reflect an inadequate understanding of the events that are required for TGF-β_1 to induce its complex tissue phenotypes. Specifically, the kinetics of TGF-β_1 induction of its tissue phenotypes are poorly understood, and, in virtually all studies, it has been assumed that each phenotype is a distinct endpoint. The possibility

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that TGF-β_1 may induce one phenotype only if it induces an earlier "different" phenotype has not been considered. As a result, the relationships between endpoints, such as apoptosis and fibrosis, have not been investigated.

IL-13 AS A MEDIATOR OF AIRWAY REMODELING

IL-13 was discovered as an IL-4–like molecule and was presumed to represent a genetic duplication of this Th2 cytokine. More recently, however, it has become clear that IL-13 and IL-4 differ significantly in their contributions to the Th2 inflammatory response, with IL-13 being a major effector at sites of Th2 inflammation (4, 6). Studies using overexpression (OE) transgenic mice played a major role in this finding. In these studies, the transgenic OE of IL-13 in these animals caused an asthma-like phenotype that included (*1*) eosinophil-, macrophage-, and lymphocyte-rich inflammation; (*2*) mucus metaplasia with goblet cell hyperplasia and the hyperproduction of neutral and acidic mucus; and (*3*) subepithelial fibrosis in the lamina reticularis that extended into the adventitia of the airway. These animals did not have significant abnormalities in baseline airway resistance. They did, however, demonstrate airway hyperresponsiveness on methacholine challenge. These studies demonstrate that IL-13 has the ability to produce many of the pathologies that are characteristic of human asthma (37). In keeping with the role of IL-13 in ILD, prolonged IL-13 elaboration also caused the airway phenotype to spread into the parenchyma. This caused a fibrodestructive disorder and premature death due to respiratory failure (38).

MECHANISMS OF IL-13–INDUCED PHENOTYPE GENERATION

A variety of interventions can be used in transgenic mice to define *in vivo* the mechanisms that are responsible for the disease-relevant phenotypes that are seen. This can be done with interventions such as antibody neutralization. Powerful results are also obtained by breeding the OE mice with mice with targeted null mutations of downstream genes. This allows one to compare the effects of the transgene in mice with (+/+), (+/−), and (−/−) downstream gene loci. These approaches have been used to characterize major aspects of the IL-13 phenotype.

Mechanisms of IL-13–induced Inflammation

To define the mechanisms of IL-13–induced inflammation, we characterized the effects of IL-13 on the elaboration of inflammation-generating chemokines and the importance of selected chemokine receptors in the pathogenesis of the IL-13–induced tissue response. Transgenic IL-13 was a potent stimulator of monocyte chemotactic protein (MCP)-1, CCL2, MCP-2 (CCL-8), MCP-3 (CCL-7), and MCP-5 (CCL12). This stimulation was not specific for MCPs because macrophage inhibitory protein (MIP)-1α (CCL3), MIP-1β (CCL4), MIP-2 (CXCL1), MIP-3α (CCL20), thymus- and activation-regulated chemokine (TARC, CCL17), thymus-expressed chemokine (TECK, CCL25), eotaxin (CCL11), eotaxin-2 (CCL24), macrophage-derived chemokine (MDC, CCL22), and C10 (CCL6) were prominently induced. The ability of IL-13 to increase lung size, alveolar size, and lung compliance; to stimulate pulmonary inflammation, hyaluronic acid accumulation, and tissue fibrosis; and to cause respiratory failure and death was markedly decreased in $CCR2^{-/-}$ mice, whereas mucus metaplasia was not altered (38). Similar decreases in inflammation, remodeling, and death were noted in IL-13 OE animals with null mutant CCR-1 loci and in IL-13 OE mice treated with neutralizing antibodies against the chemokine C10/CCL6 (39). CCR2 deficiency did not decrease the basal or IL-13–stimulated expression of target matrix metalloproteinases (MMPs) or cathepsins but did increase the levels of mRNA-encoding α_1-antitrypsin; tissue inhibitor of metalloproteinase-1, -2, and -4; and secretory leukocyte proteinase inhibitor. In addition, the levels of bioactive and total TGF-β_1 were diminished in CCR-$2^{-/-}$ animals. Treatment with anti-C10 (or the null mutation of CCR-1) decreased the ability of IL-13 to stimulate the production of MCP-1/CCL2, MIP-1α/CCL3, MMP-2/CXCL1, MMP-9, and cathepsins K, L, and S, and the ability of IL-13 to inhibit α_1-antitrypsin. These studies demonstrate that IL-13 is a potent stimulator of a large number of chemokines in the lung and highlight a chemokine cascade in which C10/CCL6 elaboration is required for the induction of MCP-1/CCL2, MIP-1α/CCL3, and proteases (MMP-2 and -9 and cathepsins) and the inhibition of α_1-antitrypsin. They also demonstrate that CCR-2 and CCR-1 play critical roles in these IL-13 responses and that CCR-2–mediated alterations in IL-13–induced fibrosis are associated with alterations in the production of TGF-β_1.

