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CRITICAL REVIEW

RADIATION PNEUMONITIS AND FIBROSIS: MECHANISMS UNDERLYING ITS PATHOGENESIS AND IMPLICATIONS FOR FUTURE RESEARCH

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Radiation pneumonitis and subsequent radiation pulmonary fibrosis are the two main dose-limiting factors when irradiating the thorax that can have severe implications for patients' quality of life. In this article, the current concepts about the pathogenetic mechanisms underlying radiation pneumonitis and fibrosis are presented. The clinical course of fibrosis, a postulated acute inflammatory stage, and a late fibrotic and irreversible stage are discussed. The interplay of cells and the wide variety of molecules orchestrating the immunologic response to radiation, their interactions with specific receptors, and the cascade of events they trigger are elucidated. Finally, the implications of this knowledge with respect to the therapeutic interventions are critically presented. © 2006 Elsevier Inc.

Radiation pneumonitis, Fibrosis, Pathogenesis, Cytokines.

INTRODUCTION

Radiation pneumonitis and subsequent radiation pulmonary fibrosis (RPF) are the two main dose-limiting factors when irradiating the lung. As such, they limit the therapeutic ratio in one of the most common and life-threatening cancers—lung cancer—and, furthermore, can complicate the quality of life of long-term survivors, such as patients who have undergone radiotherapy (RT) for breast cancer or Hodgkin's disease. Finally, radiation pneumonitis can be the most devastating complication of total body RT.

Although the advent of more sophisticated RT techniques, such as conformal RT or intensity-modulated RT, can permit dose escalation by limiting the normal tissue complication probability, radiation pneumonitis and RPF have not been eliminated, and therapy for these entities presents a challenging problem for ameliorating the survival rates and the quality of life of these patients.

Although intensive research in the past few decades has revealed many interesting aspects of the underlying mechanisms, we are far from proposing a reliable pathogenetic model. This is mainly because, first, it is not evident that the results from research addressing the idiopathic conditions leading to lung fibrosis can be extrapolated to radiation pneumopathy. Second, despite the plethora of reports using different animal models and fibrogenic agents, it is unclear whether the biologic pathways described also apply to radiation pneumonitis and RPF.

This critical review has combined the existing laboratory and clinical research evidence in an attempt to provide a background of the mechanisms underlying the pathogenesis of radiation pneumonitis and RPF.

TERMINOLOGY

The term *radiation pneumopathy* refers to a continuing process triggered after lung RT. This comprises two distinct, still tightly connected, abnormalities (1). One is radiation pneumonitis, an early inflammatory reaction involving alveolar cell depletion and inflammatory cell accumulation in the interstitial space that occurs within 12 weeks after lung RT. The second is a late phase of radiation fibrosis, considered until recently as irreversible, that consists mainly of fibroblast proliferation, collagen accumulation, and destruction of the normal lung architecture (2). Between them, an intermediate exudative phase may exist in patients in whom acute pneumonitis fails to resolve completely.

Morgan and Breit (2) have challenged these concepts of radiation pneumopathy by introducing the term *sporadic radiation pneumonitis*, compatible with a diffuse lymphocytosis involving the whole lung. Although this suggestion is important and clinically relevant, it has not yet gained universal acceptance and the present review has focused on the classic term.

INCIDENCE, CLINICAL CHARACTERISTICS, MEASUREMENT

The incidence of radiation pneumopathy varies widely among reports. Differences in radiation technique, aware-

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ness, method of reporting, and the evaluation of the symptoms themselves may account for this variability. The scoring of radiation pneumonitis is difficult, because coexisting medical conditions challenge the reliability of laboratory measurements. Also, with combined modality therapy with cytotoxic agents steadily being incorporated into clinical RT practice, a greater incidence of severe radiation pneumonitis is inevitable (3). It is beyond any doubt that the incidence of radiation pneumopathy increases with concurrent drug administration. A recent study reported that taxane-based adjuvant chemotherapy, a standard regimen for high-risk breast caner patients, increases the incidence of radiation pneumonitis to 35% (4).

The development of radiation pneumonitis depends on treatment-related factors, such as the radiation dose, volume of lung irradiated, fractionation schedule, and use of concurrent chemotherapy (5); patient-related factors, such as preexisting lung disease, poor pulmonary function, smoking (6), and unknown genetic predisposition, including impaired function of DNA repair and growth factor genes (7, 8).

Radiation pneumonitis is an entity difficult to assess. The rate and severity of symptoms increase when large lung volumes are included or high doses (>50 Gy) are applied, especially when combined with chemotherapy. The early radiation clinical syndrome consists of nonspecific respiratory symptoms, such as low-grade fever, mild cough, or mild dyspnea, which usually resolve after common doses of corticosteroids and antibiotics. The acute pneumonitis phase occurs within a time frame of 12 weeks after RT. Meticulous assessment shows that 50–90% of patients undergoing irradiation to the lung develop radiographic and pulmonary function test abnormalities (9, 10).

Lung fibrosis develops insidiously in the previously irradiated field and stabilizes during the first 1–2 years (9). Clinically, its manifestations depend on the amount of lung parenchyma undergoing fibrosis, which defines the amount of nonoxygenated shunting blood to the systemic circulation. The symptoms comprise a varying degree of exertional dyspnea and, in late stages, orthopnea, cyanosis, respiratory failure, and cor pulmonale. For reasons that remain unknown, patients irradiated for caudally located tumors have a greater risk of radiation pneumonitis (11).

Distinct patterns of radiation-induced findings on CT have been previously described (12). High-resolution CT is the modality of choice when evaluating interstitial lung diseases and would therefore also be the ideal method for radiation fibrosis. A perfusion scan made with ^{99m}Tc and a ventilation scan made with ^{133}Xe or ^{127}Xe are also useful (9).

