Chapter X: Late Radiation Effects

Section c: Lung Fibrosis

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Irradiation Damage to the Lung: Radiation Pulmonary Fibrosis:

Late effects of irradiation damage to the lung are common principles with radiation late effects in many anatomic sites. A common factor in irradiation late effects to the lung is a phenomenon of loss of organ function, and replacement of functioning tissue with scar tissue (fibrotic tissue) leading to the phenomenon of irradiation pulmonary fibrosis. There is much interest in causes of irradiation fibrosis separate from understanding late radiation effects. The condition of idiopathic pulmonary fibrosis (IPF) has been a concern for decades, particularly with physicians treating patients with autoimmune diseases including scleroderma, and other autoimmune diseases, which have as a component of the disease process, the deposition of fibroblasts. Pulmonologists, who treat patients with anthracosis (coal-miners lung), asbestosis (inhalation of asbestos particles resulting in an escalating fibrotic reaction), into inhaled chemical toxins (chemical workers exposed to chlorine, or other solvent inhaled toxins), approach the management of their patients very similar to the approach to radiation pulmonary fibrosis. In all of these medical conditions, there is loss of functioning lung tissue, and replacement of functioning lung with fibrotic tissue.

Loss of lung function is a chronic problem in patients with other causes of loss of normal lung volume. Chronic Obstructive Pulmonary Disease (COPD) remains the major cause of lung damage in general medicine. Cigarette smoking associated COPD, which results from the chronic irritation of alveolar cells and cells in distant bronchials with their production of matrix metalloproteinases inflammatory cytokines, produces oxidative stress, continuous production of free radicals including superoxide and hydrogen peroxide to directly damage pulmonary tissue. In addition, the inflammatory response includes production of chemokines by damaged tissue, which call neutrophils, macrophages, and other inflammatory cells to the lungs. In the case of lung infection, inflammatory cells are responding appropriately to a foreign pathogen. In the case of cigarette smoking or other inhaled toxins, the damage response of normal tissues is associated with continual irritation of the tissue caused by a non-biologic stimulus. The response of the immune system to this form of inflammation is indistinguishable from a response to a biologic pathogen. Both biologic and non-biologic causes of chronic inflammation irritating pulmonary tissue is replaced by a sequence of cellular occupants, first inflammatory cells, then fibroblasts proliferate in the alveolar space and replace functioning lung with fibrosis (scar tissue).

Recent experiments in two animal models have provided great insight into the pathophysiology of radiation pulmonary fibrosis.

Studies with Bleomycin lung have been carried out to document the method by which this chemotherapy drug (known to cause pulmonary fibrosis in patients treated for Hodgkin's Disease, or other lymphomas) causes local pulmonary damage, which elicits migration into the lungs of bone marrow stromal cells to proliferate and replace normal lung with fibrosis of bone marrow origin (9). In radiation pulmonary fibrosis in C57BL/6 mice, migration into the lungs of bone marrow origin fibroblasts has also been demonstrated, although in this latter case, the contribution to the fibrosis of bone marrow origin cells is relatively small compared to that caused by proliferation of in situ/resident fibroblast progenitors (5). In both Bleomycin lung and radiation pulmonary fibrosis, there is a clear genetic determinant that cause radiation late effect.

C3H/HEN, BALB/C, and other mouse strains do not develop pulmonary fibrosis. In contrast, C57BL/6J mice show a distinct late radiation effect associated with increase in mRNA for inflammatory cytokines that follows a period of return to normal pulmonary function after the acute radiation side effect has subsided. In the model of radiation pulmonary fibrosis an interesting phenomenon has been the observation that endothelial cell associated mRNA and proteins remain elevated during the "latent period" between the acute and radiation fibrotic phase (10). A mechanism by which these particular RNAs remain elevated is unknown, however, it seems to be a biomarker for a persistent radiation effect in tissues that appear normal on histopathologic examination during the latent period.

The critical role of TGF-β radiation pulmonary fibrosis has been recommended in several model systems. Most notably, SMAD3-/- (C57BL/6 background) (6) were demonstrated to resist radiation pulmonary fibrosis, as well as fibrosis of subcutaneous tissues, kidney, and other organs (14). Fibroblast migration into the lungs of SMAD3-/- mice can be significantly reduced. Furthermore, SMAD3-/- bone marrow stromal cells (which resist stimulation by TGF-β) do not migrate into irradiated lungs of wild type mice of the same mouse strain (6).

Prevention, Amelioration, and Treatment of Radiation Pulmonary Fibrosis:

Improvement in the local control, and ultimately long-term survival of patients, who received thoracic radiotherapy, there has been increasing interest in developing therapeutics to minimize or prevent further radiation damage to the lung. A recent observation has been the role of antioxidant therapy. Clinical trials of antioxidants N-Acetyl-Cysteine (free radical scavenger) and Amifostine, both idiopathic pulmonary fibrosis and radiation pulmonary fibrosis met with incomplete success. Clinical trials follow successful reports of the use of these agents in preventing fibrosis in mouse models. A recent report of another water-soluble antioxidant, the DMSO analog, MMS350 (10), demonstrated a mechanism specific reduction in radiation pulmonary fibrosis in C57BL/6J mice, by decreasing migration from the bone marrow into the lungs of luciferase labeled bone marrow stromal cells. These studies were carried out in chimeric mice in which bone marrow origin fibroblast progenitors could be distinguished from those resident fibroblast progenitors in the irradiated lung.

