5. INFLAMMATION AND FIBROSIS

David J.P. Bassett, Ph.D. and Deepak K. Bhalla, Ph.D.
Department of Occupational and
Environmental Health Sciences
Wayne State University
Detroit, MI 42807

INTRODUCTION

Inflammatory cells represent an important component of the pulmonary defenses, but they also play a critical role in the pathogenesis of lung disorders such as adult respiratory distress syndrome (Patterson et al., 1989), asthma (Bigby and Nadel, 1988), silicosis (Lugano et al., 1984; Sjostrand et al., 1991; Li et al., 1992), interstitial pulmonary fibrosis, and asbestosis (Sibille and Reynolds, 1990; Rochester and Elias, 1993; Gee and Mossman, 1995). Because of the generally recognized adverse effects of airway inflammation and contribution of inflammatory cells to lung fibrosis, the factors involved in inflammatory reactions, mechanisms of toxicity and development of chronic lung disease have received considerable attention. The initial steps in the cascade of events, that ultimately result in the development of chronic lung disease, include stimulation of resident cells, release of chemotactic agents and recruitment of inflammatory cells. These events can be set in motion by an intrapulmonary insult in the form of an acute or chronic inhalation exposure to either environmental or occupational pollutants.

Over the last decade there has been a rapid expansion of understanding of the molecular and cellular mechanisms by which such exposures result in lung inflammation. However, there still remain many unanswered questions concerning factors that determine whether such lesions resolve to a normal structure or progress from a sustained aveolitis to an irreversible potentially life-threatening fibrotic condition. Pulmonary fibrosis is an interstitial disease of the parenchymal tissue, characterized by a failure of normal repair processes of the lung that results in the collapse of alveolar structure, derangement of the epithelial and basement membrane architecture, and by the accumulations of inflammatory cells, fibroblasts and the structural protein collagen (Kuhn, 1995). In many cases, a diffuse pulmonary fibrosis can develop over a period of several years with no clear indication of the etiological cause (idiopathic pulmonary fibrosis - IPF). In other cases, the lesions can be very site specific and can be directly related to either a major acute lung injury or prolonged low level exposures to fibrogenic agents. The major experi-

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mental models that have been used to examine the inflammatory determinants of fibrogenesis have included the use of the anti-neoplastic drug bleomycin (Thrall and Scalise, 1995), radiation (Pickrell and Abdel-Mageed, 1995), and animal inhalation exposures to oxygen, ozone, and such mineral dusts as silica and asbestos (LeMaire, 1995). These exposures have provided methods to separately examine the effects of acute injuries to endothelium, airway epithelium, and the results of direct macrophage activation. In addition, continued exposure and sensitization to antigenic dusts from bacteria, fungi, animal proteins, organic chemicals and beryllium can cause delayed hypersensitivity pneumonitis that is characterized by a sustained mononuclear cell infiltration and the formation of granulomas. Failure of these lesions to resolve can lead to scar tissue formation or extensive interstitial fibrosis (Rose and Newman, 1993). Recent advances in molecular and cellular biology have also provided a greater understanding of the mechanisms and risk factors associated with fibroproliferative disorders, that are required for improving current methods of diagnosis, prevention, and clinical management (Ward and Huninghake, 1998).

This Chapter details the mechanisms of inflammation, discusses various factors that have been identified in the development of fibrosis in the lung, and highlights some of the cells and their respective functions in the fibrogenic process.

INFLAMMATION

Since chronic inflammation presents a risk of tissue damage through the release of toxic mediators, i.e., proteases and oxygen free radicals, by activated inflammatory cells, an understanding of the mechanisms responsible for the recruitment of inflammatory cells and their activation constitutes an important subject for the appreciation of injury process. The mechanistic studies implicate cell activation, cellular mediators, chemotactic factors, extracellular matrix components, cytoskeleton and cell adhesion molecules in inflammation and lung injury. The flowchart in Figure 1 shows the interactive nature of these various components and provides an overview of the sequence of inflammatory reactions which involve stimulation of resident cells, release of chemotactic agents and toxic mediators, cytoskeleton-dependent mobility of neutrophils and adhesion molecule-regulated neutrophil-endothelial cell interactions, followed by neutrophil entry into the lung. This sequence of events takes into account that: 1) the airway epithelial and inflammatory cells, upon activation, release chemokines and products of arachidonic acid metabolism; 2) extracellular matrix components, such as fibronectin, play a critical role in the inflammatory process by providing a surface for attachment and spreading of inflammatory cells, an event that causes stimulated release of chemokines and other cellular mediators; 3) the cellular mediators can cause a wide range of pathophysiologic changes, including activation of vascular neutrophils, which is associated with upregulation of cell adhesion molecules and reorganization of cytoskeletal components so as to facilitate neutrophil motility and interaction with endothelial cells prior to their entry into the lung; and 4) activated inflammatory cells contribute, through the release of toxic products, to lung injury and so amplify the direct deleterious effects of toxic agents of gaseous, particulate, and agents of fibrous or microbial origin.