A common spirometry test is essential in evaluating the forced vital capacity, forced expiratory volume in 1 s, and forced expiratory volume in 1 s/forced vital capacity ratio. The changes corresponding to fibrosis would attribute to the spirometry results a rather restrictive pattern of changes, although an obstructive pattern may coexist because of conditions such as smoking. An examination of the static lung volumes would also be useful, but the most essential test for estimating the severity of pneumonitis and/or fibrosis is the diffusing capacity test for carbon monoxide (9). The clinical, functional, and radiographic changes due to radiation pneumonitis can be scored according to the Late Effects on Normal Tissues–Subjective, Objective, Management, and Analytic (LENT-SOMA) criteria and thus can be evaluated in a reproducible and commonly understandable method (13). Pulmonary function tests show a decline in 6 months after RT and continue to decline beyond 1 year. The 6-min walk test has been proposed as a safer method to assess for radiation pneumonitis compared with common pulmonary function tests (14).

PATHOGENETIC MECHANISMS

The process of radiation pneumopathy is undoubtedly one of the most thought-provoking radiobiologic phenomena. Interstitial inflammation leading to, or accompanying, lung fibrosis is a complex process involving proinflammatory and profibrotic cytokines produced by damaged and activated cells of the interstitium and the alveolus, leading to the loss of normal architecture within the lung (15). Furthermore, similar genetic and molecular changes, such as those observed after RT, have also been observed after bleomycin infusion, suggesting that such changes belong to a group of nonspecific reactions to tissue injury (16). In an effort to distinguish in a more understandable way the different actions and various pathways leading to post-RT fibrosis, the different roles of the factors in this procedure are grouped and presented in this article.

Interplaying cells

The peripheral lung parenchyma is composed of respiratory bronchioles, alveolar ducts, and alveoli. The alveolar wall is covered by endothelium, which is connected to the epithelium by way of a common basement membrane. The interalveolar interstitial space is composed of fibroblasts, alveolar macrophages, and extracellular matrix (ECM) (17).

There are two types of alveolar epithelial cells. Type I pneumocytes are squamous epithelial cells that cover ≥90% of the surface of the alveolar epithelium. When injury occurs, they are the first to be affected and to undergo apoptosis, leading to the accelerated proliferation of Type II epithelial cells (i.e., Type I precursors), to repopulate the alveolar epithelium.

Type II pneumocytes (granular pneumocytes) are cuboidal cells that synthesize and secrete the pulmonary surfactant, which covers the alveolar surface and regulates the alveolar surface tension (18). Type II cell hyperplasia represents a nonspecific marker of alveolar injury and repair. These cells respond by increasing alveolar surfactant production during the first 2–6 weeks after RT (9).

The capillary endothelium is of the continuous type; endothelial cells are linked to each other by tight junctions. The basement membrane of capillaries is fused with that of the alveolar epithelium to constitute a single alveolar-capillary membrane. Gas transfer occurs from the intra-alveolar

air, across the Type I cells, the fused basement membrane, and, finally, across the endothelial cell to the vascular lumen. The lymphatic endothelium is abundant along the brochovascular structures but is absent from the alveolar walls.

Two types of fibroblasts exist within the interstitium, which, under normal conditions, are inconspicuous in adults. These are the common fibroblast, which is located parallel to the epithelium and is intimately associated with the fiber elements of the ECM, and the myofibroblast, which is stellate and is oriented perpendicular to the alveolar wall (19). The myofibroblast is capable of contraction.

Alveolar macrophages play an important role in alveolar physiology. Activated macrophages produce a variety of cytokines with mitogenic or chemotactic properties for neutrophils and lymphocytes and also act directly on fibroblasts and endothelial cells.

The ECM of the alveolar wall consists of a basement membrane and an interstitial matrix among fibroblasts and vessels. The ECM contains proteoglycans, fibronectin, laminin, entactin, and Type IV and Type VII collagen (20). Alveolar epithelial and endothelial cells, as well as fibroblasts, synthesize and maintain the basement membrane. Alteration of the quantity and quality of the ECM occurs during radiation pneumopathy as a result of activation of collagen-synthesizing genes by fibroblasts. Early elevation of the collagens I/III/IV and fibronectin occurs and persists until 8 weeks after lethal radiation (16). The most dramatic elevation concerns collagen IV, which is an important component of the basement membrane of the endothelium.

Histopathologic features of radiation pneumopathy

The data available on the histopathologic changes after RT of human lung are limited, because patients undergoing lung RT are unlikely to undergo diagnostic thoracotomy and autopsies are rarely performed. The existing data have mostly come from animal models.

The early histopathologic finding after RT is described as diffuse alveolar damage (21). This includes edema of the alveolar walls due to increased vascular permeability and exudation of proteins in the alveolar space (22). Vessel thrombosis may also occur, with focal necrosis and subsequent organization; intra-alveolar hemorrhage may also be present. Infiltration with inflammatory cells (inflammation) is evident and, at least in experimental models, subsides rapidly within days (23). Depletion of Type I pneumocytes is accompanied by hyperplasia of Type II pneumocytes, in the context of the alveolar epithelium regeneration process (23). Fibroblast proliferation and increased collagen accumulation in the interstitium, as well as in the intra-alveolar space, are key pathogenetic features. Within a few weeks, thickening of the alveolar septa and fibrosis are evident (9). Although not confirmed in humans, the phase of fibrosis is accompanied by a second onset of leukocytic infiltration (24). Fig. 1 shows the pathologic features of radiation pneumonitis schematically.

