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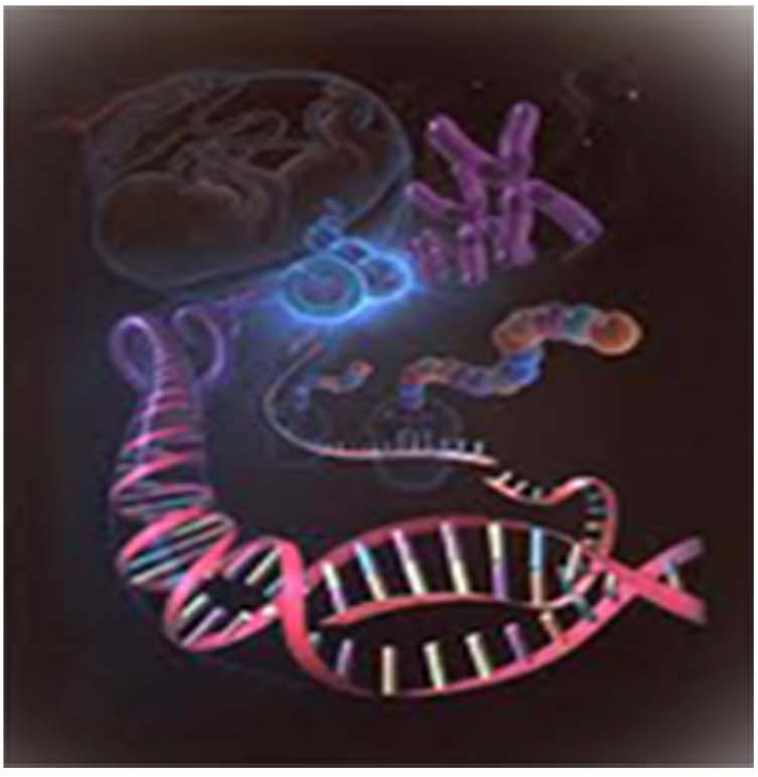

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Review Article

TRANSFORMING GROWTH FACTOR-BETA1 AS LUNG INJURY BIOMARKER: A REVIEW

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Lung injury, induced by chemicals, radiotherapy or others agents is marked by enhancement in the expression of transforming growth factor beta1 (TGF-β1) as a result of inflammation. TGF-β1 appears to be the major profibrogenic cytokine which regulates the production and deposition of the major extracellular matrix molecules (ECM) and has been reported to be involved in a host of cellular responses. Therefore, it has been attempted in this review to evaluate the role of TGF-β1 as a biomarker of lung injury, both at acute and late phase, in different models of lung injury. The up-regulation of TGF-β1 mRNA expression and its corresponding protein in lung tissue subsequent to infliction of tissue injury has been established by various investigations involving RT-PCR analysis, Western blotting involving TGF-β1 antibody, immunohistochemical and immunolocalisation studies, etc. Increase in the expression of TGF-β1 at the acute phase triggers the tissue repair mechanisms involving the synthesis of ECM proteins and other cellular mediators of tissue repair, a hallmark of development and progression of fibrosis. Experimental evidences reviewed in this article indicate that TGF-β1 is probably the main biomarker of lung injury induced by various agents and appears to contribute very significantly in the development of fibrosis. Additionally, experimental evidences also suggest that it may not act alone but rather by activation or in combination with other proinflammatory molecules in response to lung tissue injury.

Keywords: Transforming growth factor beta1 (TGF-β1), Acute lung injury (ALI), Acute respiratory distress syndrome (ADRS), Radiation-induced lung injury (RILI), inflammation, Fibrosis

INTRODUCTION

Transforming growth factor beta 1 or TGF-β1 is a polypeptide member of the transforming growth factor beta superfamily of cytokines. It is a 25-kDa disulfide-linked homodimeric cytokine that has been closely associated with both acute and chronic inflammation (Blobe *et al*., 2000). TGF-β1, when activated, is an anti-inflammatory cytokine produced by a variety of cells including epithelial cells (Magnan *et al*., 1994). Early at a site of developing inflammation, TGF-β1 upregulates endothelial adhesion molecules and

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functions as a potent chemotactic agent for the extravascular recruitment of leukocytes (Fava *et al.*, 1991; Reibman *et al.*, 1991; Buhling *et al.*, 1999). TGF-β1 has also been shown to inhibit inducible nitric oxide (NO) synthase at the onset of inflammation (Vodovotz, 1997), possibly maintaining blood pressure and blood flow that could promote the delivery of circulating effector cells to a site of developing inflammation (Kubes *et al.*, 1991; Pellacani *et al.*, 2001).