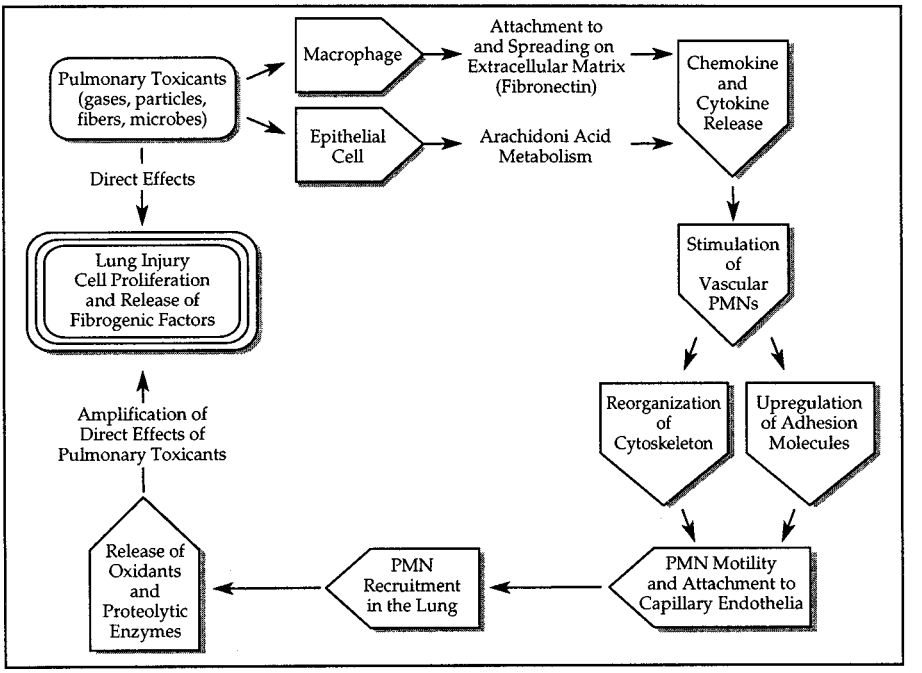

Figure 1. Sequence of inflammatory reactions leading to lung injury and release of fibrogenic factors.

In the context of our current understanding, the inflammatory response represents the end result of a set of complex reactions involving cellular components and mediators that are functionally interrelated and act in concert. The studies discussed below recognize sequential processes, multiple pathways and cytokine/ adhesion molecule specificities in the induction of inflammation.

Macrophage Activation

Alveolar macrophages represent a resident cell type in the lung and compose the majority of the inflammatory cell population in healthy individuals. These cells, along with neutrophils, constitute an alveolar source of degradative enzymes, including a large number of lysosomal enzymes and oxidants capable of inducing lung injury. The slow release of these secretory products of macrophages is contained by active detoxification processes in the lung. However, under abnormal conditions, excessive release of degradative secretions results in pathophysiologic processes leading to chronic lung disease, as discussed later in this Chapter.

In addition to their role in the generation of toxic mediators, microbial killing and tissue damage, macrophages are a source of a number of cytokines and chemokines that serve to recruit and activate other inflammatory cells, including neutrophils, lymphocytes and fibroblasts. It is, thus, apparent that the inflammatory response is the outcome of a series of events involving stimulation of resident cells, release of chemotactic agents and toxic mediators, and mobility and adhesion of

inflammatory cells leading to their recruitment from blood into the lung. The initial stages of this process involving macrophages are accompanied by changes in the cell surface properties relevant to their role in the inflammatory processes. Macrophage functions affecting the release of proinflammatory cytokines and the development of inflammation are stimulated as the macrophages adhere to various surfaces. The adhesion of these cells to plastic or other matrices results in the stimulated release of a neutrophil chemotactic factor (Merrill et. al., 1980), enhanced phagocytosis of opsonized bacteria (Newman and Tucci, 1990) and induction of gene expression of cytokines, interleukin-1 (IL-1), IL-8, and tumor necrosis factor-α (TNFα) (Fullbrigge et. al., 1987; Haskill et. al., 1988; Standiford et. al., 1991). These studies illustrate the early changes in the macrophage behavior following cell stimulation and suggest a relationship between cell adhesion and induction of immunological defenses in the lung. Similar changes could be produced following macrophage stimulation as a consequence of exposure to pulmonary irritants. Ozone exposure has been shown to augment macrophage adhesion to epithelial cells in culture, to stimulate macrophage motility towards a chemotactic gradient (Bhalla, 1996) and to reduce their recovery by bronchoalveolar lavage, perhaps due to their increased adhesion to alveolar walls following oxidant exposure (Pearson and Bhalla, 1997). Macrophages are inherently adhesive cells. A further increase in their adhesive potential is likely to promote their attachment to the airway lining for anchorage dependent functions, to other cell types for cell damage and to inhaled particles or microbes for phagocytosis. The importance of macrophage adhesion in lung injury relates to the suggested anchorage requirement for the induction of full effector functions (Mulligan et. al., 1993). The studies demonstrating an involvement of the integrins in macrophage activation (Arnaout, 1990) and cell accumulation in chronic inflammatory disorders (Malizia et. al., 1991; Argenbright and Barton, 1992) support the suggestion that the cell surface molecules modulate macrophage functions. The absence of regular respiratory burst of the phagocytic cells from patients genetically deficient in CD11/CD18 epitope and a concomitant increase in the frequency of bacterial infections (Hogg, 1989) further suggests that the release of toxic mediators and phagocytic functions may be cell adhesion molecule-dependent processes.

Fibronectin

Fibronectins, dimeric glycoproteins of 500 kDa molecular weight, are produced in the lung by a variety of cell types, including macrophages, fibroblasts, endothelial and epithelial cells, and neutrophils, which accumulate in the lung during inflammation and injury (Ruoslahti, 1988; Limper and Roman, 1992). These are regarded as multifunctional proteins having binding sites for various cell types and molecules, including collagen, fibrin and heparin (Dean, 1989). They are found in most body fluids and as a component of extracellular matrix (McKeown-longo, 1987; Ruoslahti, 1988). Fibronectins play an important role in a variety of biological processes, including cell adhesion, motility, and cell stimulation (Stanislawski et al., 1990). A widely recognized function of fibronectins is their role in tissue repair resulting from recruitment of cells to sites of tissue injury and promotion of

wound healing. The cell recruitment and tissue repair functions of fibronectins are supported by the studies demonstrating their ability to recruit fibroblasts *in vivo* and cause enhanced migration of monocytes and neutrophils *in vitro* (Rennard et al., 1981; Denholm et al., 1989; Everitt et al., 1996).