Radiation interference with gene expression

Quantitative and qualitative changes of the expression of genes after RT (25, 26) lead to the overproduction of a large number of cytokines and growth factors by irradiated cells that act in an autocrine and paracrine fashion and give birth to the finally recognizable histopathologic changes and clinical syndromes. A recent report has suggested that such changes in gene expression are a consequence of an increased mRNA translation rather than increased transcription (27). This phase of the direct induction of growth factor, adhesion molecule, and cytokine overexpression by ionizing radiation is probably the first event that triggers the subsequent cascade of radiation pneumopathy.

Triggering inflammation

Evidence has suggested a central role for inflammation in the initiation and establishment of radiation-induced pneumopathy (28). Local recruitment of inflammatory cells, including macrophages, is modulated by various cytokines and chemokines. The inflammatory leukocytes then adhere to adhesion molecules on the endothelial cells of the microvasculature. This is followed by lymphocytic and macrophage transmigration into the interstitium. The production of a variety of cytokines leads to the activation of fibroblasts and of the endothelium, leading further to the initiation of additional paracrine and autocrine loops between fibroblasts, endothelia, and macrophages.

A major conceptual advance has occurred in the area of immune responses—the unveiling of the role of T helper Type 1 and T helper Type 2 lymphocytes in the immune response (29). T helper Type 1 lymphocytes seem to facilitate generation of interleukin (IL)-2 and interferon (IFN)-γ, resulting in enhanced cellular immune responses. T helper Type 2 lymphocytes are associated with production of IL-4 and IL-10, which facilitate immunoglobulin production. T helper Type 2 responses appear to prevail in progressive lung inflammatory responses, with development of pulmonary fibrosis. In contrast, T helper Type 1 responses appear to resolve without a progressive and functionally disabling outcome (24).

Cytokine and growth factor release

Cytokines and growth factors are pleiotropic and have a wide range of activities. They are expressed after injury and are nonspecific for the radiation injury. The measurement of changes in cytokine levels may eventually prove useful in predicting the risk of radiation-induced complications. However, cytokines are also derived from tumor and may confuse the results when investigating a surrogate marker for radiation pneumonitis.

MOLECULAR CONTRIBUTORS IN RADIATION PNEUMOPATHY

Transforming growth factor-β

Transforming growth factor (TGF)-β is a key cytokine in the fibrotic process that induces phenotypic modulation of

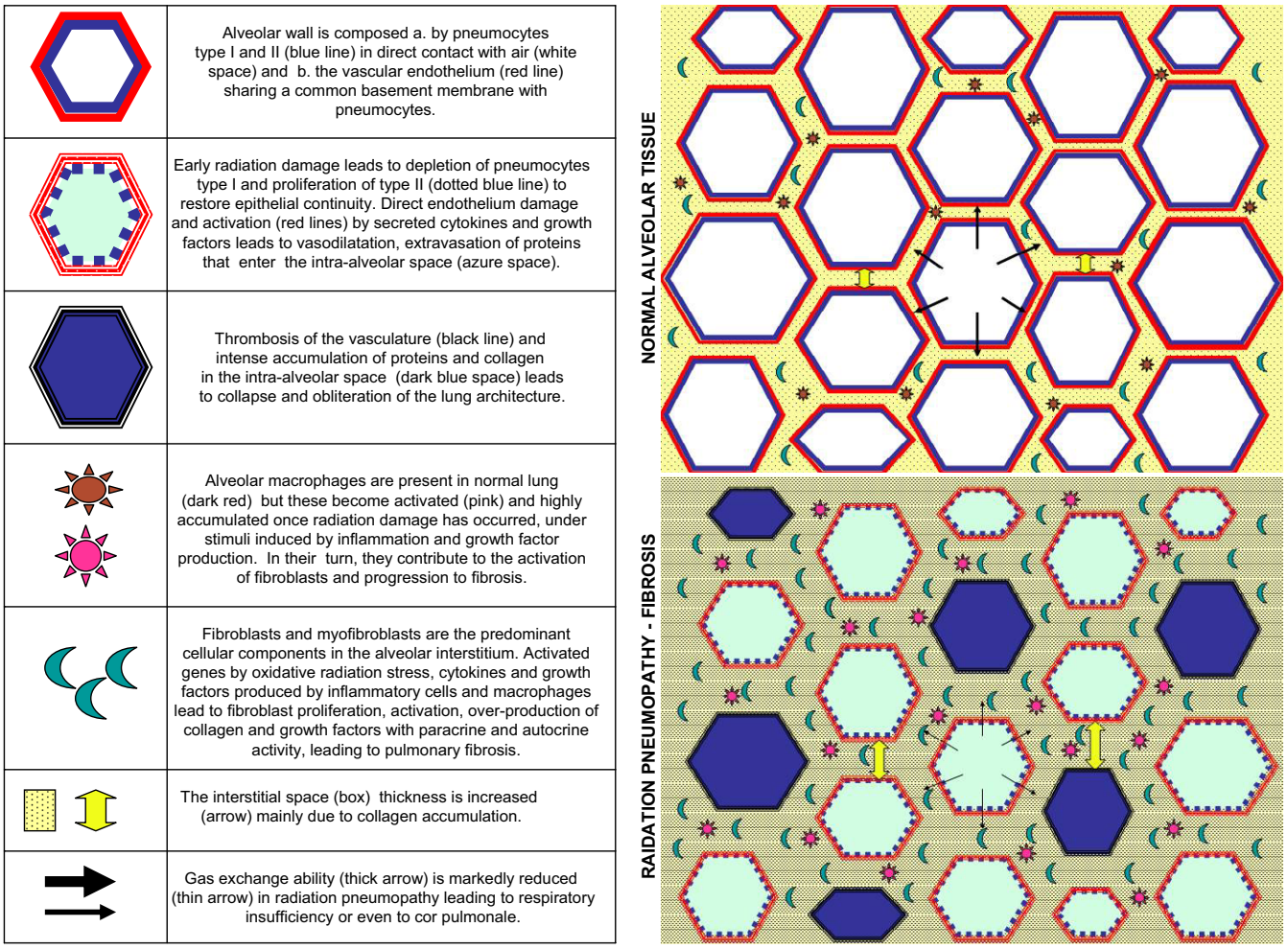

Fig. 1. Schematic representation of radiation pneumopathy.

human lung fibroblasts to myofibroblasts (30, 31). TGF-β is a potent stimulator of collagen protein synthesis (32). TGF-β gene expression has been shown to increase dramatically at 1–14 days after RT, in parallel with changes in fibroblast gene expression of collagens I/III/IV and fibronectin (33, 34). Although inflammatory cells are the source of TGF-β, pneumocytes and fibroblasts also contribute to TGF-β production.

