Interstitial Pulmonary Fibrosis
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Interstitial pulmonary fibrosis (IPF), also known as fibrosing alveolitis, may be defined as a condition characterized by inflammation of the walls of the respiratory airspaces of the lungs that proceeds to fibrosis or the laying down of an excess of connective tissue. Fibrosis need not be present at every stage of the process. The inflammatory response may be granulomatous or nongranulomatous. IPF may be considered a subset of the interstitial lung diseases, which may be defined as inflammation, primarily involving the respiratory airspace walls, that may resolve completely or may go on to fibrosis (Fig 1).

Interstitial pulmonary fibrosis is produced in humans by many known etiologic agents, such as exposure to asbestos or silica dust and hypersensitivity pneumonia from exposure to organic dust. About 15% of cases are associated with the connective tissue-vascular disorders, which are generally believed to be of autoimmune etiology. In yet other cases, or idiopathic IPF, the etiology is totally unknown. The adult respiratory distress syndrome ultimately results in pulmonary fibrosis if the lung damage does not resolve and may be considered a form of IPF.

The nature of the initial injury to the lungs in animal models of acute interstitial lung disease,1 IPF due to the inhalation of known particulates such as asbestos2 and silica,3 and animal models that focus on granulomatous responses and the immunologic mechanisms underlying them4-5 are covered elsewhere. I review the animal models that do not fit into the above categories. My main goal will be to identify some general principles of IPF regardless of etiology and to consider the factors that might determine why some lung injuries heal without fibrosis, while others result in grossly disordered lung architecture with the accumulation of a large excess of connective tissue. Although the answers are just starting to come in on this question, I believe that insights gained during the past decade are pointing to important new directions for research.

I review work on animal models of IPF that have been produced by bleomycin,6-7 butylated hydroxytoluene,8-9 cyclophosphamide,10-11 ionizing irradiation,12-14 N-nitroso-N-methylurethane,15 paraquat,16-18 phorbol myristate acetate,19-20 treatments giving rise to toxic oxygen products,21-22 and combined injuries.19-20 A genetic model, the moth-eaten mouse, has been described that first develops inflammation in its lungs and subsequently develops mild focal fibrosis.23 This model, which provides the possibility of determining the exact genetic mechanism that gives rise to inflammation and fibrosis, may lead to understanding the relatively small number of familial cases of human IPF. Chronic interstitial inflammation with mild fibrosis has also been produced in mice by infection with sublethal doses of A/PR8/34 influenza virus.24 This model suggests that as virologists gain experience with unusual viral infections, it may be worth their while to return to virologic studies of patients with idiopathic IPF.

**Stereotyped Response of the Lung to Injury**
Animal models of IPF have been produced mostly in rodents, although rabbits,25 dogs, and primates have been used to a limited extent. A review of these models leads to the inescapable conclusion that, with minor variations, a wide variety of injuries is manifest in a similar or stereotyped way in the lungs. There is frequently little abnormality for hours after the perturbation. Pulmonary edema and hemorrhage generally follow. The injury is further manifest by necrosis of type I alveolar epithelium and the entry into the lungs of inflammatory cells, which initially are predominantly neutrophils. Alveolar capillary endothelial cell injury may precede type I alveolar cell injury or may occur concomitantly. Within a few days, neutrophils recede, and macrophages and lymphocytes become the predominant inflammatory cell. Lymphocytes persist for long periods in the lungs in foci of fibrosis. Within a few days of the injury, dedifferentiation, replication, and spreading of alveolar type II cells occurs to repair...
the loss of type I alveolar epithelium. At about the same
time, a granulation tissue response develops with budding of
capillaries and increased numbers of fibroblasts. Within a few
weeks of the injury, as the inflammatory response subsides,
excess connective tissue is laid down focally within the lungs,
causing gross disruption of lung architecture. This process
may continue for several weeks. As repair of the lung
proceeds, the least severely damaged areas of lung may be
restored to a normal or nearly normal appearance.