The plasma contains a large amount of fibronectin and serves as a source of some fibronectin in tissues, where it promotes tissue repair following injury (Deno et al., 1983). In the adult lung, plasma fibronectin is found in association with the basal lamina, in alveolar lining fluid and in the interstitial spaces (Torikata et al., 1985). By providing a substrate for cell attachment, it promotes cell adhesion, cell migration and wound repair (Yamada, 1989; Hynes, 1990a and b), and mediates cell-cell and cell-substratum interactions (LaFleur et al., 1987). The heterodimer integrin $\alpha 5\beta 1$ serves an important role in binding monocytes to receptors on endothelial cells and to extracellular matrix fibronectin in the lung. The increased fibronectin secretion during the repair phase following lung injury is believed to influence the macrophage ability to attract neutrophils through an increased release of inflammatory cytokines. The baseline expression of fibronectin is fairly restricted in the adult lung, but its expression increases after tissue injury and inflammation. Increased concentrations of fibronectin in the bronchoalveolar lavage fluid is reflective of enhanced inflammatory activity in inflammatory lung diseases (Rennard et al., 1981). As discussed below, the inflammatory cell recruitment is mediated by several cytokines, and the products released by recruited inflammatory cells serve as chemotactic agents for migration and influx of additional mononuclear cells and fibroblasts. As reviewed by Limper and Roman (1992), lung injury in fibroproliferative disorders is accompanied by disruption of the alveolar epithelial barrier, and influx of plasma proteins, inflammatory cells, mesenchymal cells and fibroblasts. The fibroblast influx is accompanied by excessive deposition of extracellular matrix proteins, including fibronectin. These events are accompanied by alveolar epithelial type II cell proliferation and migration, leading to septal thickening and development of fibrosis.

Increased fibronectin levels have been found in the BAL of patients with interstitial and inflammatory lung disorders such as asthma, chronic bronchitis, idiopathic pulmonary fibrosis and sarcoidosis (Rennard and Crystal, 1981; Vignola et al., 1996), in the BAL of cigarette smokers (Villiger et al., 1981), in the BAL of sheep and humans exposed to asbestos (Begin et al., 1986) and in the lungs of animals exposed to ozone (O_3), an environmental pollutant (Pendino et al., 1994; Gupta et al., 1998). Increased fibronectin secretion has also been observed in rat parietal pleural mesothelial cells stimulated by asbestos fibers (Kuwahara et al., 1994) and in macrophages following exposure to nickel and cobalt chloride (Roman et al., 1990). The increased fibronectin could subsequently mobilize a set of events leading to lung inflammation. An ability of fibronectin to induce interleukin-1β (IL-1β) production (Graves and Roman, 1996), stimulate neutrophil migration (Everitt et al., 1996), and polymerize actin-cytoskeleton in neutrophils (Yang et al., 1994), represent functions that favor neutrophil recruitment in the lung. It is thus apparent that the extracellular matrix protein, fibronectin promotes conditions conducive to cell adhesion and recruitment of inflammatory cells. Extracellular matrix proteins are also important for their role in fibroblast migration, proliferation and collagen production. An understanding of the functions of fibronectin, therefore, offers an

opportunity for delineation of inflammatory mechanisms and recognition of its role in the development of fibrosis.

Cytoskeleton

Cytoskeletal elements are critical to a variety of cellular functions, including maintenance of cell shape, phagocytic uptake of particles, cytoplasmic transport of molecules and cell motility in response to chemotactic signals. Neutrophil alignment in response to a chemotactic gradient involves increased assembly of actin (Sheterline and Rickard, 1989) and a concomitant change in cellular morphology from a round cell with evenly distributed surface microprojections in a resting cell to an elongated shape with microvillar concentration at the trailing end and membrane ruffling at the advancing end characteristic of a motile cell. These changes represent a behavioral response to a chemotactic stimulus. While little actin is polymerized in resting neutrophils (Sheterline et al., 1984, 1986), the motility of neutrophils is accompanied by actin assembly at the advancing ruffled end, its contraction as a dorsal sheath and its disassembly at the trailing end, which often contains a network of contractile filaments. The reorganization of contractile proteins in motile neutrophils involves their preferential distribution into specific regions of the cells so as to promote cell motility. Cytoskeletal changes could also impact cell adhesion to and migration on extracellular matrix in the lung. The reorganization and regional distribution of these cytoskeletal proteins in response to a chemotactic signal, therefore, appear critical for cell motility as the cells infiltrate lung parenchyma.

Cell motility also involves an active interplay between various cytoskeletal elements and cell adhesion molecules expressed at the cell surface. Cells are generally attached to extracellular matrix by α, β-integrins and to each other by calcium-dependent adhering molecules, cadherins. The surface distribution of adhesion molecules, receptor mobility in the plane of cell membrane and transmembrane interactions constitute events that play a vital role in cellular functions such as phagocytosis, activation, attachment and mobility of leukocytes. It is suggested that CD11/CD18 expressed on the leukocyte surface is generally inactive and requires activation for binding to its ligand (Hynes, 1992; Lollo et al., 1993). This activation is associated with mobility of cell surface receptors and their redistribution into aggregates or patches, which in turn facilitates ligand recognition and binding. Consistent with this suggestion are the studies demonstrating an increase in CD11/CD18 binding to ICAM-1 when the receptors are clustered (van Kooyk et al., 1994) and an increase in the diffusion rate of CD11/CD18 following phorbol myristate acetate (PMA)-induced activation of leukocytes (Kucik et al., 1996). Cytoskeleton indirectly plays a key role in this activation process. Both cadherins and β-integrins are attached by their COOH-terminal to cytoplasmic proteins. Prominent among these linkages are associations with the cytoskeletal proteins, including talin, vinculin, filamin, tensin (or α-actinin) and actin (Burridge et al., 1988). It is suggested that cell activation is associated with a brief dissociation of receptor-cytoskeleton linkage, permitting receptor clustering and cell adhesion (Kucik et al., 1996; Lub et al., 1997). A disruption of this linkage, formation of

receptor clusters and reformation and redistribution of receptor-cytoplasmic linkages appear essential for cell motility and tissue infiltration.

Cell Adhesion Molecules

The lung is regarded as an active site of neutrophil margination (Doerschuk et al., 1990). As blood flows through pulmonary microvasculature, the leukocytes roll and stick to the endothelial cells and become static for a period of less than one to several seconds (Kuhnle et al., 1995). These conditions permit extended contact of leukocytes with endothelial cells and facilitate leukocyte entry into the lung in response to stimuli generated by airway irritation and initial injury. Leukocyte rolling on the capillary endothelium is mediated by cell adhesion molecules belonging to the selectin family including L-selectin expressed on the surface of neutrophils and E- and P-selectins on endothelial cells (von-Adrian et al., 1991; Springer, 1994). Subsequent attachment and tighter binding of neutrophils to endothelia is mediated by neutrophil LFA-1 (CD11a/CD18) and MAC-1 (CD11b/CD18) β_2-integrins and counter receptor intercellular adhesion molecule-1 (ICAM-1) on endothelial cells. While this simplified model describes the early events of inflammatory cell recruitment from systemic circulation, the mechanism of neutrophil recruitment from the pulmonary microcirculation and the precise role of cell adhesion molecules in lung inflammation constitute an area of active investigation.