TGF-β1 has also been reported to stimulate collagen and fibronectin production in fibroblasts (Romberger *et al.*, 1992; Borthwick *et al.*, 2009), process which is known to occur in epithelial repair. These findings were further supported by the observations that TGF-β1 play a key role in the repair of normal tissue injury and it has been implicated as a key cytokine in the induction of fibrosis in many organs, including the lungs (Rube *et al*., 2000). In deed this cytokine has been implicated as a "master switch" in induction of fibrosis in many tissues and organs (Sime and O'Reilly, 2001). Support to role of TGF-β1 in fibrogenesis also came from the findings that Dupuytren's disease, a progressive fibrosing disorder in humans, is prevented by different agents by inhibiting TGF-β1 (Knobloch *et al.*, 2009). Similarly, it has also been demonstrated that radiation-induced pulmonary injury and fibrosis in rats can be ameliorated by inhibition of TGF-β1 (Serin *et al.*, 2007; Anscher *et al.*, 2008). This is further supported by an earlier observation that SOD and SOD mimetics also inhibit TGF-β1 and prevent fibrosis (Vujaskovic *et al.*, 2002). Apparently, TGF-β1 appears to be the major profibrogenic cytokine which regulates the production and deposition of the major extracellular matrix molecules (ECM) and has been reported to be involved in a host of immune responses (Schuppan *et al*., 2003; Presser *et al.*, 2013). Therefore, an attempt has been made in this review to evaluate the role of TGF-β1 as a biomarker of lung injury, both at acute and late phase, induced by different agents in different models of lung injury.

INVOLVEMENT OF TGF-β1 IN INFLAMMATORY AND FIBROTIC RESPONSE IN LUNGS INDUCED BY EXTERNAL AGENTS

The role of TGF-β1 in inflammation and fibrosis has been reported in several models of lung injury, primarily induced by chemical agents and ionizing radiations.

Models of Chemically-Induced Lung Injury

Bleomycin-Induced Injury

The investigation on the ability of bleomycin (BLM) to induce pulmonary injury started probably around early 1980's. In one of such studies it was shown that intratracheal administration of BLM induces acute alveolitis and interstitial inflammation, which was characterized by the recruitment of leukocytes within 1 week and pulmonary edema in hamsters (Chandler *et al*., 1983). Since then several works has been carried out indicating the ability of BL to induce lung injuries and the involvement of TGF-β1 in the acute and late phases of the injury. The role of TGF-β1 in BLM-induced injury has been reported in several studies. Previous *in vitro* and *in vivo* studies indicated that human alveolar macrophages release proinflammatory cytokines after BLM administration (Scheule *et al*., 1992). Furthermore, it was thought that alveolar macrophages, following stimulation by BLM-induced injury, secrete a large quantity of TGF-β1 and thereby induce the lung fibroblasts in the

alveolar interstitium to synthesize collagen, resulting in pulmonary fibrosis in rats and mice (Khalil *et al*., 1993; Zhao *et al*., 2002). These suggest that BLM-activated macrophages may function as one of major sources of chemical mediators in the pulmonary inflammation/fibrosis loop. Indeed, the majority of inflammatory cells recovered from bronchoalveolar lavage fluid (BALF) were macrophages, which were of an order of magnitude higher in number than those of neutrophils and lymphocytes (Yamauchi et al., 2011). It was also shown in this study on mice that the level of interleukine-17A (IL-17A), another inflammatory cytokine, went up in response to inflammation. Since, it was already known that IL-17A is required for BLM-induced pulmonary fibrosis (Wilson *et al*., 2010) and it was proposed here that IL-17A and TGF-β1 play a cooperative roles in the development of fibrosis (Yamauchi *et al.*, 2011). This finding supports previous report that has shown that BLM-induced (0.08 units of BLM) injury resulted in reduction in TGF-β1 in BALF and consequently decreases pulmonary fibrosis and improvement in survival in mice (Nethery *et al*., 2005). It was also shown that BLM (8 mg/kg)-induced pulmonary fibrosis was significantly reduced in mice lacking L-selectin expression, intercellular adhesion molecule-1 (ICAM-1) expression, or both (Hamaguchi *et al*., 2002). It was observed that collagen deposition was inhibited in both L-selectin and ICAM-1 deficient mice when compared with wild-type normal mice. Reduction in pulmonary fibrosis was associated with reduced accumulation of leukocytes, including neutrophils and lymphocytes. Decreased mRNA expression of proinflammatory cytokines and TGF-β1 resulted in the inhibition of collagen deposition. It was reported that L-selectin and ICAM-1 play a critical role in pulmonary fibrosis by mediating the accumulation of leukocytes, which regulate the production of proinflammatory cytokines and TGF-β1.

In another study in mice, BLM induced a marked pulmonary expression of CCL11 (C-C motif chemokine) and CCR3 (C-C motif chemokine receptor), known to be involved in recruitment of inflammatory cells and the development of lung fibrosis (Huaux *et al.*, 2005). It was shown in this study that CCL11-deficient mice developed significantly reduced pulmonary fibrosis characterized by reduced expression of TGF-β1. It was also reported that increased lung expression of CCL11 significantly enhanced BLM-induced lung fibrosis and production of TGF-β1. These effects were also associated with pulmonary infiltration of eosinophils and neutrophils. In contrast, mice treated with neutralizing CCR3 antibodies developed significantly reduced pulmonary fibrosis, eosinophilia, neutrophilia, and expression of the profibrotic cytokine. Similar study was carried out on another chemokine, CCL18, which is constitutively expressed at high levels in the lung (Pardo *et al.*, 2001) and was known to increase in the lungs of patients with pulmonary diseases characterized by T-cell involvement and collagen deposition (Prasse *et al.*, 2006). In one of the study, the combine effect of over expression of CCL18 and BLM injury was evaluated for the severity of lung inflammation and fibrosis in mice (Pochetuhen *et al*., 2007). Combine effects of CCL18 over expression and BLM injury were observed on pulmonary inflammation, particularly on various regulators of fibrosis such as T-cell infiltration, tumor necrosis factor-alpha (TNF-α), interferon-gamma (IF-γ), etc. Moreover, increase

