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Short Communication

Overexpression of Granulocyte-Macrophage Colony-Stimulating Factor Induces Pulmonary Granulation Tissue Formation and Fibrosis by Induction of Transforming Growth Factor-β1 and Myofibroblast Accumulation

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We have previously reported that transfer to rat lung of the granulocyte-macrophage colony-stimulating factor (GM-CSF) gene leads to high expression of GM-CSF between days 1 and 4 and granulation tissue formation followed by an irreversible fibrotic response starting from day 12 onward. In the current study, we investigated the underlying mechanisms. We found that GM-CSF overexpression did not enhance production of tumor necrosis factor-α in a significant manner at any time after GM-CSF gene transfer. However, the content of transforming growth factor-β1 in broncboalveolar lavage fluid was markedly induced at day 4 and appeared to be maximal around day 7 and remained high at day 12. Macrophages purified from broncboalveolar lavage fluid 7 days after GM-CSF gene transfer spontaneously released significant quantities of transforming growth factor-β1 protein* in vitro. *After peak transforming growth factor-β1 production was the emergence of α-smooth muscle actin-rich myofibroblasts. Accumulation of these cells was most prominent at day 12 within the granulation tissues and they were still present in fibrotic areas between days 12 and 24 and diminished markedly afterward. Thus, we provide the first* in vivo *evidence that tumor necrosis factor-α may be dissociated from participation in a fibrotic process in the lung and GM-CSF may play a more direct role in pulmonary fibrogenesis at least in part through its capability to induce transforming growth factor-β1 in macrophages and the subsequent emergence of myofibroblast phenotypes. This GM-CSF transgene lung model is useful for a stepwise dissection of both cellular and molecular events involved in pulmonary fibrosis. (Am J Pathol 1997, 150:59–66)

Pulmonary fibrosis may result from many types of lung injury or inflammation, characterized by accumulation of both fibroblastic cells and extracellular matrix proteins.[1] The molecular events underlying pulmonary fibrosis still remain poorly understood. A number of soluble molecules including tumor necrosis factor (TNF)-α and transforming growth factor (TGF)-β, have been implicated in these processes. Among these molecules, TNF-α is thought to act as one of the early triggers for the fibrotic cascade whereas TGF-β1 serves as a direct growth factor modulating fibroblast proliferation and extracellular matrix production.[2–5] Indeed, the ability of TNF-α or

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TGF-β1 to induce fibrotic responses has been shown in a number of tissue-specific genetic transgenic models in which overexpression of these cytokines is driven by a tissue-specific promoter[6–8] and in other transgene models.[9,10] Moreover, expression of both TNF-α and TGF-β1 is often discerned in fibrotic models and certain forms of human pulmonary fibrosis.[11–13] TGF-β1, in addition to its effects on tissue remodeling, has been shown to induce the emergence of α-smooth muscle actin (α-SMA)-expressing myofibroblasts both *in vitro* and *in vivo*.[14] The early emergence of α-SMA-expressing myofibroblasts in wound healing and tissues undergoing fibrosis suggests an important role for these cells in the evolution of fibrotic events.[15–17] In this respect, the majority of cells expressing procollagen mRNA in a model of bleomycin-induced pulmonary fibrosis have recently been demonstrated to be α-SMA-positive myofibroblasts.[18]

Although it is usually up-regulated during fibrotic responses, TNF-α is not conceived to be a molecule capable of direct induction of TGF-β, myofibroblast phenotype, and matrix production *in vivo*.[19] This is because TNF-α itself serves as a rather nonspecific alarm proinflammatory cytokine induced in either early or chronic stages of inflammatory conditions with or without fibrotic sequelae in the lung and other sites[20,21] and, unlike TGF-β, TNF-α has weak or negative effects on fibroblast proliferation and matrix production and by itself is unable to induce the myofibroblast phenotype *in vitro* or *in vivo*.[16,21,22] These data argue for the crucial involvement of additional intermediate molecule(s) of yet unknown nature in the switch-on of TGF-β and α-SMA phenotypes. Interestingly, granulocyte-macrophage colony-stimulating factor (GM-CSF) has been found to induce fibrotic responses with accumulation of α-SMA-expressing myofibroblasts after chronic subcutaneous administration,[22] and this cytokine is up-regulated in a number of fibrosing pulmonary conditions.[23] However, the mechanisms underlying GM-CSF-induced myofibroblast phenotype in the skin and the precise role of GM-CSF in pulmonary fibrosis still await to be clarified. We have recently described an experimental model whereby GM-CSF is shown to induce pulmonary fibrotic reactions by using an adenoviral vector-mediated gene transfer technique.[24] These findings support the notion of GM-CSF as a fibrogenic cytokine in the lung. In the current study, we have investigated the mechanisms by which GM-CSF induces fibrotic reactions in this model by examining the association between induction of GM-CSF, TNF-α, TGF-β1, and α-SMA-rich myofibroblasts, granulation tissue formation, and fibrosis.