Further studies are required to determine which steps in the initiation of radiation pulmonary fibrosis can be used as targets for anti-fibrotic drugs. Mouse model studies have suggested that toll-like receptor 4 (TLR4) upregulation is associated with initiation of late radiation lung damage, so a targeted agent for this molecule may be a research approach (10).

Irradiation Induction of Secondary Cancers

It has been known for decades that high dose irradiation to the apex of the lung for the treatment of Pancoast tumor can lead to cure of this varying squamous cell carcinoma of the lung. However, the doses of irradiation used in the range of 70 cGy have been known to induce soft tissue sarcomas.

Recently, there is data on the late effects of irradiation in Hodgkin's lymphoma patients, who receive mantle irradiation for cure of this disease burden usually in the mediastinal and hilar

lymph nodes. In many of these patients followed for 20 or more years, breast cancer has been diagnosed in women. Furthermore, recent evidence that radiotherapy of breast cancer using tangential techniques and in which a volume of lung is in the treatment target area, may present 10 – 20 years after curative radiotherapy with lung cancer. While there is no consensus as to the lowest dose or fractionation scheme or volume, which may result in irradiation induction of lung cancer in either Hodgkin's disease patients or breast cancer patients treated with radiotherapy, there is growing evidence that second cancers induced in the irradiated volume can be detected decades after curative radiotherapy.

Methods for Studying Radiation Lung Damage:

Because of the multiplicity of cell phenotypes in the lung and the role of cells migrating into the lungs from the bone marrow and peripheral blood, histopathologic studies and immunohistochemistry techniques are critically required to understand lung radiation damage.

Investigators, who study rat models of irradiation damage, use breathing rate, number of respirations per minute as a measure of acute radiation toxicity (15-16). Breathing rate has not been shown to be effective in mouse models of irradiation as markers of acute or chronic radiation damage.

Histochemical assays for radiation damage included assays for neutrophils (staining myeloid peroxidase), macrophages, and monocytes (staining for monoclonal antibody surface markers), assays for subsets of T, B, NK, and dendritic cells (monoclonal antibody surface markers), and assays for damage to endothelial cells.

Markers of radiation damage to the lung have included in situ assays for apoptosis (apotag) damage induced by inflammatory cytokine, measuring DNA double strand breaks (H2AX assay), and assays for upregulation of inflammatory cytokines in the lung. Western Blot analysis has been used to show elevated levels of TNF-α, TGF-β, IL-1 in lung tissue as biomarkers of acute radiation damage (3).

The production of chimeric mouse models for study of cell migration. The methodologies for these assays have been published previously (6).

Analysis of Cell Phenotypes in the Irradiated Lung and Immune Responses:

Modern histopathologic and immunohistopathologic techniques require analysis of the different phenotypes of cells in the irradiated lung. The airway consists of bronchi, complex bronchial branching, bronchials, and determination of distant bronchials into alveolar spaces. Within the alveoli are several type of cells: neuroendocrine cells, alveolar type I and alveolar type II cells, as well as Clara cells. Each of these types of epithelial cells has a specific function in stem cell or differentiated cell progeny. The work of Jeffrey Whisett (11) and Barry Stripp (12) define the role of lung stem cells and the development of branching of bronchial tree in the developing fetus. The roles of specific gene pathways and signaling cascades in communication of damaged signals between cells within the functioning lung has been a subject of intense investigation over the past 3 decades. The role of neurons and endothelial cells in the developing lung and injury

response has also been a subject of focus. Within histopathologic sections of rat or mouse lung at varying times after thoracic irradiation, alveolar cell and endothelial cell swelling can be quantitated. Leakage of specific cytokines and transudates through epithelial cells into the alveolar space can be quantitated. Dual staining techniques are available to determine cells in the complex of phenotypes or injury to each stage of the organ response to irradiation. Simultaneous apotag stain for apoptosis and immunostaining for (for example) endothelial cells can determine whether these cells represent the first target of injury after irradiation or whether the first target is in the bronchial epithelium. Infiltration of the lung with inflammatory cells represents a second of the phenotypes that can be individually scored. Two techniques are available for such studies. Using lungs and perfusing the airway with saline in the technique of broncho-alveolar lavage can be used to collect immunocytes that have infiltrated the alveolar space. A technique can also remove within blood components from the radiation injured lung, allowing a better quantitation of mRNA and proteins in lung parenchyma. A method of Corti has been used to separate cell phenotypes by preparing single cell suspensions of lung using adherence techniques and cell sorting techniques to separate endothelial cells, from broncho-alveolar cells, from pulmonary macrophages. This technique has been very helpful for determining where (for example) gene therapy vectors are located after intravenous or inhalation delivery of a radioprotective transgene (13).