IL-13 Regulation of Vascular Endothelial Cell Growth Factor

Vascular remodeling is well described in the asthmatic airway. The mechanisms responsible for the increase in airway vascularity, however, are poorly defined. To address this issue, studies were undertaken to determine if IL-13 regulated the production of vascular regulating growth factors such as vascular endothelial cell growth factor (VEGF). These studies demonstrated that IL-13 is a potent stimulator of VEGF accumulation in the murine lung. Oxidant injury (induced by 100% O_2) augmented this inductive response. The 164-amino acid isoform was the major VEGF moiety in bronchoalveolar lavage (BAL) fluid from transgenic mice in room air. The 120- and 188-amino-acid isoforms accumulated in mice during oxidant injury. These studies suggest that VEGF elaboration is a downstream consequence of IL-13 and that VEGF may be responsible for the vascular abnormalities in the asthmatic airway.

To further address this issue, the double-construct, inducible OE system was used to target $VEGF_{165}$ to the murine airway (40). In these studies, VEGF increased bronchial vascularity and vascular permeability. VEGF also caused mucus metaplasia, airway remodeling, and airway hyperresponsiveness on methacholine challenge.

These studies suggest that VEGF is a multifunctional contributor to asthma pathogenesis. Because respiratory syncytial virus is also a potent stimulator of VEGF elaboration by epithelial cells (41), they also suggest that VEGF may be one of the missing links between the innate immune responses induced by viral infection and the adaptive response that is characteristic of allergen-driven Th2 inflammation.

Mechanisms of Airway Fibrosis

***Role of TGF-β_1*.** Tissue fibrosis is a prominent feature in the remodeled asthmatic airway. Because IL-13 is a major effector in airway Th2 responses, studies were undertaken to define the mechanisms by which IL-13 might engender this fibrotic response. We hypothesized that the effects of IL-13 could be mediated by its ability to interact with members of the TGF-β family. To test this hypothesis, we compared the regulation of TGF-β_1 in lungs from wild-type mice and mice in which IL-13 OE causes pulmonary fibrosis. IL-13 selectively stimulated TGF-β_1 in transgenic animals, and macrophages were a major source of TGF-β_1 production and deposition in these tissues. IL-13 also activated TGF-β_1 *in vivo*. This activation was associated with decreased levels of mRNA encoding latent TGF-β_1 binding protein and increased mRNA encoding urinary plasminogen activator, MMP-9, and CD44. Comparable alterations in thrombospondin-1, integrin β_6, and CD36 were not appreciated. TGF-β_1 activation was abrogated by the plasmin/serine protease antagonist aprotinin.

It was also decreased in the progeny of crosses of CC10–IL-13 mice and MMP-9 null mice but was not altered in crosses with CD44 null animals. IL-13–induced fibrosis was also significantly ameliorated by treatment with the TGF-β antagonist, soluble TGF–βRII-Fc. These studies demonstrate that IL-13 is a potent stimulator and activator of TGF-β_1 *in vivo*. They also demonstrate that this activation is mediated by a plasmin/serine protease and by an MMP-9–dependent and CD44-independent mechanism. Lastly, they demonstrate that the fibrotic effects of IL-13 are mediated, in great extent, via its ability to regulate the TGF-β_1 pathway (7).