In humans, thoracic RT is associated with persistently elevated plasma TGF-β1 levels at RT completion (35). However, it is the persistence of an abnormal level at the end of treatment that indicates the degree of normal tissue injury. Plasma TGF-β1 levels have been successfully used for the stratification of patients into low-, intermediate-, and high-risk groups for radiation pneumonitis development (36). However, lung cancer patients often have elevated plasma TGF-β_1 levels because of tumor TGF-β_1 production (37). A decrease in TGF-β_1 expression may, therefore, occur as the tumor regresses. Serial plasma TGF-β_1 determinations and dose–volume relationships are needed to identify patients at low risk (38).

TGF-β_1 is secreted as a biologically inactive complex that is activated by various elements, such as matrix proteins and proteases, in response to tissue damage (39, 40). Latent TGF-β_1 activation can also directly occur by way of DNA-damaging agents and free radical production from RT (41). TGF-β_1 increases extracellular matrix production and decreases its degradation as a result of its effect on the expression of proteases and protease inhibitors (42).

For experimental purposes, radiation-sensitive and nonsensitive strains of mice have been produced. TGF-β_1 and TGF-β_3 are both elevated in the sensitive host (16). In rats treated with an adenoviral-mediated soluble TGF-β Type II receptor, fibroproliferative changes were markedly reduced after RT compared with the controls (43).

Fibroblast growth factor family

Several members of the fibroblast growth factor (FGF) family stimulate DNA synthesis in endothelial and epithelial cells. They also protect endothelial cells from radiation-induced damage by inhibiting apoptosis by way of triggering signal transduction mediated by protein kinase C receptors (44).

FGF-7/keratinocyte growth factor (KGF) is produced by mesenchymal cells and exerts its activity exclusively on

epithelial cells expressing KGF receptors (FGFR2-IIIb) (45). KGF induces Type II cell proliferation and differentiation. TGF-β antagonizes the stimulatory effect of KGF on Type II pneumocytes (46). FGF-10 also exerts mitogenic activity exclusively on epithelial cells (47). Both factors have a central role in embryonal lung morphogenesis (48). KGF neutralizing antibodies suppress surfactant production by lung epithelium (49). KGF levels increase after exposure of lungs to bleomycin, reaching a peak within 2 weeks (50). Various cytokines (IL-1, TNF-α, TGF-α, and platelet-derived growth factor [PDGF]) have a regulatory effect on KGF expression (51). Glucocorticoids suppress the production of KGF by fibroblasts, and pretreated mice have reduced induction of fibroblast KGF, which questions the value of cortisone in treating the early phase of radiation pneumonitis (52).

TGF-β regulates the autocrine induction of basic FGF (bFGF), resulting in activation of the ERK mitogen-activated protein kinase pathway and induction of the activator protein-1 binding, a nuclear factor that regulates expression of a variety of genes involved in fibrosis (53). bFGF promotes fibroblast growth and differentiation and is produced by endothelial cells within hours after radiation exposure (54, 55). Serum concentrations of bFGF (together with TNF-α and IL-6) are consistently greater in patients undergoing lung RT (56). bFGF also has chemotactic and mitogenic properties for endothelial cells and stimulates secretion of collagenase and plasminogen activator (57).

Tumor necrosis factor-α

TNF-α is produced by activated macrophages during the fibrotic process and has proinflammatory and immunoregulatory effects (58). It stimulates fibroblast proliferation, secretion of extracellular matrix proteins, production of collagenase, and secretion of other proinflammatory cytokines such as IL-1 and IL-6. Thoracic RT of mice resulted in an increased presence of macrophages producing TNF-α in bronchoalveolar lavage fluid, but this reaction settles within 4 months, suggesting that TNF-α has probably a role in the early phase of radiation pneumonitis (59). Treatment with recombinant TNF-α receptor, blocking TNF-α activity, results in resolution of fibrotic lesions in irradiated lungs of mice (60).

Hypoxia and vascular endothelial growth factor pathway

Radiotherapy to the hemithorax in rats using a large fraction of 28 Gy results in marked pulmonary hypoxia 6 weeks after RT that reaches a maximum within 6 months, as shown by the intense localization of pimonidasole in lung tissues (61). Because hypoxia is known to favor generation of reactive oxygen species, upregulate TGF-β, and promote collagen formation, postradiation hypoxia may be an important factor in the tissue environment. Hypoxia slows down the degradation of hypoxia-inducible factors (HIF) 1α and 2α in cells, leading to activation of a variety of genes encoding VEGF, erythropoietin, lactate dehydrogenase 5, and $>$50 other genes involved in angiogenesis, glycolysis, and apoptosis regulation (62). Upregulation of HIFs and of VEGF is evident in the pulmonary endothelium in severe pulmonary hypertension, implying an important role in pulmonary pathophysiology (63).