Materials and Methods

Adenoviral Vectors and Animal Model

Construction and characterization of a replication-deficient adenoviral vector expressing murine GM-CSF (AdE1GM-CSF) have been previously described.[24] This vector or a control vector AdE1dl70-3 at a dose of 1×10^9 plaque-forming units was instilled intratracheally to the lung of Sprague-Dawley rats weighing 220 to 280 g after a standard procedure we have previously described.[24] At the end of 1, 2, 4, 7, 12, 18, 24, and 36 days after gene transfer to the lung, rats were anesthetized and bronchoalveolar lavage (BAL) was performed.[24] Lungs were fixed by perfusion with 10% buffered formalin and paraffin embedded. Routine histopathological assessment was performed on hematoxylin and eosin (H&E)-stained sections.

Cytokine Measurement of BAL Samples

Concentrations of TNF-α in BAL fluid were determined by enzyme-linked immunosorbent assay (ELISA) specific for both murine and rat TNF-α with a sensitivity of 15 pg/ml (Genzyme, Cambridge, MA). TGF-β1 was measured by using an ELISA kit for human TGF-β1 (R&D Systems, Minneapolis, MN). This kit detects TGF-β1 across species, and all samples were activated before measurement after the standard procedure recommended by the manufacturer.

Ex Vivo Studies for Spontaneous Release of TGF-β1 by Alveolar Macrophages

Total BAL cells were obtained from rat lung 7 days after intratracheal instillation of AdE1dl70-3 or AdE1GM-CSF as described above. These cells were then washed and plated into 24-well plates (200,000/well) in RPMI 1640 medium containing 10% fetal calf serum (FCS) and 1% penicillin/streptomycin. After 2 hours of incubation, nonadherent cells were removed by three washes with phosphate-buffered saline and the adherent macrophages were cultured in RPMI containing 1% penicillin/streptomycin with 0.1% FCS or without FCS. Supernatants were collected at 48 hours and assayed for TGF-β1 protein by ELISA as described above.

Northern Hybridization for Rat TNF-α

Total RNA samples were extracted from lungs collected at various time points after GM-CSF gene

Table 1. *Relationship of Fibrogenic Cytokines, Macrophage Accumulation, Emergence of α-SMA-Positive Myofibroblasts, Granulation Tissue Formation, and Fibrotic Reactions in Rat Lung after GM-CSF Gene Transfer*

Days	GM-CSF	Monocytosis	TNF-α	TGF-β1	α-SMA	Granulation tissue	Fibrotic reactions
1	+++	+	−	−	−	−	−
2	++++	++	−	+	−	−	−
4	+++	+++	−	+++	−	−	−
7	++	++++	±	++++	+	+	−
12	+	++	−	+++	++++	++++	++
24	−	+	−	++	++	+	+++
36	−	+	ND	+	+	−	++++

The amount or the intensity of various parameters is designated as insignificant (−), mild (+), moderately significant (++), significant (+++), and very significant (++++), relative to different time points. ND, not done.

transfer as previously described.[24] These RNA samples (25 μg/lane) were size separated in a 1% formaldehyde gel and transferred onto a nylon membrane (Pall Biosupport Corp., East Hills, NY). The blots were then hybridized with a murine TNF-α cDNA probe (a 300-bp fragment within the coding region), stringently washed, and exposed to Kodak XAR film for 5 days. We have previously demonstrated that this cDNA probe hybridizes well to the rat TNF-α mRNA.[25]

Immunohistochemical Staining for α-SMA

Lung tissue sections were deparaffinized in toluene for 5 minutes twice, dipped up and down in 95% ethanol 20 times, and soaked in ethanol/formol for 5 minutes and in running water for 10 minutes. Sections were then treated with Tris-buffered saline containing 0.3% hydrogen peroxide for 30 minutes to block the endogenous peroxidase and washed twice for 5 minutes each in Tris-buffered saline. The following staining procedure was carried out at room temperature by using reagents included in a Vectastain Elite ABC mouse IgG kit (Vector Laboratories, Burlingame, CA). Briefly, sections were treated with the blocking serum 1 for 30 minutes and incubated overnight with the first antibody monoclonal mouse anti-α-SMA (Sigma Chemical Co., St. Louis, MO) or the control mouse IgG1 antibody (Sigma) at a 1:800 dilution. The slides were then incubated with the sera 2 and 3 (Vectastain kit) for 60 and 30 minutes, respectively, followed by exposure to a substrate/chromogen solution for 8 minutes. The final immunoreactive product was identified as a red/brown-colored deposit. Slides were counterstained using hematoxylin solution. The control antibody always gave rise to no immunoreaction.