Mulligan et al. (1992, 1993a and b, 1998) have investigated the role of specific integrins in inflammatory reactions under different conditions. In these studies endothelial injury in pulmonary capillaries and neutrophil accumulation following complement activation by cobra venom factor was dependent on CD11a/CD18, CD11b/CD18, and ICAM-1, but there was no requirement for E-selectin, TNFα, IL-1, or IL-8. On the other hand, lung injury and alveolar accumulation of neutrophils in the IgG immune complex model was dependent on CD11a/CD18, but not on CD11b/CD18. In the IgA immune complex lung injury model, endothelial injury involved ICAM-1 and macrophage chemotactic protein-1, but not TNFα, IL-1 or IL-8. Lung injury produced by anti-glomerular basement membrane antibody was subject to the presence of neutrophils and required β_1- and β_2-integrins, L- and E-selectins and ICAM-1 for emergence of injury. These studies serve to demonstrate differential integrin and cytokine specificities for different inducers of lung injury and inflammation. Doerschuk et al. (1990) and Burns et al. (1994a) reported that *Streptococcus pneumoniae* caused an increased expression of CD11/CD18 in neutrophils in pulmonary capillaries. However, *Escherichia coli* endotoxin-induced neutrophil migration resulted in a decrease in L-selectin and an increase in CD11/CD18 expression only after the neutrophils had moved across endothelial cells. These studies further characterize β_2-integrin specificity and dynamics, reveal their vital contributions, and emphasize unique roles of neutrophil subsets involved in specific inflammatory reactions. Besides their role in inflammatory reactions, β-integrins seem to play a more direct role in development of pulmonary fibrosis. Monoclonal antibodies to CD11a and CD11b were effective in preventing silica or bleomycin-induced collagen deposition in mice (Piguet et al., 1993a).

Intercellular adhesion molecule-1 (ICAM-1) is constitutively-expressed on endothelial cells, epithelial cells, fibroblasts and mesenchymal cells (Dustin et al., 1986; Wegner et al., 1990, 1996). Its expression is substantially increased by cytokines, following microbial infections, in inflammatory lung disorders and upon exposure to pulmonary toxicants. Increased ICAM-1 levels have been reported in the BAL of patients with sarcoidosis (Shijubo et al., 1994). An adherence of neutrophils to fibroblasts in culture by β_2-integrins (Schock and Laurent 1991) and the release of fibroblast replication factors by neutrophils (Schock and Laurent, 1989) suggest a role for these adhesion molecules in cellular interactions and the role of neutrophils in fibrosis. Studies demonstrating increased levels of ICAM-1 the BAL of O_3-exposed rats (Gupta and Bhalla, 1998), in lungs by hyperoxia (Piedboeuf et al., 1996), and in cultured type II epithelial cells (A549) by respiratory syncytial virus (Patel et al., 1995), provides the basis for a role of this molecule in directing neutrophil mobility within the lung. A redistribution of ICAM-1 along the intercellular contacts of type II epithelial cells in culture following hyperoxia (Kang et al., 1993) and increased expression of ICAM-1 on epithelial and endothelial cells in response to inflammatory stimuli (Tosi et al., 1992; Burns et al., 1994b) support the idea that ICAM-1 plays a role in directing neutrophils. Another adhesion molecule, platelet-endothelial cell adhesion molecule-1 (PECAM-1), which is structurally related to ICAM-1 and belongs to Ig gene superfamily (Newman et al., 1990), is concentrated at the junctions between endothelial cells (Muller et al., 1989). PECAM-1 appears to play a role in neutrophil transmigration and therefore it seems to constitute a molecule responsible for neutrophil "traffic control" functions as opposed to the role of ICAM-1 in "neutrophil directionality". Antibodies to PECAM-1 have been effectively used in inhibiting stimulated neutrophil entry into the lungs, peritoneum and skin (Vaporciyan et al., 1993).

Cytokines and Chemokines

Inflammatory and epithelial cells are capable of producing a wide range of mediators with multiple functions and potential for progressive inflammation and excessive tissue injury. Macrophages activated *in vitro* by cytokines or following exposure to pollutant gases and particles, as well as macrophages recovered from patients with pulmonary diseases such as sarcoidosis, have been shown to release increased amounts of chemotactic mediators, suggestive of their role in inflammation and fibrosis (Hunninghake, 1984; Pierce et al., 1996; Stringer et al., 1996; Bhalla and Gupta, submitted for publication). Although IL-1 and TNFα have been studied for their chemotactic activity and inflammatory response in the lung (Leff et al., 1993), there is evidence to suggest that the neutrophil chemotactic activity of macrophage supernatant may be dissociated from IL-1 and TNFα activity (Georgilis et al., 1987; Schroeder et al., 1987), and antibodies to IL-1 and TNFα were not effective in blocking O_3-induced inflammatory response or airway permeability (Pearson and Bhalla, 1997). However, the potency of these antibodies in blocking O_3-induced *in vitro* adherence of macrophages to alveolar epithelial cells in the same study (Pearson and Bhalla, 1997) represents an observation consistent with the suggested role of TNFα and IL-1 in triggering the upregulation of cell adhesion

molecules (Bochner et. al., 1995; Mulligan et al., 1993c; Schleimer at al., 1992). Therefore, TNFα and IL-1 appear to play a role in neutrophil margination and attachment to endothelia through cell adhesion molecules, but other cytokines, especially IL-8 in humans, and cytokine-induced neutrophil chemoattractant (CINC) and macrophage inflammatory protein-2 (MIP-2) in rats could present major stimuli for neutrophil motility and entry into the lung. The importance of IL-8 is supported by the studies demonstrating IL-8 release by epithelial cells at inflammatory sites and in patients with chronic lung diseases such as idiopathic pulmonary fibrosis and ARDS (Carre et al., 1991; McElvaney et al., 1992; Miller et al., 1992). TNFα and IL-1 may be involved indirectly, as they both induce production of proinflammatory IL-8 (Strieter et al., 1989; Smart and Casale, 1994; Liu et al., 1996) and cause upregulation of adhesion molecules (Albelda, 1991; Tosi et al., 1992). IL-8, in turn, causes shedding of LECAM, upregulation of β2 integrins and migration of neutrophils (Huber et al., 1991). Anti-IL-8 antibodies have been found to be effective in inhibiting neutrophil migration across cultured monolayers (Bittleman and Casale, 1995) and blocking IgG immune complex-induced inflammatory reactions in the rat lung (Mulligan et al., 1993d), thereby suggesting a role for the pro-inflammatory cytokines in lung inflammation.