Role of adenosine. Elevated levels of adenosine are found in BAL fluid from patients with asthma, and aerosol adenosine exposure induces bronchospasm in patients with asthma and chronic obstructive pulmonary disease but not in normal control subjects. Because adenosine is a nucleoside that is generated in response to cellular stresses such as chronic inflammation, we hypothesized that adenosine accumulation, alterations in adenosine receptor expression, and/or adenosine–IL-13 autoinduction are critical events in the pathogenesis of IL-13–induced tissue pathologies. To test this hypothesis, we characterized the effects of transgenic IL-13 on the levels of adenosine, adenosine deaminase (ADA) enzymatic activity, and the expression of adenosine receptors in the murine lung (42). We also characterized the ability of adenosine to induce IL-13 in lungs from ADA null mutant mice. IL-13 induced a pulmonary inflammatory and remodeling response that eventuated in respiratory failure and death. During this response, IL-13 caused a progressive increase in adenosine accumulation; inhibited ADA enzymatic activity and mRNA accumulation; and augmented the expression of the A_1, A_{2b}, and A_3 but not the A_{2a} adenosine receptors. IL-13 also induced the production and activation of TGF-β_1 in this murine system. ADA enzyme therapy diminished the IL-13–induced increase in adenosine. This alteration inhibited IL-13–induced inflammation and chemokine elaboration and prolonged the survival of IL-13–transgenic animals. ADA enzyme therapy also caused a marked decrease in pulmonary remodeling, a marked decrease in tissue fibrosis, a decrease in alveolar destruction, and a significant decrease in the induction and activation of TGF-β_1. Adenosine levels are also markedly increased in lungs from partially ADA null mutant mice. In these mice, the elevated levels of adenosine stimulated the production of IL-13. These studies demonstrate that adenosine is increased and that adenosine signaling is modified in lungs from IL-13 OE transgenic mice. They also demonstrate that this increase in adenosine plays an important role in IL-13–induced inflammation, fibrosis, and alveolar remodeling and demonstrate that these effects are mediated, at least in part, by the ability of adenosine to induce the production and activation of TGF-β_1. Last, they demonstrate that IL-13 and adenosine can stimulate one another in an amplification pathway that may contribute to the nature, severity, progression, and chronicity of IL-13–mediated and/or Th2-mediated disorders.

Role of IL-11. Exaggerated quantities of IL-11 and IL-13 are found in close approximation in asthmatic tissues. We hypothesized that IL-11, signaling via the IL-11Rα–gp-130 receptor complex, plays a key role in IL-13–induced tissue responses. To test this hypothesis, we compared the expression of IL-11, IL-11Rα, and gp130 in lungs from wild-type mice and IL-13 OE transgenic mice. We simultaneously characterized the effects of a null mutation of IL-11Rα on the tissue effects of transgenic IL-13 (43). These studies demonstrate that IL-13 is a potent stimulator of IL-11 and IL-11Rα. They also demonstrate that IL-13 is a potent stimulator of inflammation, fibrosis, hyaluronic acid accumulation, myofibroblast accumulation, alveolar remodeling, mucus metaplasia, and respiratory failure and death in mice with wild-type IL-11Rα loci and that these alterations are ameliorated in the absence of IL-11Rα. Last, they provide insights into the mechanisms of these processes by demonstrating that IL-13 stimulates CC chemokines, MMPs, mucin genes, and gob 5, and stimulates and activates TGF-β_1 via IL-11Rα–dependent pathways. These studies demonstrate that IL-11Rα plays a key role in the pathogenesis of IL-13–induced inflammation and remodeling.

TRANSGENIC MODELING OF TGF-β_1

The studies noted previously demonstrate that IL-13 induces tissue fibrosis via its ability to stimulate and activate TGF-β_1 (7). They also demonstrate that the alterations in this fibrotic response induced by the manipulation of chemokine receptors, IL-11, and/or adenosine are associated with the proportionate alterations in TGF-β_1. These studies led to the hypothesis that TGF-β_1 generation and activation can be a major final common pathway of tissue fibrosis in the respiratory tract. To address this hypothesis, studies were undertaken to determine if we could generate airway and parenchymal remodeling and fibrosis by overexpressing TGF-β_1. To avoid the vagaries of TGF-β_1 activation, we overexpressed bioactive TGF-β_1. We initiated these studies using the constitutive transgenic system described by our laboratory (37). With this system, we never got viable transgene-expressing animals. This is in accord with the demonstration that the overexpression of a similar TGF-β_1 construct using the surfactant apoprotein-C promoter results in fetal lethality due to a block in branching morphogenesis (44). We followed this with studies using the double transgenic inducible system described by our laboratory. The low level of basal transgene leak in this system was also sufficient to cause fetal lethality. To allow this otherwise fetal lethal transgene to be adequately expressed in the mature murine lung, we developed a novel triple transgenic system based on the tetracycline-controlled transcriptional suppressor (tTS) and the reverse tetracycline transactivator (45). In this system, tTS binds to the transgenic apparatus in transgenic mice on normal water and suppresses gene expression. When the animals are given doxycycline water (dox) the tTS is released, allowing the activating reverse tetracycline transactivator to bind and transgene activation. In the absence of dox, these mice did not express transgenic TGF-β_1 on BAL ELISA or lung RNA RT-PCR (35 cycles) evaluations. They did, however, manifest inducibility with transgenic TGF-β_1 readily noted after dox administration. These levels were maintained for the duration (up to 3 mo) of dox administration and returned to undetectable levels within 48 h of the removal of dox from the animal's drinking water.