Results

Examination of TNF-α Production after GM-CSF Gene Transfer to the Lung

We have previously shown that the GM-CSF transgene product is released in rat lung most actively between days 1 and 7 after intratracheal delivery of AdE1GM-CSF, followed by a progressive fibrotic reaction (summarized in Table 1). As TNF-α induction is often seen to be associated with fibrotic tissue responses, we investigated whether this cytokine was markedly induced in rat lung by GM-CSF overexpression. BAL samples collected at different times after GM-CSF or control vector delivery were analyzed for TNF-α content by ELISA. There was no or only minimally detectable levels of TNF-α protein measured throughout the course of 24 days, and no marked differences were found between GM-CSF and control vector-treated animals (Figure 1).

To verify these data on protein release, total RNA samples from lung tissues obtained at the same time points were analyzed for TNF-α mRNA by Northern analysis. Consistent with the protein data, little TNF-α message was detected in these samples even after extended film exposure times, and there was no significant differences between GM-CSF-expressing or control vector-treated lungs. As control, rat lung tissue challenged with endotoxin expressed a remarkable message for TNF-α (Figure 2).

Examination of TGF-β1 Production after GM-CSF Gene Transfer to the Lung

Because TGF-β1 has been regarded as a fibrogenic growth factor directly involved in the fibrogenic process, we next examined the possible contribution of this cytokine to GM-CSF-induced pulmonary fibrosis. To address this issue, the content of total TGF-β1

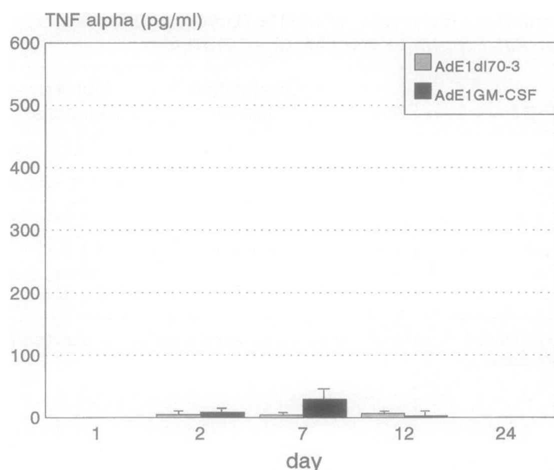

Figure 1. *TNF-α concentrations in BAL fluid. BAL samples were collected from rats receiving either the control vector AdE1dl70-3 or AdE1GM-CSF at different time points. The samples were measured by ELISA and the results expressed as means ± SEM from three to five animals. The difference between two treatments at day 7 is not statistically significant* (P = *0.4*).

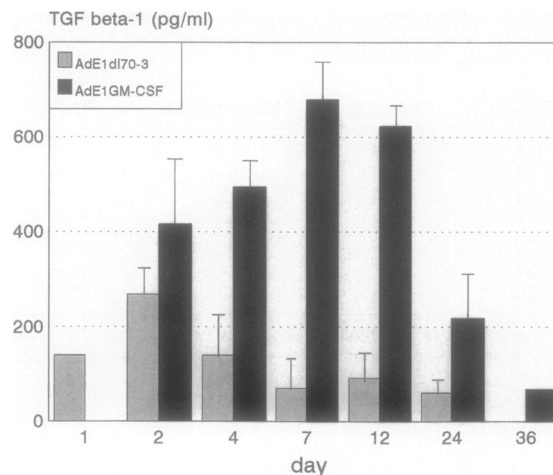

Figure 3. *TGF-β1 concentrations in BAL fluid. BAL samples were collected as described in Figure 1 and measured for total TGF-β1 protein by ELISA. Results are expressed as means ± SEM from three to five animals for most time points.*

protein in BAL fluids was determined by ELISA. As shown in Figure 3, markedly increased amounts of TGF-β1 were noticed in BAL fluid from rats receiving AdE1GM-CSF but not the control vector. The peak levels appeared to be around day 7 after GM-CSF gene transfer. The levels of TGF-β1 significantly decreased by day 24 and continued to decline toward the control levels by day 36.

Examination of Spontaneous Release of TGF-β1 by Macrophages from the Lung after GM-CSF Gene Transfer to the Lung

As GM-CSF has been shown to activate functional activities of monocytes/macrophages *in vitro* including cytokine responses, survival, and proliferation, and in our model, macrophages underwent local expansion and proliferation as assessed morphologically[24] (Table 1), we wondered whether these macrophages were activated by GM-CSF to release TGF-β1, thus contributing to enhanced TGF-β1 production as detected in BAL fluid. To test this, macrophages were purified from BAL fluid collected at day 7 after GM-CSF gene transfer as day 7 was the time when there was a peak accumulation of TGF-β1 protein in BAL fluid. These cells were then cultured with or without very low concentrations of FCS to avoid the interference from TGF-β present in FCS, and the spontaneous release of TGF-β1 to the supernatants was measured by ELISA. Significant spontaneous release of TGF-β1 protein was found in macrophages from rats receiving GM-CSF vector but not the control vector. The release appeared to be further enhanced in the presence of low concentrations of FCS (Figure 4).