Apart from hypoxia, VEGF upregulation may occur by TGF-β stimulation through Smad3 signaling (64). Because VEGF induces VEGF receptors in fibroblasts and myofibroblasts (65, 66), TGF-β–mediated VEGF production by these cells may trigger an autocrine stimulus leading to fibrosis, as has been confirmed in patients with Crohn's disease (67).

Hepatocyte growth factor

Hepatocyte growth factor (HGF) is a membrane-spanning tyrosine kinase, a product of the c-met gene, with angiogenic properties. It is present in fibroblasts, epithelial cells, and alveolar macrophages (68) and has a role in the alveolar and bronchial morphogenesis in rats (69). After injury, HGF levels increase in the whole lung, supporting the reparative process (70). Continuous infusion of HGF in mice attenuates the bleomycin-induced lung damage and, furthermore, administration of HGF after establishment of bleomycin fibrosis reverses the fibrotic process (71). As TGF-β strongly suppresses the HGF expression in human fibroblasts (72), impaired HGF-mediated reparatory activity is expected during radiation pneumopathy. This has been documented in idiopathic pulmonary fibrosis (73).

Platelet-derived growth factor

PDGF isoforms have an important role in stimulating the proliferation and migration of myofibroblasts during fibrosis. PDGF action is directed to PDGF-α and -β receptors with tyrosine kinase activity that are present on the surface of stimulated fibroblasts (74). Fibrotic activity of TGF-β and bFGF seem to depend on PDGF profibrotic activity. Irradiation of human lung endothelial cells results in upregulation of all isoforms (A, B, C, and D) of PDGF, and subsequent coculture with human fibroblasts induces phosphorylation of the fibroblast PDGF receptors and proliferation (24). Radiation-induced expression of PDGF and phosphorylation of PDGF receptors persist even in the late phase of radiation-induced fibrosis.

INTERLEUKINS

The ILs originate from lung macrophages, as well as from a variety of nonmacrophage cellular sources (e.g., alveolar epithelial cells, fibroblasts, mast cells). Studies of TNF-α and IL-1, the early response cytokines, established the primacy of these mediators in the upregulation of lung vascular adhesion molecules. The IL-8 family of cytokines has chemotactic activity for leukocytes and angiogenic activity. They also induce collagen synthesis and cell proliferation (28). Although most cytokines are thought to be proinflammatory, a family of anti-inflammatory interleukins also exists (75). IL-4, IL-6, IL-10, and IL-13 have powerful anti-inflammatory effects, apparently related to their ability to

suppress TNF-α production and consequently inhibit upregulation of endothelial adhesion molecules, such as intercellular adhesion module-1 (ICAM-1).

IL-6 is an acute phase proinflammatory cytokine produced by activated alveolar macrophages, T helper lymphocytes, lung fibroblasts, and Type II pneumocytes (76). Although IL-6 induces apoptosis of normal lung fibroblasts, it prevents apoptosis of activated fibroblasts in patients with idiopathic pulmonary fibrosis, contributing to the fibrotic process (77). Measurement of circulating IL-6 may reflect the inflammatory state of the lung. High pretreatment or posttreatment levels of IL-6 correlated with the development of radiation pneumonitis in humans (78). IL-6 is thought of as the major cause of lymphocytic alveolitis in the pneumonitic process of lung injury (79).

IL-1β, as is TNF-α, is another major product of activated macrophages. RT to alveolar macrophages *in vitro* results in increased production of both IL-1α and IL-1β (80). Furthermore, IL-1 has been shown to stimulate IL-6 production by human lung fibroblasts (81). In studies of radiation-induced injury to the brain, neutralizing antibodies against either IL-1β or TNF-α prevented expression of ICAM-1 (82). However, studies with radiation fibrosis-resistant and fibrosis-prone mice have indicated that IL-1β may have a protective effect (83).

IL-10 is a product of the T helper Type 2 subset of helper lymphocytes. One of its main functions is to downregulate inflammation. The expression of mRNA for IL-10 increases in a radiation dose-dependent manner and may also explain some of the immunosuppressive effects of ionizing radiation (84). Interleukin-10 inhibits radiation-induced transendothelial cell migration by leukocytes in mice through the inhibition of ICAM-1 expression (85).

Endothelial cell adhesion molecules

Direct upregulation of endothelial adhesion molecules in the alveolar endothelium seems to be an important early event that leads to lymphocyte and white cell attachment and transendothelial migration into the alveoli and interstitium, thereby initiating inflammation (75). For instance, the β2-integrin family (CD11/CD18) of molecules on leukocytes is reactive with ICAM-1 in a manner that promotes leukocyte adhesion to the endothelium. E- and P-selectins of endothelial cells are reactive with sialyl Lewis^x-containing molecules present on surfaces of leukocytes (28). Radiation to mice lungs to 12 Gy results in a sharp increase of lung tissue ICAM-1 mRNA, vascular cell adhesion molecule (VECAM), and p-selectin (86). Thoracic RT induces ICAM-1 and E-selectin in the pulmonary endothelium (87).

Endogenous oxidative stress—nitric oxide and nitric oxide synthase

Radiation-induced oxidative damage to the lung is a process that persists for several months after the initial radiation exposure (88). The biologic effects of ionizing radiation begin with the direct generation of reactive oxygen species, leading to cellular damage, followed by activation of a cascade of genes. In contrast, exposure of endothelial cells to ionizing radiation results in a ninefold increase in the transcription of the inducible nitric oxide synthase (NOS) and release of nitric oxide (89), a potent vasodilator that contributes to the extravasation of proteins and facilitates inflammatory cell accumulation. Inducible NOS is strongly expressed in pneumocytes and alveolar macrophages in idiopathic fibrosis, and peroxynitrite, a potent oxidant produced by the rapid reaction of nitric oxide and superoxide, is highly present in such lungs (90). In bleomycin-induced fibrosis, inducible NOS expression is highly increased in alveolar and bronchiolar epithelia and in inflammatory cells (91). Recent data have also shown that nitric oxide induces stabilization of HIF-1α through a mechanism involving free radicals, so that upregulation of NOS by radiation may subsequently trigger the HIF/VEGF molecular cascade (92).