Although IL-8 has not been cloned in rat, newly emerged chemokines, MIP-1, MIP-2 and CINC have been studied for their chemotactic potential. Studies in the last few years have revealed an induction of MIP-2 and KC mRNA expression in rat macrophages and lungs by lipopolysaccharide and vanadium compounds (Watanabe et al., 1991; Huang et al., 1992; Gupta et al., 1996; Pierce et al., 1996), MIP-1 and MIP-2 mRNA expression in rat alveolar macrophages by mineral dust (Driscoll et al., 1993a), and MIP-2 and CINC mRNA expression in mice and rat lungs by O_3 (Driscoll et al., 1993b; Koto et al., 1997). In the recent studies of Yuen et al. (1996), MIP-2 and KC mRNA expression in rats exposed to silica correlated with neutrophilic inflammation and chemotactic activity, even though the cytokine expression was only transient while the inflammation persisted for 10 days. While these results support the role of MIP-2 and CINC as important cytokines, they also recognize a complex mechanism involving multiple cytokines in the induction of lung inflammation and tissue injury.

A large number of cytokines are constitutively-expressed in the lung, but their production is upregulated in connective tissue disorders. It is suggested that an abnormal production of cytokines maintains connective tissue buildup preceding tissue remodeling (Kovacs, 1991; Kovacs and Dipietro, 1994). Interleukin-2 (IL-2)-stimulated cytokines were found to cause fibroblast proliferation, and leukocyte supernatant effectively induced increased expression of type 1 procollagen and fibronectin mRNA (Kovacs et al., 1993). Other cytokines have been implicated in activation and proliferation of mesenchymal cells leading to increased secretion and deposition of extracellular matrix. Transforming growth factor-β (TGFβ) is regarded as a fibrogenic cytokine involved in the development of pulmonary fibrosis. An increase in the production of TGFβ has been reported both in an animal model of pulmonary fibrosis (Khalil and Greenberg, 1991; Westergren-Thorsson et al., 1993) and in lung tissues from patients with idiopathic pulmonary fibrosis (Khalil et al., 1991; Broekelmann et al., 1993). TNFα and IL-1 are believed to play important roles in silica-induced fibrosis. Higher levels of TNFα mRNA were found in lungs

from patients with idiopathic pulmonary fibrosis than in normal lungs (Piguet et al., 1993b). Furthermore, an anti-TNFα antibody, a TNFα antagonist and an IL-1 receptor antagonist prevented silica-induced hydroxyproline increase and collagen deposition in mice (Piguet et al., 1990, 1993c; Piguet and Vesin, 1994).

PROGRESSION TO FIBROSIS

Following the onset of toxic exposure, lung inflammatory reactions, and endothelial, epithelial and interstitial repair processes usually proceed in an orderly fashion and under normal circumstances would be expected to result in resolution of the initial lesion. Understanding the pathogenesis of interstitial fibrosis is therefore dependent on the identification of factors that alter these normal repair mechanisms. The site and extent of the initial interaction, the characteristics of the associated injury and inflammation, and the persistence of the offending agent can all influence the pathogenic outcome (Doherty et al. 1993). Continuing exposures to the same or different agents have the potential to continue to cause injury, perpetuate the inflammatory condition, and as illustrated by studies with oxidants, interfere with endothelial, epithelial, and interstitial repair processes (Haschek and Witschi, 1979; Haschek et al., 1983; Last et al., 1993; Choi at al., 1994; Riley and Poiani, 1995). A sustained level of inflammatory cells within the lung together with high levels or altered patterns of mediator production, oxidant metabolism, and proteinase release all appear to be common determinants of fibrogenesis. Extensive damage to extracellular matrix components, either as a result of the initial or continuing injury, has also been associated with the loss of a suitable template for normal epithelial replicative repair, and subsequent infiltration and proliferation of fibroblasts can lead to the excessive collagen synthesis and deposition associated with fibrogenesis.

Fibrogenesis is clearly a very complex process that involves every cell type within the lung alveolar septum and within the extracellular matrix in which these cells and resident and infiltrating inflammatory cells operate. These different cell-types are subject to a series of mechanisms that control, stimulate, and/or inhibit molecular, cellular, and biochemical processes. It is therefore not surprising that in addition to a wide range of occupational and environmental risk factors associated with the development of fibrosis, studies have also been conducted to seek evidence of genetic factors that might render individuals more susceptible to fibrogenesis (Marshall et al., 1997).

FIBROGENESIS

Fibrogenesis can proceed from a wide range of different inflammatory scenarios (Figure 2). In the case of acute damage to either lung endothelium or airway epithelium, brought about for example by septic shock or by accidental exposure to reactive substances such as phosgene or methyl mercaptan, an exudative phase associated with edema fluid and neutrophil accumulation is followed by a period of proliferative repair activity. It is during this latter period that continuing toxic exposures might either interfere with epithelial and endothelial repair or result in the

maintenance of high numbers of inflammatory cells within the lung, representing possible factors that determine whether complete repair takes place or fibrogenesis is initiated. In the case of chronic exposures to mineral dusts, the resident alveolar macrophages are thought to be kept at a high level of activation, resulting in the sustained release of proinflammatory cytokines that promote neutrophil, monocyte-macrophage, and lymphocyte accumulations. Sustained levels of these cells within the interstitium in an activated state have the potential to enhance the production of fibronectin, and the release of factors such as insulin-like growth factors (IGF), platelet-derived growth factor (PDGF), TGFs that have been identified with promoting fibroblast migration and proliferation. Inhalation exposures to antigenic material results in a complex series of inflammatory and immunological events whereby alveolar macrophages and T-lymphocytes release proinflammatory cytokines (including IL-1, interferon-γ (IFNγ), and colony stimulating factors) that promote lymphocyte and monocyte recruitment and proliferation that can lead to a mononuclear cell infiltrate/granuloma (Rose and Newman, 1993; Semenzato and Agostini, 1993). Under certain circumstances the cells of these lesions release chemoattractants for monocytes and neutrophils, and fibronectin and growth factors that cause fibroblast proliferation and enhancement of collagen deposition associated with the fibrogenesis found in sarcoidosis (Semenzato and Agostini, 1993) and delayed hypersensitivity disorders (Rose and Newman, 1993).