With irradiation pneumonia, endothelial cell injury is
much more prominent than type I epithelial cell injury,
although the latter does occur.24 Macrophages first decrease
and then increase, and the neutrophil response is much less
prominent in this example of IPF probably because of the
neutropenia that is usually part of whole-body irradiation in
small animals.43

**Inhomogeneity of the Response**

Regardless of how the injury is produced, whether via the
airways or the blood vessels, the lesion is focal in its
distribution, even though widespread within the lungs.
Some areas of the lung are severely damaged, while others
are relatively spared or remain normal. The reasons for this
characteristic inhomogeneity are not known. I believe that
the explanation is related to the extensive vasomotor and
bronchomotor functions of the lung, which so exquisitely
match ventilation and perfusion in this organ. This homeo-
static mechanism is necessary, because the lungs are subject
to the buffetting of gravity and many other factors that give
rise to wide variations in pressure and flow in its blood and
gas compartments. The presence of this homeostatic mechan-
ism likely represents one factor that makes it impossible for
all alveolar walls to be equally exposed to an injurious agent,
whether the agent is blood- or air-borne. Varying degrees of
solubility of airborne gases and of penetration of inhaled
particles are other factors that account for the inhomogeneity
of interstitial lung diseases.

**Physiology**

The composite picture of disordered physiology in experi-
mental IPF is that of a restrictive ventilatory defect with
hypoxemia. The volume of air in the lungs at a standard
transpulmonary pressure, such as 25 cm H2O, is decreased,
as are the other compartments of lung volume. The compli-
ance of the lungs in mid-volume range is diminished. Thus,
the volume-pressure curve is often described as shifted down
and to the right. However, it must be recognized that there is
only a weak correlation between abnormalities of volume-
pressure relationships and the severity of fibrosis as deter-
mined by lung collagen content.46 Airflow obstruction is
absent or at most minimal in the forced expiratory flow-
volume curve. The diffusing capacity for carbon monoxide is
decreased, and arterial blood gases in awake animals show
hypoxemia without hypercapnia. Increased contractility of
parenchymal lung strips has been observed in bleomycin-
induced IPF.57 This increased contractility of the lung ap-
ppears to be clearly related to increased contractile protein
observed in the connective tissue cells known as myo-
fibroblasts, which are part of the granulation tissue response
in this model.

**Biochemistry**

Much new information has been gained on the biochem-
istry of interstitial pulmonary fibrosis from a study of animal
models. Total lung protein, DNA, collagen, elastin, and
glycosaminoglycans are increased.51,54-56 The total amounts
of these substances in the lungs must be compared with the
total amounts in the lungs of the controls. Since lung protein
and DNA are also increased, the ratios of collagen, elastin, or
glycosaminoglycan to protein or DNA, or concentration data
(amount of a substance per unit weight of lung) may show
little or no alteration from the control values at many stages of
the process.

In the early stages of the lesion, the increase in total protein
reflects the leakage of plasma proteins into the lungs. Later,
an increase in the total number of cells and of connective tissues is the major reason for the increase in total protein. The increase in DNA reflects an increased number of cells, whether from inflammatory cells that have infiltrated the lungs or from resident cells which have undergone replication. Increases in RNA, calcium, cAMP and cGMP indicate the activation of many cells that are present and participating in the repair process.

**Connective Tissue Synthesis**

In *vivo* and lung organ culture studies show that the rates
of collagen synthesis are increased at the end of the first
week, although striking increases in total lung collagen
amounts are not noted until the third to fourth week after injury.54-56 Increases in lung collagen synthesis may persist
8-12 weeks. One study has indicated decreased degradation
of newly synthesized collagen.54 Lung elastin synthesis is
increased.55 Studies with radiolabeled precursors indicate
that glycosaminoglycan synthesis is also increased and that
there is a preferential production of dermatan sulfate.56-57

Increases in the enzymes that play a role in connective
tissue production have been observed. For example, there
are increases in glucosamine-6-phosphate synthetase, which
is important in glycosaminoglycan synthesis58 and in prolyl
hydroxylase and lysyl oxidase, which are important in col-
lagen and elastin synthesis.55,59 There is an increase in the
ratio of type I to type III collagen in the new connective
tissue that is produced.60 Type V collagen appears to remain
normal in amount.