Immunohistolocalization of α-SMA after GM-CSF Gene Transfer to the Lung

Accumulation of myofibroblasts is considered to be a key step during wound healing and fibrosis. The hallmark of myofibroblast phenotype is α-SMA expression,[14,16] and among all cytokines examined,

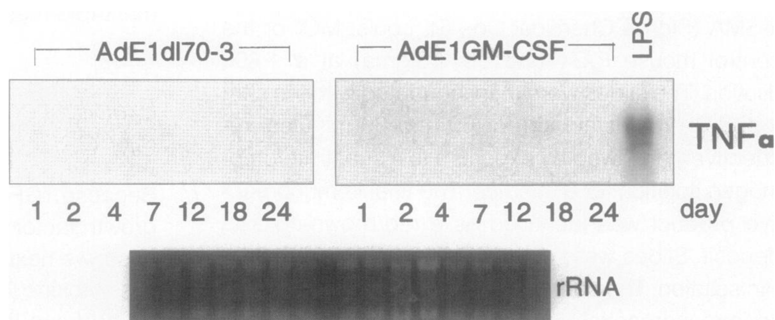

Figure 2. *TNF-α mRNA expression in lung tissue. Total RNA was extracted from lung tissues after GM-CSF gene transfer or after control vector delivery at different time points and subjected to Northern hybridization. The RNA from rat lung challenged with lipopolysaccharide for 6 hours was used as a positive control.*

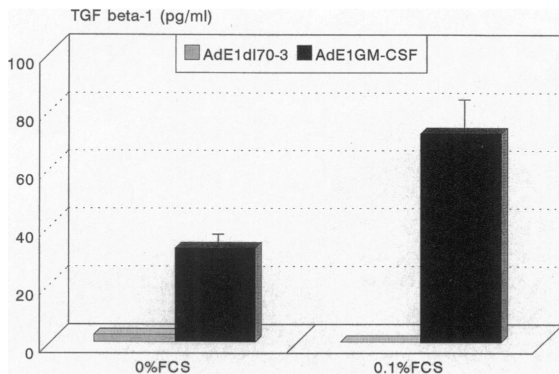

Figure 4. *Spontaneous release of TGF-β1 by alveolar macrophages* in vitro. *Alveolar macrophages were isolated from BAL fluid collected at day 7 after AdE1dl70-3 or AdE1GM-CSF delivery to the lung and cultured without or with 0.1% FCS. The supernatants were collected at 48 hours and assayed for total TGF-β1 by ELISA. Results are expressed as means ± SEM from triplicate determinations.*

only GM-CSF and TGF-β1 have been found to induce this phenotype at the skin site *in vivo*.[14,16] To examine whether there was any emergence of myofibroblast-like cells with the evolution of fibrotic responses after GM-CSF overexpression in the lung, immunohistostaining was performed on lung tissues using a specific monoclonal antibody against α-SMA. Between days 1 and 7, in both GM-CSF- and control vector-treated tissues, the immunoreactivity was largely localized to the bronchial or vascular smooth muscle layers. Figure 5a shows α-SMA staining in the vasculature with surrounding macrophage granulomatous lesions seen in the lung overexpressing GM-CSF. By day 12, although the pattern of staining remained similar to that in earlier time points in control tissues, there was a significant accumulation of α-SMA-positive fibroblastic cells in lung tissues of rats receiving GM-CSF vector, particularly in the areas of granulation tissue. These cells were