in pulmonary levels of active TGF-β1 was correlated with the increase in collagen levels, leading to fibrosis. Furthermore, the involvement of TGF-β1 in BLM (2.2 mg/kg)-induced lung inflammation and fibrosis was demonstrated in rat model where it has been shown that the mRNA expression profiles of fibrotic markers including TGF-β1 increases at day 3 and 14 accompanied by procollagen and fibronectin increase in expression indicative of fibrotic lesion. Histological staining of collagen corroborated fibrosis development (Chaudhary *et al*., 2006).

Several other studies in BLM-induced lung injury in mice resulting in inflammatory responses mediated by higher level of expression of TGF-β1 and triggering of fibrotic changes in the lung have also been reported (Gharaee-Kermani *et al.*, 1996; Ortiz *et al*., 1998; Xu *et al.,* 2009; Rhee *et al*., 2011; Yamauchi *et al.*, 2011; Mackinnon *et al.*, 2012).

Cigarette Smoke-Induced Injury

Cigarette Smoke (CS) is known to be the most important risk factor for CS-related pulmonary pathophysiology (Pauwels *et al.*, 2004). Chemokine C-X-C motif receptor 3 (CXCR3) along with its specific ligand CXCL10/interferon (IFN)-inducible protein-10 (IP-10), highly expressed in bronchiolar epithelial cells and airway smooth muscle cells, play a significant role in mediating T-cell recruitment into the airways and lung parenchyma (Grumelli *et al*., 2004). In a study to investigate the role of CXCR3 in CS-induced pulmonary injury, CXCR3 gene-deficient (CXCR3-/-) mice were used (Nie *et al.,* 2008). Mice were exposed to the mainstream of the tobacco smoke of 5 cigarettes, 4 times a day with a 30 min smoke-free interval for 3 consecutive days. The control mice received filtered air. The animals were killed at 2 h after the last CS exposure. Analysis of bronchoalveolar lavage fluid and mRNA and protein levels in the lungs have shown that in comparison to their wild-type counterparts, the CXCR3-/- mice showed reduced inflammation, as revealed by fewer inflammatory cells, particularly cytotoxic CD8+ T cells. The mRNA level and the corresponding protein level of inflammatory and chemotactic cytokines, including TGF-β1, were significantly lowered in the CXCR3-/- mice. It was concluded that CXCR3 regulates acute lung inflammation induced by CS via the recruitment of CD8+ T cells into the airways and the lungs to trigger the inflammatory response cascade with the overexpression of some inflammatory and fibrotic cytokines and chemokines such as TGF-β1 after CS exposure.

Cadmium Chloride (CdCl2)-Induced Injury

In one study involving conventional histological, immunohistochemical and Western blot analysis of rat lung antigens, has shown that 3 days of CdCl2 (2.5 μmol/l) exposure in combination with TGF-β1 (1 ng/ml) to rat lung slices induced lung injury *in vitro* (Kasper *et al*., 2004). It was characterized by extracellular matrix accumulation and myofibroblast trans differentiation, type I cell injury with loss of type I cell antigens, increased apoptosis of pulmonary cells and activation of microvascular endothelial cells.

Silica-Induced Injury

In a study on the role of cluster of differentiation 36 (CD36) in the development of silica-induced lung fibrosis, silicosis induction by intratracheal injection of 10 mg silica per rat was used to observe both the inhibition of L-TGF-β1 activation and the antifibrotic effect obtained by lentiviral vector (Lv-shCD36) silencing of CD36 expression (Wang *et al.*, 2009). The lung epithelial growth

inhibition assay, hydroxyproline assay, pathological and immunohistochemical examinations revealed the inhibition of silica-induced lung fibrosis. The lentiviral vector (Lv-shCD36) silenced expression of CD36 in alveolar macrophages (AMs) obtained from bronchoalveolar lavage fluid (BALF) and the activation of L- TGF-β1 in the BALF was inhibited by Lv-shCD36. The resultant hydroxyproline content and the degree of fibrosis of silica+Lv-shCD36 treated groups was significantly lower than in other experimental groups without Lv-shCD36. Expectedly, the expression of collagen I and III in the silica+Lv-shCD36-treated group was significantly lower than in the other experimental groups. It was reported that silencing expression of CD36 can result in the inhibition of L- TGF-β1 activation in a rat silicosis model, preventing the development of silica-induced lung fibrosis. Similar result was obtained in different studies on rats and mice and was proposed that down regulation of the expression of the TGF-β1 along with other mediators of lung injury results in decreasing lung injury induced by silica (Wang *et al.*, 2010; Gao *et al.*, 2011).