**Miscellaneous Findings**

There is an increase in total and specific superoxide
dismutase activity in bleomycin-induced pulmonary fibrosis
in rats.56 Increases in connective tissue mast cells and in
histamine content of the lungs have also been observed in
this model.52 There is an increase in non-smooth muscle
actin, with an increased degree of its polymerization similar
to the finding in other tissues that are undergoing remodel-
ing.54,55

**Mechanism of Fibrosis**

Several studies have been done on animal models in the
hope of casting some light on the pathogenesis of IPF. Phan
and Thrall61 showed the presence in neutral salt-soluble
extracts of normal lung of a heat- and trypsin-sensitive,
>100,000 molecular weight substance capable of inhibiting
collagen and noncollagenous protein synthesis by lung organ cultures. Bleomycin-treated lung extracts were less effective in inducing this inhibition. Clark et al. showed that lung explant conditioned medium from normal and bleomycin-treated hamsters, increased prostaglandin E, production, and intracellular CAMP in human and hamster lung fibroblast cultures. Cell growth and collagen production were diminished. The bleomycin-treated lung conditioned medium had the greater effect.

I believe that the initiation of connective tissue synthesis occurs in response to injury to nonconnective tissue portions of the lungs and not to injury of the connective tissue itself. Enzymatic injury of the connective tissue framework of the lungs with bacterial collagenase or with pancreatic or human neutrophil elastase causes tissue injury, hemorrhage, edema, and cellular injury, but does not result in pulmonary fibrosis. Although the connective tissue synthetic machinery of the lungs is initiated by these injuries, the process stops when total lung collagen is normal or at most only slightly elevated. Unpublished data from our laboratories show that in the cadmium chloride-induced model of IPF, radiolabeled elastin present at the beginning of the injury is not degraded. That is not to say that there may not be considerable degradation of newly formed connective tissue, with connective tissue production exceeding connective tissue degradation.

Figure 2 summarizes the stereotyped events of the response of the lung to injury in schematic form. An arrow is drawn from inflammation to injury. Inflammation is composed of many redundant processes that ensure the entry into the lung and the activation of neutrophils, macrophages, and lymphocytes. The arrow is meant to indicate that the activated cells of the inflammatory response may contribute to further injury of lung cells. The processes of inflammation may also affect the connective tissue matrix of the lungs and the cells that lay it down, stimulating the production of new connective tissues or perhaps impairing the orderly laying down of newly formed connective tissues. The key question, restated, is why do some lungs heal with fibrosis, whereas others are restored to a virtually normal state?

**Lung Cells and Fibrosis**

**Neutrophils:** There can be little question that neutrophils play a key role in the initial injury to the lung. It is much less clear that they play a role in the fibrogenic process. Indeed, depletion of the lungs of rats or hamsters of neutrophils by giving them antineutrophil serum prior to the administration of intratracheal bleomycin gives rise to an increase in lung collagen content and in collagen synthetic rates. Beige mice, which are unable to degranulate their giant neutrophils of granules in normal fashion but can generate toxic oxygen radicals, have an increased synthesis and accumulation of collagen in their lungs after intratracheally given bleomycin.

**Macrophages:** Macrophages secrete a factor that increases the production of PGE by fibroblasts; this prostaglandin down-regulates collagen production. Macrophages from bleomycin-treated hamsters secrete more of this factor than macrophages from normal controls. The factor may serve to limit collagen production in bleomycin-induced IPF. Phorbol myristate-induced IPF is inhibited by catalase but not by neutrophil depletion or superoxide dismutase, suggesting that hydrogen peroxide generated by macrophages is the major mechanism of injury that leads to fibrosis in this animal model. Although there is ample evidence for fibroblast macrophage interactions, there is as yet little in vivo evidence that macrophages interact with fibroblasts to cause their movement to the most injured areas of the lungs or to influence the amounts of protein, collagen, or elastin that they synthesize.

**Lymphocytes:** Although the precise role of lymphocytes in the fibrogenic process is still far from clear, there is some evidence from animal model studies that they do have some effect. One group found athymic nude mice, which are T-cell deficient, no different from white mice in induction of fibrosis by bleomycin administration. Another group showed lower cellular infiltration, fibroblast proliferation, collagen synthesis, and connective tissue accumulation after intratracheal bleomycin in nude than in euthymic controls. BALB/c mice are low fibrosis responders to bleomycin. Pretreatment of these mice with a nonpulmotoxic dose of cyclophosphamide increased their fibrogenic response. Repletion of cyclophosphamide-treated mice with normal spleen cells but not T-cell-depleted spleen cells reconstituted the low fibrogenic response.