PHENOTYPIC ANALYSIS OF TGF-β_1 TRANSGENIC MICE

To define the effects of TGF-β_1 in the fully formed lung, 6-wk-old, triple-transgene positive [herein referred to as transgene (+)] animals and their transgene (−) littermate controls were randomized to receive normal water or dox. At intervals thereafter, animals were killed, and the lungs were analyzed. These studies revealed a complex phenotype with early apoptosis followed by inflammation, fibrosis, α-smooth muscle actin (+) cell accumulation, and alveolar remodeling. Each is described below.

Apoptosis

After as little as 12 h of TGF-β_1 induction, a TDT-mediated nick-end labeling (TUNEL) (+) cell death response could be readily appreciated in lungs from transgene (+) but not transgene (−) animals. Many of the TUNEL (+) cells were epithelial cells, as evidenced by their histologic location and by double-labeling

experiments. This apoptosis peaked after 48 h of dox administration and decreased despite continuous dox administration and TGF-β_1 elaboration. This demonstrates that TGF-β_1 induces an early wave of epithelial apoptosis that decreases with continuing TGF-β_1 elaboration.

Inflammation

Within 2 d of the administration of dox, a prominent inflammatory response was noted. In BAL, this manifests as an increase in total cell and macrophage recovery. In the tissues, this effect was seen after 2 d and increased in intensity over 10 d. This response was largely due to an increase in macrophages. However, a modest increase in eosinophils could also be appreciated.

Fibrosis

The induction of TGF-β_1 caused an airway and parenchymal fibrotic response. On trichrome stains, this response could be appreciated after as little as 2 d of dox administration, predominantly in the subepithelial and adventitial regions of the airway. With longer periods of dox administration, this fibrotic response could be appreciated in the parenchyma. In accord with these findings, lung collagen content continued to increase over a 2-mo period of dox administration.

α-Smooth Muscle Actin Accumulation

To determine if TGF-β_1 caused alterations in myocytes and/or myofibroblasts, α-smooth muscle actin immunohistochemistry was undertaken. Within 10 d of dox administration, a prominent increase in α-smooth muscle actin (+) cells was appreciated. After 1 mo of dox administration, prominent increases in airway muscle mass could be appreciated. At all time points, transmission electron microscopy demonstrated that TGF-β_1 increased the accumulation of myofibroblasts and true myocytes.

Alveolar Remodeling

After prolonged periods of dox administration, alveolar remodeling could be readily appreciated. This response was patchy in nature and associated with alveolar septal thickening and areas of septal rupture. In many areas, it had the appearance of honeycomb lung. It was pronounced enough to be detected via morphometric assessments of chord length (the distance between alveolar walls). Increases in chord length could be appreciated after 7 d of dox administration and were most prominent with longer periods of TGF-β_1 elaboration.