Libby Amphibole-induced Injury

Pulmonary and histopathological changes were investigated following Libby amphibole (LA) exposure in a rat model (Danielle *et al*., 2011). Rat respirable fractions of LA and amosite (aerodynamic diameter <2.5 μm) were prepared by water elutriation, the technique that separates amphibole particles with aerodynamic diameters smaller than 2.5 μm (within the target respirable range for rodent species) from larger fibers according to a sedimentation settling velocity based upon the particle density and radius (Webber *et al.*, 2008). Male rats were exposed to single doses of either saline (SAL), amosite (0.65 mg/rat), or LA (0.65 or 6.5 mg/rat) by intratracheal instillation (IT). In this study, the animals were placed into 4 groups: saline (SAL, control, 0.0 mg/rat), amosite (0.65 mg/rat; positive control), low-dose LA (0.65 mg/rat), and high-dose LA (6.5 mg/rat) for each necropsy time point (1, 3, or 7 days, 2 week, and 3 months after IT instillation). TGF-1β assayed in the lung tissue and mRNA expression analysis using RT-PCR indicated that it was up-regulated at different time points in LA- and amosite-exposed groups. Moreover, the mRNA level of pro-collagen-1 went up particularly at 3 months time point. Histopathological examination corroborated these observations and showed notable thickening of interstitial areas surrounding the alveolar ducts and terminal bronchioles.

Models of Radiation-induced Lung Injury

Radiation-induced pulmonary injury has been reported in many species including humans (Kong *et al.*, 2005; Haston *et al*., 2007). These injuries are primarily manifested in the form of radiation pneumonitis and fibrosis resulting in impaired lung function (Haston *et al*., 2007). The acute phase is marked by inflammation of the lung characterized by increased synthesis of proinflammatory mediators such as TGF-β1 and others (Linard *et al.*, 2004; Chen *et al.*, 2005; Fleckenstein *et al.*, 2007; Rodemann and Blaese, 2007).

Radiation Therapy-Induced Injury

Analysis on variations of the cytokine levels including TGF-β1 in lung cancer patients during radiation therapy (RT) with the occurrence of symptomatic Radiation Pneumonitis (RP) has been widely explored. In one of these studies, 34 lung cancer patients with histologically proven

lung cancer, received three-dimensional conformal radiation therapy (3D-CRT) above 45 Gy (Kim *et al.*, 2009). Peripheral blood sample, collected from the patients at six points-before, at the beginning, in the middle of, at the end of RT and 2 and 4 weeks after RT, were analyzed for TGF-β1 and other cytokines by performing enzyme-linked immunosorbent assay (ELISA). By serial measurement of cytokines level, only the TGF-β1 level showed a correlation to the symptomatic RP. None of the other cytokines were correlated with the risk of RP. The mean pre-treatment TGF-β1 level did not differ between RP and non-RP groups. However, during the period of radiation treatment, the TGF-β1 level began to increase at the end of RT in the RP group and became significantly higher 4 weeks after RT. It was found that significant associations between the changes of TGF-β1 during the time course of the RT and the risk of developing RP. It was concluded from this study that the changes of TGF-β1 could be correlated with RP and the incorporation of the biological parameters into the dosimetric data could be useful for predicting symptomatic RP. This corroborates earlier reports on the role of plasma TGF-β1 as a marker for the development of symptomatic radiation pneumonitis (Anscher *et al.*, 1998). In this study on patients with lung cancer, a normal plasma TGF-β1 by the end of RT was observed in patients who would not develop pneumonitis than those patients who did develop pneumonitis. Similar studies on the status of TGF-β1 was carried out to identify reliable biomarkers for radiation pneumonitis and to identify individuals at risk for pneumonitis before or during the early stage of thoracic RT for malignancy (Anscher *et al.,* 1997; Chen *et al.*, 2002). Analysis of plasma specimens for circulating cytokine changes before, during, and up to 12 weeks after radiation indicated an increase in TGF-β1 along with other cytokines during and after RT. Similarly, assessment of the effects of thoracic RT on the concentrations of TGF-β1 in BALF in lung cancer patients indicated that the TGF-β1 concentrations in the BALF recovered from the irradiated areas was significantly increased by thoracic RT (Barthelemy-Brichant *et al*., 2004). The increase in TGF-β1 levels was greater in the group of patients who developed severe pneumonitis. In the BALF from the nonirradiated areas, the TGF-β1 concentrations remained unchanged reaffirming that this cytokine may contribute to the process leading to a radiation-induced injury in human lung tissue.

In another study involving RT, the relationship between loss of heterozygosity (LOH) at the mannose 6-phosphate/insulin-like growth factor 2 receptor (M6P/IGF2R) gene locus and the development of radiation-induced lung injury have been investigated (Kong *et al*., 2001). Here the development of radiation pneumonitis after thoracic RT was evaluated and it was reported that the loss of the M6P/IGF2R gene strongly correlates with the development of radiation pneumonitis. Moreover, patients with LOH are much more likely to have elevated plasma TGF-β1. It was concluded that loss of the M6P/IGF2R gene may predispose patients to the development of radiation-induced lung injury mediated by TGF-β1.