Matrix metalloproteinases

One category of potential biochemical markers of tissue injury is matrix metalloproteinases (MMPs). MMPs are a family of proteolytic enzymes involved in the degradation, turnover, and remodeling of basement membrane and extracellular matrix proteins (93). MMP-9 (gelatinase B, Type IV collagenase) is capable of degrading Type IV collagen, laminin, elastase, and fibronectin in the lung interstitial matrix. MMP activity *in vivo* is regulated by naturally occurring tissue inhibitors of metalloproteinases. Tissue inhibitors of metalloproteinases form physiologically irreversible complexes with all types of activated MMPs. MMP-9 is produced by macrophages, eosinophils, neutrophils, and lung epithelial cells and is capable of digesting components of basement membranes. However, in a recent study, measurement of MMP-3 and MMP-9 did not correlate with radiation fibrosis (94). Although the levels of certain MMPs increase during radiation lung injury (95), the role of these molecules remains unclear.

CD95/Fas, Fas ligand, and apoptosis

Fas/CD95 and Fas-ligand activation have been linked to chronic lung injury, consistent with a fibrotic response in the lungs. Immunohistochemical studies have shown that in idiopathic lung fibrosis, Fas-ligand protein is present in infiltrating lymphocytes and granulocytes, and expression of Fas is upregulated in bronchiolar and alveolar epithelial cells (96, 97). Radiation can directly induce Fas expression in cells (98); therefore, inflammatory cells expressing Fas ligand (macrophages and T cells) may directly promote apoptosis of pneumocytes, initiating the early-response phase of radiation pneumonitis. A role of activated fibroblasts in the induction of apoptosis of alveolar epithelial cells has been also postulated in *in vitro* studies (99).

Genetic predisposition

That only a certain percentage of patients treated at a certain dose level develop radiation pneumopathy suggests that a genetic predisposition, defining increased lung sensi-

tivity or altered responsiveness to radiation, exists among individuals. Research in the field, although promising in providing tools for the identification of patients susceptible to radiation fibrosis, has been limited.

The presence of a proline allele at codon 10 of the TGF-β_1 gene is associated with increased production of TGF-β_1 (100). TGF-β polymorphisms, however, seem not to be related to idiopathic pulmonary fibrosis (101). In another study, the −509T and +869C alleles were linked with a ≤15 times greater incidence of radiation-induced severe breast fibrosis (102). Similarly, Asn/Asp and Asn/Asn genotypes at codon 1853 of the ATM gene have been linked with Grade 3 fibrosis in breast cancer patients treated with RT (103).

Loss of heterozygosity at the mannose-6-phosphate insulin-like growth factor 2 receptor (M6P/IGF2R) locus has been found to predispose patients to radiation-induced lung injury (8). Furthermore, such patients are much more likely to have elevated plasma TGF-β levels. Thus, loss of the M6P/IGF2R gene may predispose patients to the development of radiation-induced lung injury.

Polymorphisms of the HIF and VEGF genes have also been identified and linked with responsiveness to hypoxic stimuli, but the role of these in radiation fibrosis has not been studied (104, 105).

PREVENTION AND THERAPEUTIC APPROACHES

Cytoprotection

The cellular antioxidant response seems to play an important role in the development of post-RT lung fibrosis. Superoxide dismutase (SOD) gene therapy studies in animals have shown a protective SOD effect from radiation lung toxicity (106). Three types of SOD exist: MnSOD (SOD2), CuZnSOD (SOD1), and extracellular SOD (EC-SOD), which is also referred to as SOD3 (87). EC-SOD is the predominant extracellular antioxidant enzyme and, in the lung, is primarily localized to Type II pneumocytes and macrophages (107). All three forms of SOD protect against radiation injury (106, 108, 109). Recently, it has been shown that SOD-mimetic small molecular weight catalytic metalloporphyrin increases the tolerance of the lung to ionizing radiation in rats (110). Administration of a liposomal Cu/Zn SOD effectively reversed radiation-induced fibrosis (111).

Thiol compounds such as cysteine and cysteamine have been used to target oxygen and oxygen-free radicals in an attempt to reduce radiation-induced damage (112). The only thiol compound of clinical relevance in RT today is amifostine (Ethyol) (113). The cytoprotective mechanism of amifostine is complicated, involving free radical scavenging, DNA protection, and repair acceleration. A reduction of the incidence and severity of radiation pneumonitis has been reported in clinical trials (114, 115). However, in a recent randomized Phase III study of patients who received chemotherapy plus hyperfractionated RT, administration of amifostine four times weekly did not reduce the incidence of radiation pneumonitis, implying that the dose and time factors of amifostine administration are important for optimal cytoprotection (116). Greater doses of amifostine were able to sustain a low incidence of radiation pneumonitis during aggressive chemoradiotherapy (117).

Amifostine administration is associated with the prevention of the decline of the diffusing capacity of the lung for carbon monoxide when given with concurrent chemoradiotherapy (118). In a study by Vujaskovic *et al.* (119), Fisher-344 rats bearing mammary adenocarcinoma received fractionated RT (5 Gy in 3 days). Reduced damage of the lung that paralleled lower plasma TGF-β levels in the rats treated with amifostine was documented. Administration of amifostine before RT also reduced the accumulation of macrophages and the expression of lung tissue TGF-β1 (120). Using a mutagen sensitivity test based on the bleomycin-induced chromosome breaks, Komaki *et al.* (121) were able to identify a subgroup of lung cancer patients with high mutagen sensitivity who experienced significantly reduced lung fibrosis when supported with amifostine.