After bleomycin injection, there is a transient increase in the proportion of B cells. The normal helper-to-suppressor ratio of 1:1 is changed to 2:1 at 30 days and 1:2 at 120 days. On the other hand, lymphocytes from bronchoalveolar lavage fluid, which are elevated at 3–14 days, retain the 1:1 helper to suppressor ratio.

**Platelets:** Very little is known about the role of platelets in IPF. Native platelet activating factor (1-O-octadecyl-2 acetyl-sn glyceryl-3 phospholyl chol) produces dose-dependent acute interstitial inflammation and subsequent fibrosis when instilled into the lungs of rabbits.
In a recent study on bleomycin-induced pulmonary fibrosis, especially when the agent is given by injection. There is also evidence of endothelial cell dysfunction after a single, low-dose intratracheal treatment with bleomycin in the absence of development of fibrosis.

Injury to the endothelial cell is the major lesion in irradiation-induced IPF and the occurrence of this form of experimental fibrosis appears to be related in a dose-dependent manner to the severity of injury to endothelial cells. Inflammatory cell infiltration is not a prominent feature of irradiation-induced interstitial pulmonary fibrosis. The slow onset of fibrosis after irradiation may be related in some poorly understood way to the minimal inflammatory response to injury, which might be in turn related to depression of bone marrow and lung macrophage response by the irradiation. There appears to be only minimal damage to type I epithelial cells in this lesion, and type II cell differentiation and replication are not prominent features of the process.

Necrosis of type I epithelial cells is a prominent feature of IPF induced by bleomycin, oxidant, parapquat, BHT, or combined injury. In contrast, in elastase-induced emphysema, type I epithelial cell injury is minimal, and this process is accompanied by minimal increase in collagen accumulation. These observations clearly indicate that in a great many animal models of IPF, injury to type I epithelium, which is so extensive that it is not susceptible to repair by dedifferentiation and replication of type II epithelial cells, in some poorly understood way triggers the granulation tissue response from which fibrosis results.

In a recent study on bleomycin-induced pulmonary fibrosis in the hamster, Vaccaro et al found no major alterations in capillary endothelial basement membranes. By six days after bleomycin administration, there was focal injury to alveolar epithelial cells and resultant denudation of epithelial basement membranes. The denuded epithelial basement membranes were folded, with lamina densa 60% thicker than in controls, suggesting active or passive retraction. Type II cell hyperplasia and repopulation of the epithelium was observed, but there was no duplication of epithelial basement membranes. Thirty and 60 days after bleomycin treatment, the epithelium was intact in many areas, inflammation had subsided, and widespread but focal fibrosis was present. At this stage, thickening and duplication of the epithelial basement membrane was observed, involving 10% of epithelial basement membrane at 30 days and 30% at 60 days. Although duplication and thickening were worse in fibrotic areas, they also occurred in normal-appearing areas of lung, suggesting that epithelial basement membrane changes may be the residual of previous injury. Areas of folded epithelial basement membrane remained at 60 days in areas of fibrosis, suggesting that alveolar walls had collapsed and had been incorporated into the interstitium of the lung. Although type I epithelial cell injury may well be the key to fibrosis in many acute lung lesions, I doubt that it always plays the starring role. Endothelial cell injury appears to be a key player in some injuries such as irradiation-induced fibrosis.

Possible Therapeutic Interventions

Figure 2 shows a schema similar to that of Figure 1. Possible sites where therapeutic intervention might prevent the development of fibrosis are indicated by breaks in the arrows with question marks beside them.