A study was also conducted on the protective effects of berberine, an alkaloid widely used as an antimicrobial and an antidiarrhoeal drug (at a dose of 20 mg/kg, once a day) on radiation-induced lung injury (RILI) in non-small cell lung cancer (NSCLC) patients treated with RT (Liu *et*

al., 2008). Here 90 patients with NSCLC were divided into two groups randomly. The trial group received radiation therapy plus berberine, and the control group received radiation therapy plus a placebo containing starch for 6 weeks. Circulating cytokines analysis on blood samples at baseline (prior to treatment) and then at 3, 6 and 12 weeks revealed that there was a significant reduction in the level of soluble intercellular adhesion molecular-1 (sICAM-1), critical to the pathogenesis of RILI, in the trial group at 6 and 12 weeks in comparison to the control. The plasma TGF-β1 level was also lower at 3 and 6 week in comparison with the control group. It was also observed that the incidence of RILI was significantly lower in the trial group at 6 weeks and 6 months than that of the control group. It was shown that berberine significantly reduced the incidence of RILI at 6 weeks and 6 months, improved basal pulmonary function (PF) and decreased the levels of sICAM-1 and TGF-β1. Similar result in NSCLC in other study whereby the level of plasma TGF-β1 was increased following RT in several patients which could have resulted in the development of symptomatic RP (De Jaeger *et al*., 2004; Rübe *et al*., 2008).

Experimental Radiation-Induced Injury

Numerous studies have been carried out on radiation-induced tissue damage to the lung in different experimental models (Marks *et al.*, 2003; Linard *et al*., 2004; Machtay *et al.*, 2006; Haston *et al.*, 2007; Christofidou-Solomidou *et al*., 2011; Kma *et al*., 2012). In one such study, mice were exposed to 13.5 Gy single-dose irradiation to the thorax (Machtay *et al.*, 2006). The animals were then administered with glycol (PEGylated) antioxidant enzymes (AOEs). Here, 100 microgram of a 1:1 mixture of PEG-AOEs {PEG-catalase and PEG-superoxide dismutase (SOD)} intravenously, 10-15 min prior to irradiation and subgroups was evaluated at different time-points for inflammation and fibrosis. At 48 h post-irradiation, control irradiated mice had significant elevations of tissue p21, Bax and TGF-β1 in lungs as revealed by semi-quantitative analysis of mRNA expression, not seen in irradiated, PEG-AOE-treated mice. Similarly, terminal deoxynucleotidyl transferase-mediated d-UTP Nick End Labeling (TUNEL) staining of lung sections was performed at 24 h post-irradiation revealed a decrease in apoptotic cells with AOE treatment. Consequently, at 4 months post-irradiation, these mice had significantly increased pulmonary fibrosis as measured by hydroxyproline content in comparison with PEG-AOE group that were similar to that of unirradiated controls correlated with enhancement in TGF-β1. This was corroborated by less pulmonary fibrosis with AOE's as observed histologically.

Laser-Induced Injury

Mammalian RUNX3, a transcription factor, regulates gene expression in several important developmental pathways (Levanon *et al*., 2003) and has been reported to closely involve in neurogenesis (Inoue *et al*., 2002; Levanon *et al.,* 2002). The relationship between lung defects caused by the loss of function of Runx3 and wound healing has indicated that Runx3 is essential for normal murine lung development (Lee *et al*., 2010). The relationship between Runx3 and TGF-β1 and their involvement in wound healing following laser exposure to the lungs has been investigated using Runx3 knockout (KO) mice (Lee *et al.*, 2010). In this study it was observed that the Runx3 KO mice that die soon after birth exhibit alveolar hyperplasia. To evaluate this observation and involvement of TGF-β1, a CO_2 laser system was used at a continuous

wavelength of 1,064 nm. After dissection of the lungs, only the left lobes were irradiated with a single beam of laser light for 2 s at an average fluence rate of 208 W/cm^2. Since TGF-β1 is also known to be a major regulator of re-epithelialization and wound closure during wound healing (Reynolds *et al.,* 2005), TGF-β1 was localized after laser irradiation in wild-type and in Runx3 KO mouse lungs at post-natal day 1 along with other wound-healing markers. Immunohistochemical studies revealed that along with other proteins, TGF-β1 was dramatically down-regulated by loss of Runx3 during lung wound healing. This was indicated by the increased localization of TGF-β1-stained cells in the wounded region in WT. Very few TGF-β1-positive cells were observed in the Runx3 KO wounded lung compared with those in WT. However, Smad3, the signal transducers and transcriptional modulators that mediate multiple signalling pathways, is up-regulated in the Runx3 KO laser-irradiated lung region. Therefore, it was proposed that the lung wound healing mechanism is inhibited in the Runx3 KO mouse, which shows abnormal lung architecture, by reduced TGF-β1 along with other mediators of lung injury and by induction of Smad3.

Other Models of Lung Injury

Bacterial-Induced Injury

The effects of either intravenous (IV) or intrabronchial (IB) treatment with TGF-β1 during bacterial pneumonia in rats have been studied (Cui *et al*., 2003). Immediately following IB *Escherichia coli* inoculation (T0), animals were administered with human recombinant TGF-β1 either via IV or IB, or via both IV and IB routes, or to receive placebo (human serum albumin, HSA) only. In this study, blood and lung analysis was done at 6 and 168 h after *E. coli* inoculation. It was shown that TGF-β1 decreased blood and lung bacteria count at 6 and 168 h. It was also shown that there was increase in serum tumor necrosis factor levels and lung injury scores at these time points. Administration of TGF-β1 resulted in an increase in lung leukocyte recruitment with improved microbial clearance in this rat model of pneumonia. However, it worsened lung injury and the overall survival was not significantly improved.