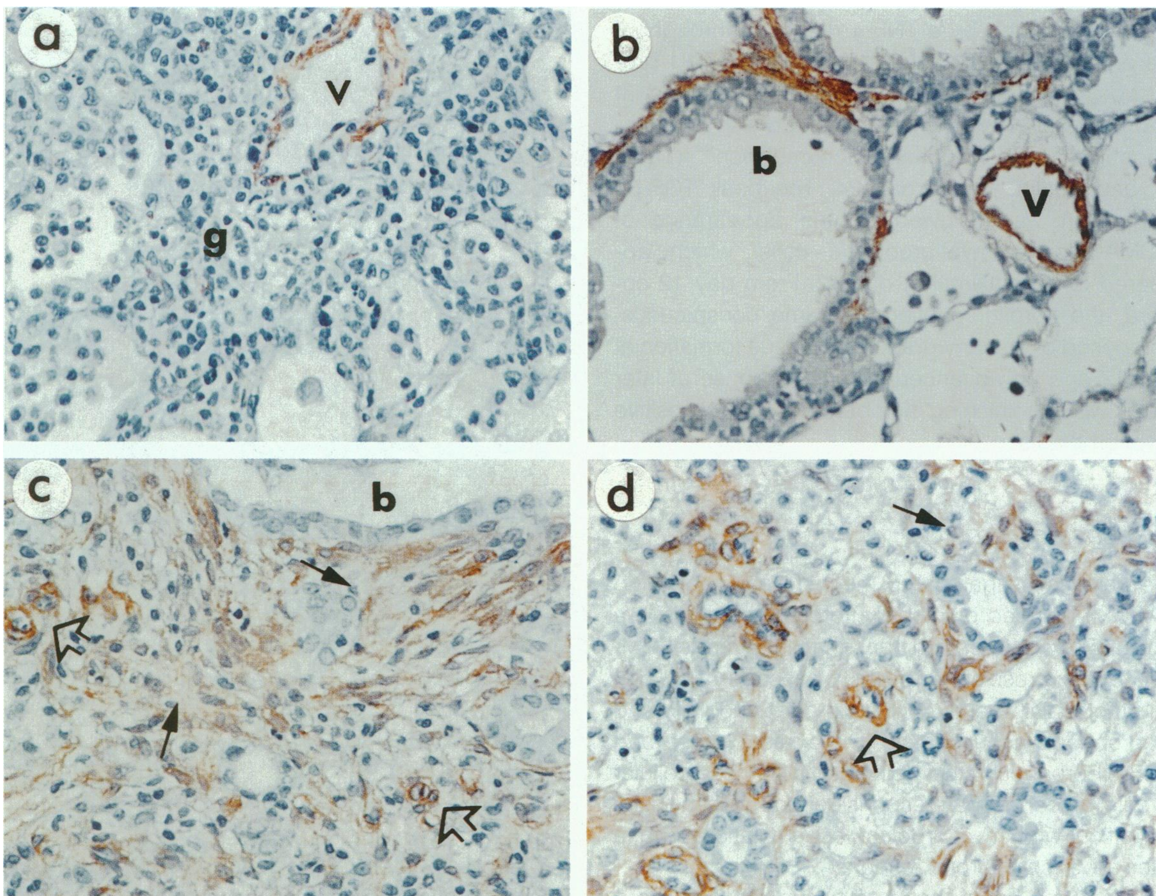

Figure 5. *α-SMA-positive cells in lung tissue. Lung tissues were fixed and processed for immunohistochemical staining using a monoclonal antibody against α-SMA. Tissue sections were then counterstained with hematoxylin.* a: *At 7 days after AdE1GM-CSF, α-SMA immunoreactivity is primarily localized to the smooth muscle layer in a vessel (v) but absent in the granulomatous lesion (g). Magnification, ×150.* b: *At 12 days after AdE1dl70-3, α-SMA staining is confined mainly to the smooth muscle layers in bronchial (b) and vascular (v) structures. Magnification, ×150.* c *and* d: *At 12 days after AdE1GM-CSF, α-SMA staining is localized to many fibroblastic cells* (solid arrows) *in granulation tissue areas, and many capillaries are also stained positive* (open arrows). *Magnification, ×200.*

topographically distinct from the smooth muscle cells stained in the microvasculature of granulation tissues (Figure 5, b–d). By days 18 or 24 after GM-CSF gene transfer when the granulation tissue largely disappeared, the overall intensity of staining decreased, but some α-SMA-positive cells were still seen in certain fibrotic areas (Figure 6a). By day 36, although there was a more intense fibroblastic response in fibrous areas, there were few α-SMA-positive myofibroblastic cells seen in these areas with only microvasculature predominantly stained (Figure 6, b and c).

Relationship between Cytokine Production, α-SMA Expression, Granulation Tissue Formation, and Fibrotic Reactions after GM-CSF Gene Transfer to the Lung

As shown in Table 1, GM-CSF was significantly released locally in the lung between days 1 and 12 after intrapulmonary gene transfer,[24] and this was not accompanied by a TNF-α response throughout the entire course of study. In contrast, TGF-β1 production markedly increased at day 4, peaked at approximately day 7, which coincided with peak eosinophilia and macrophage accumulation[24] (Table 1), and declined from day 24. The peak TGF-β1 production was followed by the appearance of α-SMA-positive myofibroblastic cells, which appeared to be maximal at day 12. From day 12 onward, the fibrotic reactions became conspicuous, composed of an early granulation tissue formation at day 12 and an advanced fibrous response at later times with diminishing numbers of α-SMA-positive myofibroblasts.

Discussion

We have investigated the mechanisms underlying GM-CSF-induced pulmonary fibrotic responses in a GM-CSF overexpression transgene lung model. GM-CSF overexpression triggered a cascade of cellular and molecular events consisting of eosinophilia and macrophage accumulation in the initial phase and TGF-β1 production that coincided with these cellular events, an early repair response of granulation tissue formation with myofibroblast accumulation, and a later advanced fibrotic response.