ANALYSIS OF GENE EXPRESSION

To begin to understand the mechanisms by which TGF-β_1 induces this phenotype, Affymetrix gene chip analysis was performed using U74 sub A chips and lungs from transgene (+) mice and transgene (−) mice that received dox for 2 d, 5 d, or 1 mo. At each time point, we compared the gene expression in transgene (+) and transgene (−) animals using Gene Spring software (Agilent Inc., Palo Alto, CA). These studies demonstrated that over 2,251 genes and/or expressed sequence tags (ESTs) were altered by at least twofold at any time point. Clustering analysis demonstrated different patterns of regulation with genes that were consistently up-regulated, up-regulated early and then normalized, up-regulated only at the later time points, consistently down-regulated, and inhibited only at early time points. An analysis of these genes revealed a number of interesting findings *vis-à-vis* the phenotypes that were induced by TGF-β_1. Prominent alterations in genes that control cell cycle and apoptosis, genes that induce tissue inflammation, genes that encode extracellular matrix and their synthetic enzymes, genes that encode proteases and antiproteases, genes that encode transcription factors, and genes that encode cytokines and growth factors and integrins were noted. Prominent examples include the up-regulation of proapoptotic molecules (e.g., caspase-7, Bid, and p53) and antiapoptotic molecules (e.g., A1, Bcl-3, p21, and 14-3-3 σ). TGF-β_1 was also a potent stimulator of a variety of chemokines, including TARC/CCL17, small inducible cytokine A-2, small inducible cytokine A-9, and eotaxin/CCL11. In addition, the receptors for TARC/CCL17, CCR4, and CCR5 were up-regulated. This provides a logical explanation for the macrophage-rich and eosinophil-rich inflammatory response that is induced by this cytokine. In accord with the fibrotic response that was induced in these animals, TGF-β_1 was a potent stimulator of tenascin-C, elastin, fibrilin-1, fibronectin-1, laminin, lysyl oxidase, and a variety of procollagen moieties. In keeping with the patchy alveolar remodeling response, TGF-β_1 also had complex effects on proteases and antiproteases with increases in a variety of MMPs (MMP-8, -9, and -12), calpain-1, and antiproteases (plasminogen activator inhibitor type I and tissue inhibitor of metalloproteinase 1). In addition, TGF-β_1 was a potent stimulator of its own gene and a variety of other members of the TGF-β_1 family, connective tissue growth factor, tumor necrosis factor, insulin-like growth factor 1, and tumor necrosis factor receptor 1. Prominent induction of early growth response gene (EGR)-1 and EGR-2 was noted when transcription factors were analyzed.

To ensure that the results of our gene chip experiments accurately reflected tissue events, confirmatory experiments were performed using a variety of approaches, including ribonuclease protection and real-time reverse transcriptase–polymerase chain reaction (RT-PCR) assays. Seventeen genes were chosen at random, including genes that were up-regulated, down-regulated, and not altered. In all cases, appropriate concordance was noted. These studies highlight the many pathways that are activated by TGF-β_1 in this *in vivo* modeling system. They also provide insights into the mechanisms by which TGF-β_1 may be inducing the exciting phenotype that has been noted.

ROLE OF EGR-1

EGR-1 is a potent transcriptional regulator of many of the genes that were altered in our gene chip and mRNA evaluations. We hypothesized that EGR-1 might play an important role in the pathogenesis of the TGF-β_1 phenotype. To test this hypothesis, we first used RT-PCR and real-time PCR to define the regulation of EGR-1 in our transgenic system. These studies demonstrated that, after as little as 2 d of dox, increased levels of EGR-1 mRNA could be appreciated in RNA from transgene (+) mice. A similar result was seen with 1 wk of dox administration. EGR-2 and -3 were also induced. The role of EGR-1 was then defined by breeding EGR-1 (−/−) mice (a gift from Dr. T. Milbrandt) (46) and the TGF-β_1 OE animals. Using the progeny of these crosses, we were able to compare the phenotypes induced by TGF-β_1 in mice with (+/+) and (−/−) EGR-1 loci. The lungs from wild-type mice and transgene (−)/EGR-1(−/−) mice on normal water or dox were virtually identical. In contrast, the TGF-β_1 phenotype was markedly altered in EGR-1 (−/−) animals. As previously reported (45), in the absence of EGR-1, the ability of TGF-β_1 to stimulate cellular apoptosis was markedly diminished. In the absence of EGR-1, TGF-β_1–induced fibrosis on trichrome evaluations, total lung collagen content and alveolar remodeling were also markedly ameliorated. These studies demonstrate that EGR-1 plays a central role in critical aspects of the *in vivo* TGF-β_1 phenotype.