Ischemia/ Reperfusion (I/R)-induced Injury

Pre-treatment with nitric oxide (NO; 10 min, 15 ppm) could mitigate the symptoms of lung I/R injury (normothermic left lung ischemia, maintained for 90 min, followed by a 5-h reperfusion period), especially the inflammatory response involving TGF-β1 and others in porcine model (Waldow *et al*., 2004). Symptoms of I/R injury such as pulmonary hypertension and decreased oxygenation were ameliorated by NO inhalation. The reperfusion-induced increases of the levels of cytokines along with TGF-β1 in plasma were reduced by NO pre-treatment.

Severe Acute Pancreatitis (Sap)-Induced Injury

Resveratrol, a potent natural antioxidant has been found to be effective in reducing the onset and progress of acute lung injury in rat model of severe acute pancreatitis (SAP) (Wang *et al.*, 2006). Resveratrol administration resulted a decrease in TGF-β1 in lungs of SAP-induced rat. In this study, SAP was induced by injecting 4% sodium taurocholate, a dosage of 0.1 mL/100 g, into the biliopancreatic duct. The control group was injecting with an equal amount of normal saline into the biliopancreatic duct. For treatment group, after induction of the SAP model, the resveratrol was used to injection at a dosage of

0.3 mL/100 g through the penis vein; at solvent group, after induction of the SAP, the same amount of tween 80 injected through the penis vein. All rats were sacrificed at 3, 6 and 12 h. Evaluation of BALF, pulmonary morphology and TGF-β1 indicated an enhancement in inflammatory cells and TGF-β1 in experimental group than that of treatment group. It was concluded that resveratrol can inhibit the expression of TGF-β1, and thereby reduce the severity of acute lung injury complicated with severe acute pancreatitis.

SARS Coronavirus (Sars-cov)-Induced Injury

It has been shown that some patients who died of severe acute respiratory syndrome (SARS) contained SARS-CoV spike (S) protein and some pro-inflammatory cytokines (PICs) including TGF-β1 in their autopsy tissues (He *et al.*, 2006). Apart from other observations, high levels of TGF-β1were expressed in the SARS-CoV-infected angiotensin-converting enzyme 2 (ACE2+) cells, but not in the uninfected cells. These results suggest that cells infected by SARS-CoV produce elevated levels of PICs which may cause immuno-mediated damage to the lungs and other organs, resulting in acute lung injury and subsequently, multi-organ dysfunction.

Fas/FasL-Induced Injury and Apoptosis

TGF-β1 and the Fas/FasL system are known to be involved in the pathological processes during lung injury at the early phase and tissue repair at the later phase (Dhainaut *et al*., 2003; Galani *et al*., 2010). TGF-β1 is also known as a potent modulator of Fas-mediated apoptosis (Sánchez-Capelo, 2005). The role of TGF-β1 regulating Fas/FasL apoptotic signaling during the two different pathological processes-acute lung injury and subsequent tissue repair was explained by a study using in human lung epithelial cell line, A549 cells (Bai *et al.*, 2011). Here, A549 cells were pre-treated with TGF-β1(2.5 ng/ml) for the intervals of 0, 6, 12, 24, and 48 h. In cells exposed to TGF-β1 for 24 h, apoptosis induced by FasL was dramatically reduced to a rate of 11.2%, and when the exposure was elongated to 48 h, apoptosis was almost completely inhibited. It was proposed in this study that TGF-β1 changed sensitivity of lung epithelial A549 cells to Fas/FasL induced apoptosis signalling in a time-dependent manner. Here it was also proposed that the pro-apoptotic effect of TGF-β1 on lung epithelium might be associated with the initiation and amplification of lung injury process at early phase, and accordingly, its anti-apoptotic effect associated with lung epithelium repair and even fibrosis at later phase. It was also reported in the same study that TGF-β1 and Fas death receptor pathway might have a synergistic effect on apoptosis in A549 cells. Negative effect of TGF-β1 on Fas/FasL apoptotic signal was only associated with down-regulation of Fas level, whereas expression of FADD, Daxx, caspase-8, JNK, the major downstream molecules of Fas/FasL pathway remained unchanged. This investigation proposed that TGF-β1-induced resistance to Fas-mediated apoptosis in human lung epithelial cells contributes to epithelial-mesenchymal transition and tissue repair after lung injury and TGF-β1 inhibits Fas-mediated apoptosis via Akt activation.