Importance of Epithelial Cell Repair

The role of lung capillary endothelium in controlling inflammatory events has been described above. The airway epithelium also has a controlling role, since it is often involved in the initial injury, inflammatory reactions and the subsequent repair processes. Failure or delay in the repair of ciliated airway epithelium, damaged by either infection or oxidant injury, can decrease lung clearance of airway-deposited and macrophage-engulfed fibrogenic agents, increasing the risk of sustained injury and inflammation, and so facilitating fibrogenesis. Loss of epithelial and endothelial integrity in acute lung injuries can result in fibronectin release into the airways and alveolar accumulation of blood clotting components that can lead to fibrin deposition (Idell, 1995). Fibrin formation from fibrinogen has been shown to be influenced by both alveolar epithelium and macrophages (McGee and Rothberger, 1985; Gross et al., 1991). Under conditions when fibrin and other exudative components are not effectively removed by inflammatory phagocytes, fibroblasts can migrate into the fibrin matrix and deposit interstitial collagens, leading to scar formation and/or progressive fibrosis.

Regeneration of damaged alveolar surface epithelium (type I) involves proliferation of the surfactant-generating type II cell and their subsequent transformation to new type I cells (Adamson and Bowden, 1974, Evans et. al., 1974, 1976). Oxidant inhibition of this proliferative repair process following butylhydroxytoluene (BHT)-induced damage has been implicated in causing uncontrolled fibroblast proliferation, collagen deposition, and fibrogenesis in mice (Haschek and Witschi, 1979; Haschek et al., 1983).

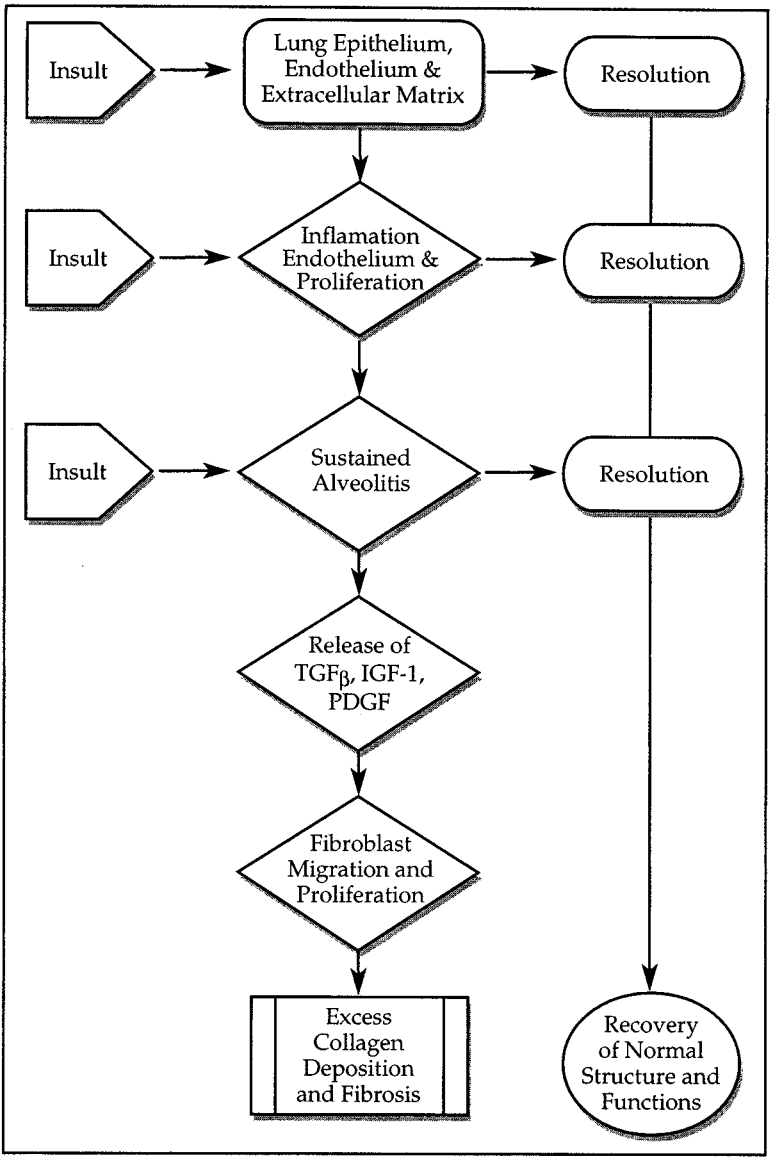

Figure 2. Sequence of cellular events leading to fibrosis.

Understanding the control of epithelial repair and the potential importance of an intact array of basement membrane components for such activity is also an important area of research interest (Kuhn et al., 1989; Sannes, 1991). Alveolar endothelial and epithelial cells have both been shown to synthesize extracellular matrix components that make up the basement membrane, that include type IV collagen, fibronectin, laminin, and proteoglycans. Severe damage and disorganization of this complex series of components that make up the basement membrane separating endothelium from epithelium in the alveolar septa, can lead to failure in

repair, alveolar collapse, and proliferation of resident fibroblasts. Fibroblast migration and proliferation might also be influenced by altered epithelial synthesis of fibronectin, prostaglandins, cytokines, and other growth factors. Transgenic mice with respiratory epithelium expressing human TGFα have been shown to contain fibrotic lesions (Korfhagen et al., 1994), demonstrating a major role of the epithelium in controlling extracellular matrix metabolism.