Growth factor inhibitors

Modulation of pneumocyte proliferation may be important in accelerating alveolar structure remodeling. Recombinant human KGF (rHuKGF), because of its unique action on epithelial cells, might be an interesting therapeutic option. After rHuKGF (palifermin, Kepivance) administration, a doubling of Type II cell proliferation occurs (122). rHuKGF administration is under clinical evaluation (45). In rats, administration of rHuKGF can protect against late radiation-induced lung injury by way of TGF-β downregulation and restoration of the integrity of the pulmonary epithelium (123).

Inhibitors of TNF-α, such as infliximab (Remicade), a chimeric monoclonal IgG1 antibody to TNF used in Crohn's disease and rheumatoid arthritis, have been shown to downregulate both bFGF and VEGF in the serum of patients (124). Administration of infliximab in a patient with lung fibrosis and pulmonary hypertension associated with advanced systemic sclerosis resulted in stabilization of lung function test results and pulmonary arterial pressures that progressively worsened after cessation of therapy (125).

TGF-β inhibition is expected to have important activity against the fibrotic process. Smad inhibitors, such as naringenin, downregulate expression and phosphorylation of Smad proteins in fibroblasts by blocking TGF-β signaling (126). Pentoxyfyllin also seems to exert an antifibrotic activity through the downregulation of TGF-β by interference with the Smad pathway (127). Relaxin, a potent antifibrotic agent and TGF-β inhibitor, also seems to exploit the Smad pathway (128). Specific inhibitors of the activin receptor-like kinase activity of the TGF-β_1 receptor, such as SB-525334, have also been developed and shown to exert important activity against lung fibrosis (129, 130). Pirfenidone is a novel drug approved for the treatment of idiopathic pulmonary fibrosis. It reduces fibroblast proliferation and collagen production by suppressing the TGF-β, TNF-α,

and other proinflammatory cytokines, including IFN-γ and IL-6 (131, 132).

Blockage of the VEGF-related pathway by VEGF tyrosine kinase inhibitors may also lead to the cessation of the autocrine or paracrine loops acting on fibroblasts and leading to the reversal of their activation status and the prevention of fibrosis or even the reversal of the fibrotic process. In a study by Ko *et al.* (133), administration of the ZD6474 VEGFR-2 inhibitor in mice 7 days before wounding led to a reduction in the degree of fibrosis. Blockage of the VEGF using a genetic approach attenuated the pulmonary fibrosis induced by bleomycin (134). TNP-470, an antiangiogenic compound, has been also shown to reduce VEGF expression and to suppress the proliferation of myofibroblasts in experimental models (135).

Administration of imatinib (Glivec) or SU9518 (blocking PDGF receptor agents) resulted in prolongation of the survival of mice receiving 20 Gy to the lungs and reduced the radiomorphologic signs of lung fibrosis on CT. At the histologic level, PDGF receptor inhibitors administered after the establishment of fibrosis showed a marked reduction of collagen accumulation and alveolar thickness (23). Imatinib has also been shown to inhibit bleomycin-induced lung fibrosis (136).

Administration of IL-10 in patients with hepatitis-related liver fibrosis resulted in the reduction of inflammation and fibrosis scores (137). IL-18 may also have a therapeutic effect (138). Fluticasone propionate, a drug widely used to reduce pulmonary inflammation in chronic obstructive pulmonary disease, has shown important suppression of the inflammatory cytokines IL-6 and IL-8 (139).

Other agents

Proteasome-blocking agents reduce collagen concentration and inhibit the tissue inhibitors of metalloproteinases, thereby enhancing the activity of MMPs, and appear as a promising approach for the treatment of fibrosis (140).

Administration of HGF reverses fibrosis induced by bleomycin in experimental animals (71), but its value in human fibrotic diseases has not been evaluated clinically. Retinoic acid, an active metabolite of vitamin A that acts on specific receptors on cells, prevents fibrosis by counteracting the activity of TGF-β by stimulating HGF-promoter activity and HGF-receptor phosphorylation (141).

Interest has recently been increased in the use of pentoxyfyllin and vitamin E in the prevention and treatment of fibrotic lesions (142). A recent clinical trial provided evidence that this combination has a significant protective effect against acute and late lung toxicity (143).

Some limited evidence has shown a protective effect for angiotensin-converting enzyme inhibitors, especially captopril and an angiotensin II Type 1 receptor blocker, against radiation-induced pulmonary injury (144). An ongoing Radiation Therapy Oncology Group trial is focusing on captopril (145). Finally, cyclooxygenase selective inhibitors may also have a role in preventing radiation pneumopathy (146).

CONCLUSIONS

In many ways, irradiated tissue responds to injury in a manner similar to that of normal wound healing. In the case of irradiated tissues, however, the wound does not heal normally but instead enters a "death spiral," containing features of hypoxia, angiogenesis, cell death, proliferation, and macrophage infiltration (147). Ultimately, this spiral leads to the total replacement of the tissue by collagen, leaving few, if any, cellular elements.

We believe that the process of acute pneumonitis and radiation fibrosis are tightly linked with each other. Radiation-induced oxidative stress and free radical generation triggers inflammation (radiation pneumonitis) and results in DNA damage in all lung cellular components, inducing mRNA translation of a variety of genes involved in epithelial/connective tissue and vascular regeneration. HIFs and inducible NOS gene activation are eventually the key events for the complex interactions among macrophages, fibroblasts, pneumocytes, and endothelium that follow. The interplay among these cells and their products at the molecular level (e.g., cytokines, growth factors, ICAM, vascular endothelial adhesion molecule [VECAM]) leads to collagen accumulation, changes in the quality of the extracellular matrix, and destruction of normal lung architecture, resulting in a prefibrotic status, of a varying duration, that may be clinically undetectable. Depending on the dose, fraction, and host factors, the prefibrotic condition may regress and never progress to fibrosis. Failure to switch off molecular events that had led to the prefibrotic step will lead to clinical-laboratory manifestation of fibrosis (radiation lung fibrosis).