Ventilation-Induced Injury

In order to study bronchopulmonary dysplasia (BPD), which occurs in association with prenatal conditions predisposing infants to inflammation and remodeling of the premature lungs, an established in utero ovine model of ventilation-induced lung injury was used (Hodges *et al*.,

2012). In this study, at day 110 of gestation, singleton fetal lambs either had sham in utero ventilation (IUV), 12 h of IUV alone, or 12 h of IUV and human amnion epithelial cells (hAEC) administration. The primary outcome, structural lung injury, was assessed 1 week later by analyzing the gene expression of tracheal aspirate fluid (TAF) cells in premature infants by mRNA quantification on real-time polymerase chain reaction. In this study, there were 3-folds increased in the level of TGF-β1 mRNA in IUV compared to control. There was also lowering compared to normal in case of hAEC. An increase in collagen in IUV to 25% from 10% in control was also observed. Even elastin went up from 5 to 15% in control and IUV, respectively. The septal crest came down from 12 to 3% in control and IUV respectively. Even the alpha smooth muscle actin content went up from 15 to 30% in control and IUV, respectively. These are known indicators of inflammatory process leading to fibrosis in premature lungs as reported in other study (Hikino *et al.,* 2012).

Analysis of biological behavior of alveolar fibroblasts during acute lung injury (ALI)/ acute respiratory distress syndrome (ARDS) in ventilation-induced lung injury was carried out using cultured cells from BALF obtained from 68 critically ill, ventilated patients (ALI n517; ARDS n531 and ventilated controls n520) (Quesnel *et al*., 2010). The patients were followed for 28 days. Analysis of TGF-β1 and collagen I protein and assessment of their mRNA expression by RT-PCR in alveolar fibroblast supernatant indicated that collagen I production was elevated in alveolar fibroblasts and correlated with TGF-β1 production. Collagen 1 production was assessed by measurement of C-terminal propeptide of type I pro-collagen (PICP) in cell culture supernatants. PICP secretion by alveolar fibroblasts was four-fold higher than that of control fibroblasts. In addition, COL1A1 and COL1A2 mRNA expression in alveolar fibroblasts was higher than in control fibroblasts and was positively correlated with collagen 1 protein secretion. It was also shown that TGF-β1 stimulated production of collagen 1 by control and alveolar fibroblasts. After stimulation, collagen 1 production remained higher in alveolar fibroblasts than in control fibroblasts. The basal levels of TGF-β1, a key factor in collagen 1 production by fibroblasts, secretion by alveolar and control fibroblasts were found to be similar. A strong correlation was found between TGF-β1 and collagen 1 production by alveolar fibroblasts at both protein and transcriptional levels. Such a correlation was not found in control fibroblasts. This result corroborated earlier reports on the significance of fibroblast in ALI/ARDS patients in the development of fibrosis (Serhan *et al.*, 2007; Tager *et al.*, 2008; Chandel *et al*., 2009).

MECHANISM OF ACTION

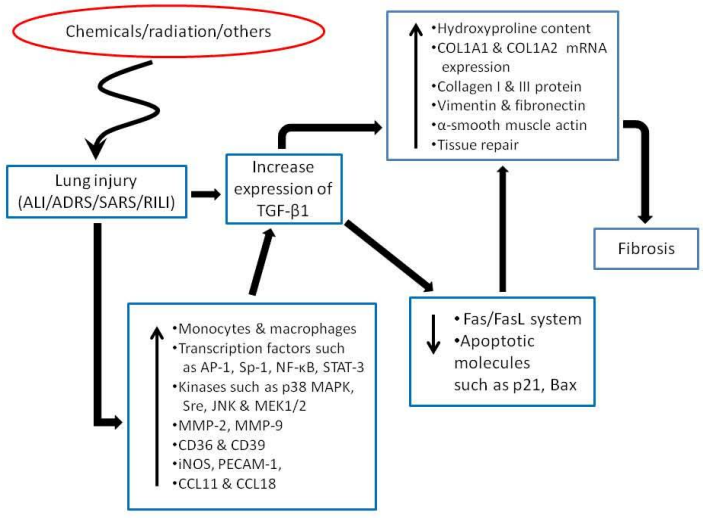
Involvement of TGF-β1 in different models of lung injury and in radiotherapy is perhaps undisputed as has been reported previously. It appears to play a central role as a mediator of inflammatory response in respiratory disorders such as ALI or ARDS or SARS or RILI and has shown to ultimately contributing to the onset of fibrogenesis. TGF-β1 has indicated to interact with multiple cellular pathways and molecules which are known to trigger the fibrotic development in tissues primarily the lungs upon injury (Rube *et al*., 2000; Huaux *et al*., 2005; Nie *et al.*, 2008; Wang *et al.,* 2008; Borthwick *et al.*, 2009; Xu *et*

al., 2009; Quesnel *et al.,* 2010; Bai *et al*., 2011; Danielle *et al*., 2011; Gao *et al*., 2011; Yamauchi *et al.,* 2011; Meng *et al*., 2012).