TNF-α has been shown to be up-regulated in a number of models of pulmonary fibrosis and in patients with certain lung fibrotic diseases.[11–13] The early involvement of this cytokine is also demonstrated in some animal models in which anti-TNF-α antibodies have been shown to inhibit the ensuing lung fibrotic response.[4] However, TNF-α is also induced or present, either acutely or chronically, in almost all other inflammatory or immune conditions in the lung and other tissue sites that assume no apparent fibrotic outcomes.[20,21,26] Importantly, TGF-β

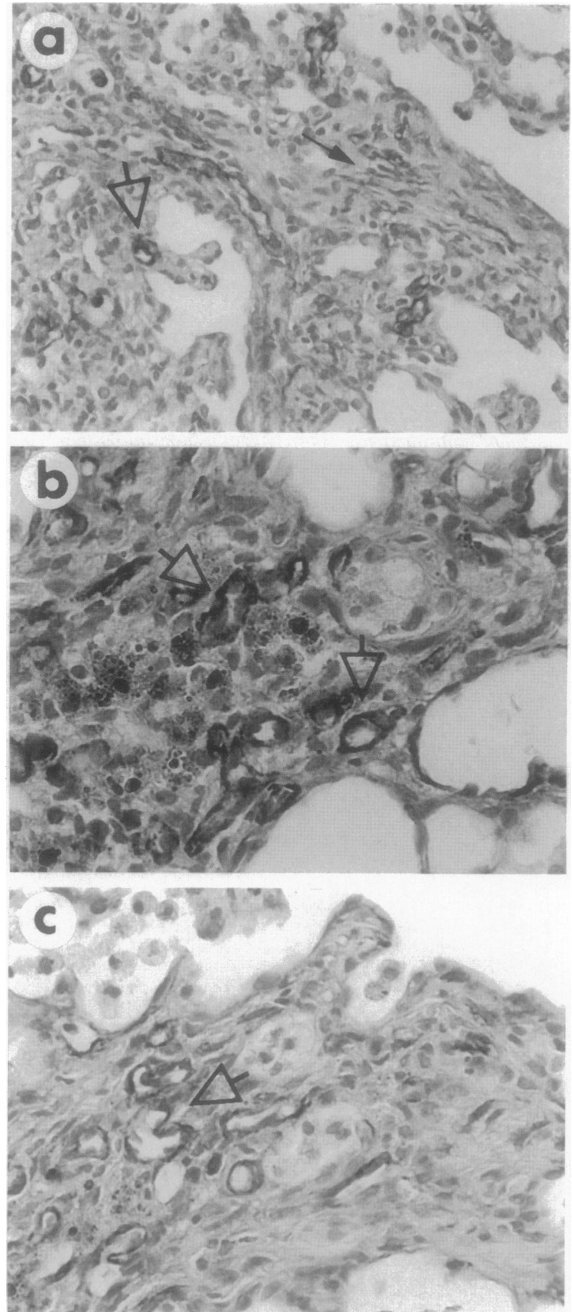

Figure 6. *α-SMA-positive cells in lung tissue.* **a:** *At 18 days after AdE1GM-CSF, α-SMA staining intensity is decreased but still seen in fibroblastic cells in fibrous areas* (**solid arrow**) *and neovasculature is noticed* (**open arrow**). *Magnification, ×150.* **b** *and* **c:** *At 36 days after AdE1GM-CSF, the number of α-SMA-positive fibroblastic cells is further diminished and the staining is localized almost exclusively to some of the neovasculature structures* (**open arrow**). *Magnification, ×150.*

elaboration remains unchanged in many of these conditions despite the presence of TNF-α.[27–29] These findings suggest that whether a fibrotic outcome will ensue is determined by a complex orchestrated interaction of multiple events or factors, perhaps including the nature of insult, the degree and the type of injury, and the portfolio of induced mediators. Although the mechanisms leading to TGF-β up-regulation *in vivo* have remained elusive, these lines of observation strongly suggest that other factors downstream of TNF-α may be imperative in the induction of both TGF-β and myofibroblastic phenotypes. This assumption is in fact further supported by findings by Rubbia-Brandt et al[22] that TNF-α as well as interleukin-1 cannot induce the emergence of myofibroblasts. Our findings suggest that GM-CSF may represent one such downstream factor, the exaggerated expression of which can virtually elicit, without apparent participation of TNF-α, the elements that are likely required for the initiation of pulmonary fibrotic responses including eosinophil infiltration, macrophage accumulation, TGF-β up-regulation, myofibroblast accumulation, and granulation tissue formation. Rubbia-Brandt and Vyalov have previously observed that GM-CSF, like TGF-β1, is able to induce local myofibroblastic accumulation in the skin after subcutaneous minipump delivery, and this induction appears to require the presence of tissue macrophages.[22,30] Yet, unlike TGF-β1, GM-CSF has no effect on myofibroblast induction *in vitro*.[22] Our current findings thus support and extend their observations, indicating that GM-CSF can induce myofibroblastic phenotype in the lung, and this induction is associated with macrophage accumulation/activation and subsequent TGF-β1 release. The early and transient appearance of α-SMA-rich myofibroblasts and the relatively sustained levels of TGF-β1 observed in our model are in agreement with the results obtained in different fibrotic conditions by others.[12,17,18] The temporal sequence of GM-CSF expression, eosinophil and macrophage accumulation, macrophage release of TGF-β1, granulation tissue formation with prominent emergence of myofibroblasts, and advanced fibrotic reactions, strongly suggests that macrophage activation, TGF-β release, myofibroblast emergence, and granulation tissue formation represent some critical early steps toward the advanced stages of pulmonary fibrosis.