EFFECT OF CASPASE INHIBITION

Our studies with EGR-1 null mutant mice demonstrated that an intervention that diminished TGF-β_1–induced apoptosis also

decreased the ability of TGF-β_1 to induce fibrosis and alveolar remodeling. This led to the hypothesis that there is an intimate relationship between the apoptosis and the fibrotic and remodeling phenotypes, with the apoptosis being required to allow the latter phenotypes to be seen. To address this hypothesis, experiments were undertaken in which apoptosis was blocked via a different mechanism, and the effects of this intervention on fibrosis and alveolar remodeling were assessed. In these experiments, transgene (+) and transgene (−) mice were randomized to receive broad-spectrum caspase inhibitor Z-VAD-fmk (intraperitoneally) or vehicle control starting 24 h before the administration of dox. After 10 d of dox administration, the animals were killed, and phenotypic analysis was undertaken. Z-VAD was a potent inhibitor of TGF-β_1–induced apoptosis. Z-VAD inhibition of apoptosis was also associated with a marked decrease in tissue fibrosis on trichrome and total lung collagen content evaluations and alveolar remodeling. Thus, in accord with what was noted with EGR-1 null mutant animals, an intervention that blocked apoptosis simultaneously decreased fibrosis and alveolar destruction. When viewed in combination, these studies support the hypothesis that TGF-β_1–induced apoptosis is a critical event in the pathogenesis of TGF-β_1–induced fibrosis and alveolar destruction. They also demonstrate that TGF-β_1 can simultaneously induce injury (apoptosis) and fibrotic responses. One can easily envision how this capacity might work to debride a wound and, by removing marginally viable cells, allow the healing response to take place on a fully healthy wound base.

REVERSIBILITY OF THE FIBROTIC RESPONSE

The reversibility of tissue fibrosis has critical consequences for the natural history of fibrotic pulmonary disorders. Unfortunately, our knowledge of the processes that control reversibility is marginal at best. This is due, in great extent, to the limited ability of the vast majority of animal models to appropriately allow the resolution phase of an injury and repair response to be evaluated. A unique feature of our inducible transgenic system is the clarity with which it allows this sort of analysis to be undertaken. Specifically, in these mice a transgene can be turned "on," and a phenotype can be engendered. The transgene can then be turned "off," and the natural history of the phenotype can be evaluated over time. To accomplish this in our transgenic system, TGF-β_1 transgene (+) and transgene (−) mice were randomized to receive normal water or dox for 10 d. At the end of this interval, they were placed on normal water and analyzed at intervals thereafter. Dox administration to transgene (+) mice caused an increase in tissue fibrosis. After 1 mo on normal water, this fibrotic response had significantly decreased. These studies demonstrate that TGF-β_1 induces a fibrotic response that is at least partially reversible when overexpressed in the murine lung.

CONCLUSIONS

These studies demonstrate that IL-13 is a potent stimulator of inflammation, remodeling, and fibrosis and highlight the important roles that the adenosine system, CCR-2–dependent inflammatory processes, VEGF, and IL-11 play in the generation of these responses (Figure 1). They also highlight the prominent role that TGF-β_1 plays in this pathway and the ability of IL-13 to stimulate and activate TGF-β_1 (Figure 1). Last, they demonstrate that TGF-β_1 induces apoptosis and fibrosis and alveolar remodeling, highlight the role of EGR-1 in this apoptotic response, and demonstrate that TGF-β_1–induced apoptosis as a critical prerequisite for its induction of fibrosis and alveolar simplification. Interventions can be envisioned in a number of locations in this pathway to control these important responses.

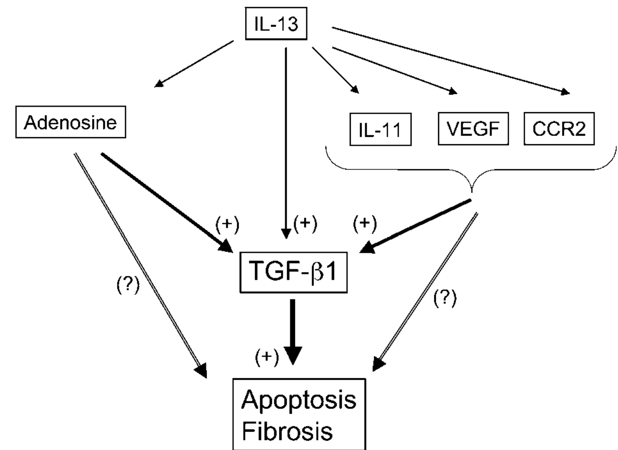

Figure 1. Proposed interleukin (IL)-13–transforming growth factor (TGF)-β_1 remodeling pathway in the lung. The ability of IL-13 to regulate TGF-β_1 directly or via altering adenosine metabolism, CCR2-induced inflammation, or the production of IL-11 or vascular endothelial growth factor (VEGF) is illustrated. The possibility that IL-13, adenosine, IL-11, or VEGF regulate fibrosis and or apoptosis via TGF-β_1–independent pathways is also illustrated.

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