The induction of inflammatory response and the role played by TGF-β1 in the development of fibrosis in tissue injury is largely dependent on its own regulation by other molecules. The general mechanism involving key pathways and molecules are summarized in Figure 1. Molecules such as transcription factors AP-1, Sp-1, Nuclear factor-kappa-B (NF-κB), STAT-3 (Gao *et al*., 2011; Presser *et al*., 2013), cellular kinases such as p38 MAPK, Sre, JNK and MEK1/2 (Bai *et al.,* 2011), CD36 (Wang *et al.,* 2009), CD39 (Yamauchi *et al.*, 2011), etc., are known to enhance the expression of TGF-β1. Consequently, its activation results into activation of PDGF (Danielle *et al*., 2011; Rhee *et al.,* 2011), fibrocytes and stromal cell-derived factor-1 (Xu *et al*., 2009), keratinocyte and hepatocyte growth factor (Quesnel *et al.*, 2010), COL1A1 and COL1A2 mRNA expression and collagen production (Borthwick *et al.*, 2009; Chandel *et al.*, 2009; Wang *et al*., 2009; Quesnel *et al*., 2010; Gao *et al.*, 2011; Yamauchi *et al.*, 2011), vimentin, fibronectin (Chaudhary *et al.*, 2006; Borthwick *et al*., 2009), α-smooth muscle actin (Chandel *et al*., 2009) leading to the development of fibrosis.

Cytokines such as IL-1β, IL-4, IL-6, IL-13, IL-17 (Chen *et al.*, 2002; Hodges *et al.*, 2012; Kolodsick *et al.*, 2004; Waldow *et al*., 2004; Huaux *et al*., 2005; Chaudhary *et al*., 2006; He *et al*., 2006; Kurzius-Spencer *et al.*, 2008; Kim *et al*., 2009; Rhee *et al*., 2011; Meng *et al.*, 2012) are known to be associated with elevated TGF-β1 level. However, IL-8, epithelial markers, KGF expression and protein (Hart *et al.*, 2005; Nie *et al*., 2008; Borthwick *et al*., 2009; Chandel *et al.*,

Figure 1: Schematic Representation Of The Activation Of Transforming Growth Factor Beta1 (Tgf-β1) Expression Following Lung Injury, Contributing To The Inflammatory Response And Ultimately Leading To The Development Of Fibrosis. Ali: Acute Lung Injury; Adrs: Acute Respiratory Distress Syndrome; Sars: Severe Acute Respiratory Syndrome; Rili: Radiation-induced Lung Injury; Mmp: Metalloproteinase; Cd: Cluster Of Differentiation; Inos: Inducible Nitric Oxide Synthase; Pecam: Platelet Endothelial Cell Adhesion Molecule; Ccl: C-c Motif Chemokines; Col: Collagen. The Up Or Down-arrow Inside The Boxes Indicate The Up-regulation Or Down-regulation Of Various Cellular Molecules And Systems, Respectively



2009; Quesnel *et al.*, 2010) have been found to associated with reduced level of TGF-β1. The role of TNF-α in modulating the expression of TGF-β1 have been slightly controversial. There are conflicting reports on its role associated with up-regulation (He *et al.*, 2006; Pochetuhen *et al*., 2007; Rhee *et al.,* 2011) or down-regulation (Borthwick *et al.*, 2009; Danielle *et al*., 2011; Gao *et al.,* 2011) of TGF-β1 expression and protein in tissue and blood plasma.

The interaction with Fas/FasL-induced apoptosis signalling have provided probably the most convincing role of TGF-β1 as a biomarker of lung injury, both for the acute phase as well as at the later phase of fibrotic development. Its administration have shown to reduced the Fas-mediated aopoptosis in lung epithelial cells following injury, characterized by increased in anti-apoptotic mediators such as caspase-8, JNK etc. (Bai *et al.,* 2011). It was reported that TGF-β1 and Fas death receptor pathway might have a synergistic effect on apoptosis. It was suggested that timely interplay of TGF-β1 and the Fas/FasL system could be the decisive factor on the outcome of cell survival or death signalling during early lung injury and later repair process of lung epithelium. Rather than allow the cell to die by apoptosis, TGF-β1 appears to trigger the repair process, characterized by increased in collagen synthesis along with other ECM proteins, leading to its accumulation and development of fibrosis.

CONCLUSION

Lung injury, in the form of ALI or ARDS or SARS or RILI induced by chemicals, radiotherapy or others agents is marked by enhancement in the expression of TGF-β1 as a mediator of the inflammatory response. The up-regulation of TGF-β1 mRNA expression and its corresponding protein in lung tissue and in blood plasma subsequent to infliction of tissue injury has been established by RT-PCR analysis, Western blotting involving TGF-β1 antibody, immunohisto-chemical and immunolocalisation studies, etc. Increase in the expression of TGF-β1 at the acute phase triggers the tissue repair mechanisms involving the synthesis of ECM proteins and other cellular mediators of tissue repair, a hallmark of development and progression of fibrotic lesions. Therefore, experimental evidences reviewed in this article indicate that TGF-β1 is probably the main biomarker of lung injury induced by various agents and appears to contribute very significantly in the development of fibrosis as a late manifestation of the tissue injury. This is further corroborated by the fact that reduction in TGF-β1 resulted in decrease in pulmonary fibrosis and improvement in survival. However, experimental evidences also suggest that it may not act alone but rather by activation or in combination with other proinflammatory molecules in response to lung tissue injury.

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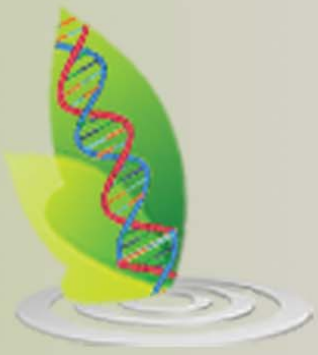

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