Involvement of Neutrophils

As noted above, the margination and infiltration of neutrophils represents an early inflammatory event in acute lung injuries. These cells have been implicated as being responsible for causing or amplifying the destruction of tissue elements as a result of their ability to generate reactive oxygen and nitrogen intermediates and to release various proteinases into the interstitial environment (Henson and Johnston, 1987). Alveolar macrophages have similar potential but are thought to have more of a controlling role in subsequent events rather than being a major destructive influence. Neutrophil accumulation alone does not necessarily result in lung damage, since macrophage-mediated recruitment of neutrophils in response to airway exposure to chemoattractants has demonstrated no associated extracellular matrix or permeability damage (Staub et al., 1985; Rheinhart et al., 1998). However, an association between neutrophil infiltration and amplification of acute lung injury have been extensively demonstrated in other types of inflammatory model (Repine, 1985; Bassett et al., 1989; Patterson et al., 1989; Sibille and Reynolds, 1990; Bhalla et al., 1992; Kleeberger and Hudak, 1992; Pino et al., 1992; Ghio and Hatch, 1996).

Stimulated neutrophils have the capacity to generate superoxide anion, hydrogen peroxide, nitric oxide, hyroxyl radicals, and hypochlorous acid as well as release such proteinases as elastase, cathepsin G, collagenase, and gelatinase (Weiss, 1989). These proteinases target specific protein elements of the extracellular matrix. Other potentially damaging components include cationic proteins, peptides, and polyamines. It is thought that under normal conditions of neutrophil chemotaxis and phagocytosis, there are sufficient antioxidant and antiproteinase activities within the neutrophil itself, potential target cells, the alveolar lining fluid, and the interstitial environment. However, excess activity and imbalances between oxidant and antioxidant mechanisms, and between proteinases and antiproteinases activities would be expected to adversely affect a neutrophil-enriched lung. For example, oxidant exposures have been associated with inactivation of the methionine residue of α1-antiproteinase. Neutrophils have a relatively short half-life within the lung airways of less than 24 - 48 hr, during which time they undergo programmed cell death (apoptosis) and are then removed by macrophages (Savill et al., 1989; Savill, 1992). It has been proposed that interference with these processes that include failure of macrophages to recognize and remove apoptotic neutrophils, could result in release of excess proteinases and myeloperoxidase from necrotic neutrophils into the lung interstitial environment.

The conditions under which neutrophils contribute to the permeability damage observed in the early stages of acute oxidant injuries remain controversial and under investigation (Repine, 1985; Bassett et al., 1989; Bhalla et al., 1992; Kleeberger

and Hudak, 1992; Pino et al., 1992; Ghio and Hatch, 1996). Their role at different stages of fibrogenesis is also not fully understood. Collagen levels during the first 7 d following bleomycin administration have been found to be enhanced in treated rats that have been neutrophil-depleted, suggesting a protective effect (Thrall et al. 1981; Clark and Kuhn, 1982; Thrall and Scalise, 1995). In contrast, sustained levels of neutrophils and hyroxyproline-enriched collagen degradation products in the lavage fluid recoveries from patients with IPF and from experimental animals undergoing progressive fibrosis, suggests a role in later events. Neutrophil recoveries in BAL have been correlated with the severity of the disease. It is interesting to note that in the case of granulomatous disease, the possibility that fibrosis might subsequently develop is indicated by the presence of a greater ratio of lavage recovered T-helper type 1 lymphocytes (T_H1) to type 2 cells (T_H2) have been discussed (Rose and Freeman, 1993; Semenzato and Agostini, 1993; Ward and Hunninghake, 1998). T_H1 cells are a source of IL-2 and IFNγ that would be expected to promote granuloma formation, while T_H2 cells release the anti-inflammatory cytokines IL-4 and IL-10 that could reduce neutrophil accumulations by their ability to influence macrophages to decrease TNFα generation. In addition to a potential role in sustaining tissue injury during fibrogenesis, neutrophil myeloperoxidase production of hypochlorous acid has been shown to activate collagenase and gelatinase enzymes (Brieland and Fantone, 1995). Neutrophil elastase has been shown *in vitro* to activate TGFβ, the main growth factor implicated in controlling fibroblast proliferation and metabolic function (Murphy and Docherty, 1992).

Macrophages as a Source of Fibrogenic Mediators

As previously noted, the determinants and mechanisms of fibrogenesis are far from clear, but the enhanced presence of fibroblasts in the progressive stages of the disease is a common feature. The macrophage has a central role in generating growth factors that promote fibroblast proliferation, migration, and collagen deposition include TGFβs, PDGF, IGFs, and granulocyte-macrophage colony stimulating factor (GM-CSF). However, it should be noted that fibroblasts also represent an important source of these factors under normal and inflamed conditions (Tremblay et al., 1995).

TGFβ1 is considered to be a major regulatory cytokine as it is generated as an inactive precursor that requires enzymatic modification for its activation. TGFβ1 has a wide spectrum of activity that includes being a chemoattractant for fibroblasts and a main stimulating factor in their synthesis of collagens and other extracellular matrix components (Khalil and OíConner, 1995). TGFβs have also been implicated in increasing matrix proteins by their inhibition of proteinase production with a concomitant stimulation of proteinase inhibitor generation (Kelley et al., 1991, 1992). In addition to alveolar and interstitial and alveolar macrophages, PDGF is also generated in the lung by endothelial and epithelial cells and the mesenchymal fibroblasts and smooth muscle cells. It has the potential to cause proliferation of fibroblasts and act as a chemoattractant, stimulating their migration into the affected region of the lung together with monocytes and neutrophils. IGFs have been associated with cell proliferation and differentiation. GM-CSF is usually associated

with stimulation of myeloid stem cell differentiation to granulocytes and monocytes-macrophages. However, in fibrogenesis, GM-CSF is thought to have a direct role by its potential to induce macrophage production of TGFβ, resulting in enhanced accumulation of myofibroblasts and fibroblasts (Xing et al., 1997).