Although the exact role of eosinophils in this cascade still remains to be clarified, these cells, perhaps together with activated macrophages, may participate by elaborating TGF-β and causing enzymatic injury to lung structures.[31] The important role of alveolar macrophages in pulmonary fibrosis has been suggested by the findings of Khalil et al that alveolar macrophages are a predominant source of TGF-β1 in bleomycin-induced fibrosis,[32] thus in support of our findings. A close relationship between alveolar macrophages immunolocalizing TGF-β1, α-SMA-expressing fibroblasts, and fibrotic lesions is also reported in a model of asbestos-induced pulmonary fibrosis.[33] Having addressed a direct role of TGF-β1 in myofibroblast differentiation, granulation tissue formation, and fibrotic reactions in the lung overexpressing GM-CSF, it cannot be ruled out that GM-CSF is also directly involved in the fibrogenic process as GM-CSF is found to be able to stimulate migration/proliferation of endothelial cells and fibroblastic cells *in vitro*.[34] Indeed, we observed a rather striking neovascularization in the granulation tissue and fibrosing areas throughout the course of study.

Taken together, our study has demonstrated that GM-CSF is able to trigger a cascade of cellular and molecular events leading to irreversible fibrotic changes in the lung, and these findings should draw attention to the role of this cytokine in the pathogenesis of a number of pulmonary conditions, particularly those with eosinophilia and fibrotic pathology.

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References

1. Crouch E: Pathobiology of pulmonary fibrosis. Am J Physiol 1990, 259:L159–L184
2. McDonald JA: Idiopathic pulmonary fibrosis: a paradigm for lung injury and repair. Chest 1991, 99:87S–93S
3. Gauldie J, Jordana M, Cox G: Cytokines and pulmonary fibrosis. Thorax 1993, 48:931–935
4. Piguet P-F: Cytokines involved in pulmonary fibrosis. Int Rev Exp Pathol 1993, 34B:173–181
5. Raghow R: Role of transforming growth factor-β in repair and fibrosis. Chest 1991, 99:61S–65S
6. Miyazaki Y, Araki K, Vesin C, Garcia I, Kapanci Y, Whitsett JA, Piguet P-F, Vassalli P: Expression of a tumor necrosis factor-α transgene in murine lung causes lymphocytic and fibrosing alveolitis. J Clin Invest 1995, 96:250–259
7. Lee M-S, Gu D, Feng L, Curriden S, Arnush M, Krahl T, Gurushanthaiah D, Wilson C, Loskutoff DL, Fox H, Sarvetnick N: Accumulation of extracellular matrix and developmental dysregulation in the pancreas by trans-