According to such a model, it is evident that pharmacologic or molecular interventions (Fig. 2) at the prefibrotic stage may facilitate the suspension of the molecular cascade that would lead to radiation fibrosis. Furthermore, it may be that blocking specific molecular pathways active in clinically detectable fibrosis can reverse the process, allowing remodeling of the damaged lung. The complexity of radiation lung pneumopathy at the molecular and cellular level, however, indicates that switching off the whole procedure would require targeting of more than one molecular pathway.

Figure 2 summarizes the biologic events, interactions among cells, and therapeutic interventions awaited to break the myth of the irreversibility of late radiation sequelae. Many targets are available to consider, and, presumably, not just one is the key for an effective prevention or treatment of radiation pneumopathy. A combination of blockers of proinflammatory and profibrotic cytokines with inhibitors of growth factors and their receptors may be necessary for a clinically meaningful effect.

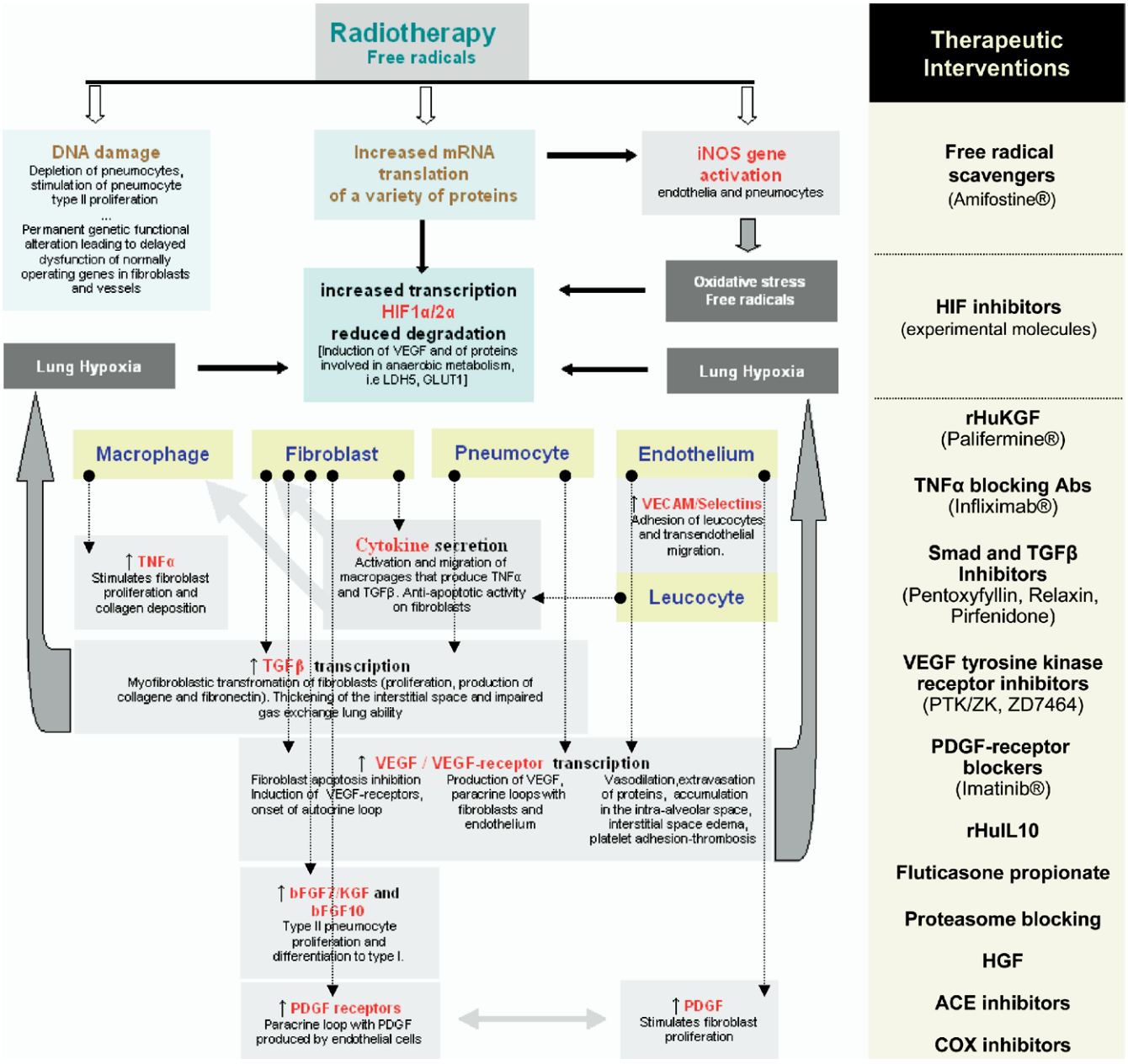

Fig. 2. Distinct roles of cells, molecules, and potential therapeutic implications for radiation pneumopathy. iNOS = inducible nitric oxide synthase; HIF = hypoxia-inducible factor; TNF = tumor necrosis factor; VECAM = vascular endothelial cell adhesion molecule; rHuKGF = recombinant human keratinocyte growth factor; TGF = transforming growth factor; VEGF = vascular endothelial growth factor; PDGF = platelete-derived growth factor; rHuIL 10 = recombinant human interleukin 10; HGF = hepatocyte growth factor; ACE = angiotensin-converting enzyme; COX = cyclooxygenase.

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