Fibroblasts and Collagen Metabolism

The disorganization and remodeling of the interstitium associated with fibrogenesis is accompanied by excessive production of structural collagens. Although epithelial and endothelial cells can produce basement membrane collagens, the major collagen in the lung is the fibrillar type I collagen with some type III. These structural proteins, together with elastin and fibronectin, are mainly produced by interstitial fibroblasts. Early studies suggested that collagen turnover in the adult lung was extremely slow. However, use of radiotracers that cannot be reutilized has demonstrated very high rates of collagen turnover that in experimental animals indicate that as much as a tenth of all lung collagen can be replaced every day. This finding has lead to the recognition that alterations in either collagen protein synthesis or collagen degradation can result in a net increase in lung collagens and fibrosis within the lung. The biochemical processes by which collagens are synthesized has been extensively investigated (Berg, 1986; Goldstein, 1991; McAnulty and Laurent, 1995; Bienkowski and Gotkin, 1995). In summary, procollagen polypeptide α chains are generated and subjected to post-translational modifications that include hydroxylation of proline residues and hydroxylation and some glycosylation of lysine residues. Triple helix formation takes place with some disulphide bond formation. Following cell excretion and protease-facilitated removal of propeptide chains, collagen molecules form into fibrils, stabilized by crosslinks involving the action of lysyl oxidase. Hydroxyproline measurements have been extensively used as a measure of lung collagen content and as an indicator of its degradation when recovered in BAL fluid, blood, and urine. Enhanced lysyl oxidase activity has been used as a marker of increased collagen deposition in animal models of fibrogenesis.

It should be noted that studies with rats (Mays et al., 1989) and isolated fibroblasts (Rennard et al., 1982) have demonstrated that a very high percentage of newly formed procollagen is most likely degraded by intracellular proteinases before it is secreted from the cells (Berg, 1986; Murphy and Docherty, 1992). Extracellular collagen and collagen fibers can be degraded by cathepsins, collagenases and gelatinases generated by neutrophils, macrophages, and fibroblasts (Murphy and Docherty, 1992). Control of the activity of these destructive enzymes is provided by a need for their activation, and by several serum and extracellularly located inhibitors such as α2-macroglobulin and tissue inhibitor of metalloproteinases (TIMP).

Heterogeneity in fibroblast populations exists between different tissues and within the same tissues when inflamed and undergoing fibrogenesis. Their metabolic activities appear to be influenced by age, hormones, mechanical stresses, and the presence of growth factors. Comparisons of fibroblasts from normal and inflamed tissues have demonstrated enhanced ability to proliferate *in vitro*, in the presence of stimulating cytokines and other growth factors such as serum proteins. Fibroblasts have also been shown to be effector cells in the fibrogenic process by their autocrine

release and response to a similar series of growth factors as those observed to be released by macrophages from fibrotic lungs (Tremblay et al., 1995). Myofibroblasts, that are identified by the presence of the contractile element smooth muscle cell actin, have also been observed in chronically-inflamed lungs, in bleomycin-induced lung fibrogenesis, and in lung biopsies from patients with IPF. The presence of these cells might represent a permanent change in phenotype, or a transient stage in the development of mature fibroblasts (Leslie et al. 1992).

SUMMARY

Lung fibrosis can result from a wide range of different types of insults that include acute exposures to reactive substances and chronic long term exposures to mineral and antigenic dusts and particulates. In the case of idiopathic fibrosis, the etiological origins often remain unknown, although in some cases the involvement of both the humoral and cellular immune systems and the formation of damaging immune complexes have been implicated in the early stages of fibrogenesis. In the case of mineral dust exposures, focal fibrotic lesions are often observed within the lung parencyhma in association with regions of normal tissue architecture. However, following an acute exposure to an oxidant, more diffuse lesions might be observed that could progress to cover a wide area of the lung parencyhma leading to a more rapid decline in pulmonary function. In spite of the diversity of pathogenesis, there are many common features that have linked the development of fibrosis with a persistent lung inflammatory state, characterized by a sustained aveolitis or mononuclear cell-enriched granuloma.

The inflammatory process involving the activation of the cellular immune system, infiltration of phagocytic cells into the lung and alterations in lung permeability, can be considered representative of protective mechanisms that: a) reduce exposure of lung cells to harmful substances; and b) orchestrate and/or initiate repair processes. However, under certain circumstances of either extreme acute exposure or sustained toxic exposure over prolonged periods of time, the inflammatory process can alternatively cause or contribute to a sustained level of lung damage or interfere with normal endothelial, epithelial, or extracellular matrix proliferative repair processes. Infiltrating inflammatory cells can cause damage by excess release of oxidants/proteinases. The neutrophil is recognized as having one of the greatest potentials to cause damage. Its continued presence within an inflamed lung has been associated with the development of fibrosis. Under a range of different pathological conditions, resident and infiltrating inflammatory cells as well as endothelial, epithelial, and mesenchymal cells of the lung release a complex series of lipid and peptide mediators and growth factors that can result in sustained tissue damage and inflammation, and subsequent migration and proliferation of fibroblasts. The associated derangement of the extracellular matrix with excess interstitial collagen synthesis and deposition represents a common pathological feature of lung fibrosis.

Detailed mechanisms associated with the inflammatory process have been examined extensively using modern molecular and cell biological approaches. The results of these studies have lead to the experimental evaluation of several different types of intervention that have reduced fibrogenesis. Such investigations have used

antibodies against key inflammatory mediator receptors, and treatments with agents that affect the function and availability of inflammatory cells. Although such studies have delineated many of the inflammatory determinants of fibrogenesis, fewer intervention studies have been conducted to probe the complex series of events associated with fibrogenic progression.

In order to improve the diagnosis, prevention and clinical management of pulmonary fibrosis, continued research is required to obtain a greater understanding of: a) the biochemical mechanisms that determine the balances between oxidant and antioxidant, and between proteinase and antiproteinase activities within the interstium of the lung; b) the relationships between extracellular matrix components that include proteoglycans, collagens, and elastin, and the epithelium and endothelium during proliferative repair activities; c) factors that influence whether inflammatory cells release pro- or anti-inflammatory cytokines; and d) the mechanisms that control growth factor generation, fibroblast migration, proliferation and collagen deposition.

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