genic production of transforming growth factor-β1. Am J Pathol 1995, 147:42–52

8. Wyss-Coray T, Feng L, Masliah E, Ruppe MD, Lee HS, Toggas SM, Rockenstein EM, Mucke L: Increased central nervous system production of extracellular matrix components and development of hydrocephalus in transgenic mice overexpressing transforming growth factor-β1. Am J Pathol 1995, 147:53–67
9. Isaka Y, Fujiwara Y, Ueda N, Kaneda Y, Kamada T, Imai E: Glomerulosclerosis induced by *in vivo* transfection of transforming growth factor-β or platelet-derived growth factor gene into the rat kidney. J Clin Invest 1993, 92:2597–2601
10. Gauldie J, Sime PJ, Xing Z, Graham FL: Adenovirus vector mediated transfer of activated (mutant) transforming growth factor-β1 cDNA to the respiratory tracts of rats causes fibrosis. Cytokine 1997 (in press)
11. Khalil N, O'Connor RN, Flanders KC, Umruh H: TGF-β1, but not TGF-β2 or TGF-β3, is differentially present in epithelial cells of advanced pulmonary fibrosis: an immunohistochemical study. Am J Respir Cell Mol Biol 1996, 14:131–138
12. Kapanci Y, Desmouliere A, Pache J-C, Redard M, Gabbiani G: Cytoskeletal protein modulation in pulmonary alveolar myofibroblasts during idiopathic pulmonary fibrosis: possible role of TGF-β and TNF-α. Am J Respir Crit Care Med 1995, 152:2163–2169
13. Phan SH, Kunkel SL: Lung cytokine production in bleomycin-induced pulmonary fibrosis. Exp Lung Res 1992, 18:29–43
14. Desmouliere A, Geinoz A, Gabbiani F, Gabbiani G: Transforming growth factor-β1 induces α-smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts. J Cell Biol 1993, 122:103–111
15. Darby I, Skalli O, Gabbiani G: α-Smooth muscle actin is transiently expressed by myofibroblasts during experimental wound healing. Lab Invest 1990, 63:21–29
16. Desmouliere A: Factors influencing myofibroblast differentiation during wound healing and fibrosis. Cell Biol Int 1995, 19:471–476
17. Vyalov SL, Gabbiani G, Kapanci Y: Rat alveolar myofibroblasts acquire α-smooth muscle actin expression during bleomycin-induced pulmonary fibrosis. Am J Pathol 1993, 143:1754–1765
18. Zhang K, Rekhter MD, Gordon D, Phan SH: Myofibroblasts and their role in lung collagen gene expression during pulmonary fibrosis. Am J Pathol 1994, 145:114–125
19. Kovacs EJ: Fibrogenic cytokines: the role of immune mediators in the development of scar tissue. Immunol Today 1991, 12:17–23
20. Fiers W: Tumor necrosis factor. FEBS Lett 1991, 285: 199–212
21. Ulich TR: Tumor necrosis factor. Cytokines of the Lung. Edited by J Kelley. New York, Marcel Dekker, 1993, pp 307–332
22. Rubbia-Brandt L, Sappino A-P, Gabbiani G: Locally applied GM-CSF induces the accumulation of α-smooth muscle actin containing myofibroblasts. Virchows Arch B Cell Pathol 1991, 60:73–82
23. Xing Z, Braciak T, Ohkawara Y, Sallenave J-M, Roley R, Sime PJ, Jordana M, Graham FL, Gauldie J: Gene transfer for cytokine functional studies in the lung: the multifunctional role of GM-CSF in pulmonary inflammation. J Leukocyte Biol 1996, 59:481–488
24. Xing Z, Ohkawara Y, Jordana M, Graham FL, Gauldie J: Transfer of GM-CSF gene to rat lung induces eosinophilia, monocytosis, and fibrotic reactions. J Clin Invest 1996, 97:1102–1110
25. Xing Z, Kirpalani H, Torry D, Jordana M, Gauldie J: Polymorphonuclear leukocytes as a significant source of tumor necrosis factor-α in endotoxin-challenged lung tissue. Am J Pathol 1993, 143:1009–1015
26. Gordon S, Keshav S, Stein M: BCG-induced granuloma formation in murine tissues. Immunobiology 1994, 191: 369–377
27. Broekelmann TJ, Limper AH, Colby TV, McDonald JA: Transforming growth factor-β1 is present at sites of extracellular matrix gene expression in human pulmonary fibrosis. Proc Natl Acad Sci USA 1991, 88:6642–6646
28. Khalil N, O'Connor RN, Unruh HW, Warren PW, Flanders KC, Kemp A, Bereznay OH, Greenberg AH: Increased production and immunohistochemical localization of transforming growth factor in idiopathic pulmonary fibrosis. Am J Respir Cell Mol Biol 1991, 5:155–162
29. Xing Z, Jordana M, Kirpalani H, Schall TJ, Driscoll KE, Gauldie J: Cytokine expression by neutrophils and macrophages *in vivo*: endotoxin induces TNF-α, MIP-2, IL-1β, IL-6, but not RANTES or TGF-β1 mRNA expression in acute lung inflammation. Am J Respir Cell Mol Biol 1994, 10:148–153
30. Vyalov S, Desmouliere A, Gabbiani G: GM-CSF-induced granulation tissue formation: relationship between macrophage and myofibroblast accumulation. Virchows Arch B Cell Pathol 1993, 63:231–239
31. Zhang K, Flanders KC, Phan SH: Cellular localization of transforming growth factor-β expression in bleomycin-induced pulmonary fibrosis. Am J Pathol 1995, 147: 352–361
32. Khalil N, Whitman C, Danielpour D, Greenberg A: Regulation of alveolar macrophage transforming growth factor-β secretion by corticosteroids in bleomycin-induced pulmonary inflammation in the rat. J Clin Invest 1993, 92:1812–1818
33. Perdue TD, Brody AR: Distribution of transforming growth factor-β1, fibronectin, and smooth muscle actin in asbestos-induced pulmonary fibrosis in rats. J Histochem Cytochem 1994, 42:1061–1070
34. Bussolino F, Wang JM, Defilippi P, Turrini F, Sanavio F, Edgell C: G- and GM-CSF induce human endothelial cells to migrate and proliferate. Nature 1989, 337:471–473