Prevention of Injury

An attempt to prevent injury is one way to approach this problem. This approach will likely require pretreatment when patients are known to be at risk. At the very least, treatment must be initiated in the very early stages of injury. For example, the injury caused by bleomycin seems to occur within the first few hours, long before the phase of edema. Lazo and Pham and Grant and Bernard showed that less than 1% of the dose of bleomycin administered intratracheally is still present in the lungs at 24 hours. The additive effect of oxygen to bleomycin injury has been shown by several studies, and it is obvious that combinations of perturbants, such as bleomycin and oxygen, should be avoided. Modification of the inflammatory response by indomethacin, methylprednisolone, lipopolysaccharide, and depletion of complement with cobra venom factor administered concomitantly with bleomycin have been shown to ameliorate the fibrogenetic response to intratracheal bleomycin in rodents. The mechanisms of action of these interventions remains incompletely defined.

Prevention of Fibrosis

Another approach has been to attempt to modify the deposition of connective tissue. Penicillamine, β-amino-propionitrile, proline analogues, and paramino-benzoic acid have been shown to be able to decrease collagen accumulation and generally to improve the volume-pressure curve in bleomycin-induced IPF. There was little effect in these studies on the inflammatory response, and in some studies survival was not affected. Their potential
toxicity is an obvious limitation to the use of these drugs in humans.

It seems to me that it is appropriate to attempt to intervene in the fibrosing process at an earlier level by more clearly understanding the interaction between fibrosis and destruction of type I epithelium or injury to endothelial cells. A reasonable approach seems to be manipulation of these areas in the cascade, either by preventing the injury to the cells or by learning what triggers them to initiate the fibrogenic response and learning how to thwart it.

**SUMMARY**

The study of animal models of IPF has demonstrated that there is a stereotypic response of the respiratory airspace walls to a wide variety of injuries. Inflammatory and immune effector cells play a major and complex role in the fibrosing process. They may contribute to the injury of the lung beyond the original insult. These cells secrete substances that play an important role in determining cell traffic in the lungs and in controlling the connective tissue-producing cells. Products derived from the inflammatory response may interfere with protection of normal lung matrix, although injury to lung matrix itself does not lead to fibrosis. Injury to endothelial cells and especially type I epithelial cells appears to play a major role in the fibrogenic response. Further understanding of the factors that injure these cells, the development of methods of protecting them from injury, and a clear understanding of their role in the fibrogenic process appear to be key to developing better methods of preventing and treating interstitial pulmonary fibrosis.

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A Molecular Basis for Bleomycin-induced Pulmonary Fibrosis

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Bleomycin is an antineoplastic agent used in the treatment of several malignancies. Its mechanism of action is not fully understood, although it is known to cause DNA damage and an inhibition of DNA synthesis. The principal and rate-limiting toxicity of bleomycin is pulmonary fibrosis. The fibrotic process includes the participation of many lung cell types, cell-cell interaction, stimulatory and inhibitory factors, immunologic changes as well as genetic-related susceptibility.1 Lung fibrosis induced by bleomycin is characterized by increased collagen synthesis and collagen deposition in lung parenchyma.

Although many descriptive studies have been done on the effect of bleomycin on collagen metabolism, studies concerned with the molecular basis by which bleomycin increases procollagen synthesis are lacking. Bleomycin increases procollagen synthesis in rat carrageenan granuloma fibroblasts,4 IMR-90 human fetal lung fibroblasts,5,6 and embryonic chick skin and chick lung fibroblasts.7 The stimulatory effect of bleomycin on procollagen synthesis in lung fibroblasts is specific, since total cellular noncollagen protein synthesis is not affected by this chemotherapeutic agent. These latter cell culture models may be used to elucidate the cellular interaction(s) by which bleomycin increases procollagen synthesis.

To determine the molecular mechanism of the bleomycin-induced increase of procollagen synthesis in fetal chick fibroblasts, total cellular RNA was isolated and hybridized to 32P-labeled cloned pBR322 plasmids containing either type I procollagen cDNA for pro a1 or pro a2 mRNA. No differences in procollagen mRNA content were noted for total cellular RNA preparations isolated from control and bleomycin-treated chick fibroblasts. The chick fibroblasts were then subfractioned into nuclei, polysomes, and the postpolysomal cytoplasm, and the RNA from each subfraction was then subfractioned into nuclei, polysomes, and the postpolysomal cytoplasm. Our data indicate that the bleomycin-induced increase of procollagen synthesis in fibroblasts results from a partitioning of type I procollagen mRNAs into polysomes. These data provide a novel mecha-