Time Tracking of Cell Populations in the Irradiated Lung by Scanning Live Animals:

A valuable technique that has recently been used to track bone marrow derived stromal cells migrating into the irradiated lung on multiple occasions in the same animals. While histochemical or fluorescent markers such as green fluorescent protein (GFP) can be used in removed lung sections from sacrificed animals, a technique by which to follow cell migration into the lung and localized distribution requires tracking cells in live animals. The luciferase reporter system has been valuable for these techniques. An example for the use of tracking the migration of bone marrow stromal cells into the fibrosis forming regions of the lung demonstrates the power of this technique and also the ability of an antioxidant radiation mitigator (MMS350) to ameliorate and reduce this migration (10).

Real Time Polymerase Chain Reaction:

This technique has been used to measure RNA transcripts in the irradiation response of whole lung, and separated cell populations. Detailed methods for the use of this technique in lung have been published recently (10).

Luminex Assay for Detection of Proteins in Subpopulations of Cells in the Irradiated Lung:

During the various stages of the pulmonary radiation response, including acute, sub-acute, and chronic, cell populations can be removed by the sorting techniques described above, or whole lung specimens in single cell suspension can be assayed for expression of proteins using the Luminex assay. This assay is described in great detail in another section of this textbook (Fetal Animal Irradiation Response).

Methodology for Total Body Compared to Thoracic Irradiation:

Shielding of the head region and abdomen for thoracic irradiation has been described previously. These experiments are carried out on a linear accelerator (clinical radiotherapy machine) using lucite positioning devices for anesthetized mice and 10 – ½ value layered lead block shielding of the head region and abdomen. Microbeam irradiation techniques and animal irradiators are now available to deliver homogeneous doses to lung only. Mouse phantoms are available, and will be described in the Physics section of this textbook (Ke Sheng, UCLA). Total body irradiation is usually delivered in a Cesium 137 gamma cell animal irradiator. Investigators should specify dose rate, which is usually around 70 cGy per minute with shielding devices in place, or up to 340 cGy per minute if filter is removed. These differences are significant with respect to the time that the animal spends in the irradiator and time has been shown to increase stress in animals and can confound radiation dose calculations and stability of dose from week to week or month to month with respect to biological outcome (See Physics section of this textbook and Basic Radiobiology Methods Section).

C3H/HeJ compared to C57BL/6J mice

Studies of irradiation-induced lung damage carried out by Franko, et al. and Travis, et al. identified genetically inbred mouse strains with a propensity for acute radiation pneumonitis (C3H/HeJ) and others avoided acute radiation effects and progressed to radiation fibrosis (C57BL/6J) (15-16). There was a suggestion from correlation with clinical studies that the propensity for late radiation fibrosis could be attributed to TGF-β (12). However, in studies of both RNA induction by irradiation and protein, it was determined that a complex pattern of proteins separated fibrosis prone C57BL/6J mice, from other mouse strains (10). Studies with F1, F2, and back-cross strains attempting to identify the chromosome location of genes responsible for radiation fibrosis in the C57BL/6J mouse strain led to the chromosome site for localization of MnSOD (SOD2), which is a mitochondrial based antioxidant manganese superoxide dismutase. Treatment of fibrosis prone mice with intratracheal or inhalation MnSOD-plasmid liposomes, significantly ameliorated the onset of fibrosis 100 days later (1-4). Radiation fibrosis was demonstrated in mouse models to be in part attributable to migration into the lungs of bone marrow origin fibroblast progenitor cells when phenotyped to bone marrow stromal cells (5, 10). A similar pattern of bone marrow stromal cell migration to the lungs was observed in Bleomycin induced pulmonary fibrosis in a mouse model (9).

Chronic Pulmonary Insufficiency and Clinical Radiotherapy

It is well known that patients, who present with lung cancer or esophagus cancer and may require 40 – 60 cGy irradiation to a target volume in the chest, experience side effects of radiotherapy, dependent upon total dose, fraction size, and volume of lung treated to reach the gross tumor volume. Patients, who have comorbid lung disease including chronic obstructive pulmonary disease, and severe bronchitis will have more difficulty with acute radiation side effects of radiotherapy. Attempts to ameliorate the side effects of pulmonary radiotherapy have been largely limited to physical parameters including use of Intensely Modulated Radiotherapy or Stereotactic Radiosurgery to minimize the lung volume treated.

Continuous Elevation of Some Cytokines Causing Some Immune Responses

Studies in a mouse model demonstrate continuous elevation of cytokines associated with vascular endothelial cells between the acute irradiation pneumonitis and the onset of late radiation fibrosis. This latent period is associated with return to normal levels of RNA for many inflammatory cytokines and stress response genes, however, the data indicated that genes associated with endothelial cell responses remained elevated in transcriptional activity during this latent period despite return to a normal morphology of the lung (10). The mechanism of continuous elevation of some gene transcripts during the latent period between acute radiation pneumonitis and late fibrosis is unknown.

Chronic Infection

After lung radiotherapy or pulmonary exposure, there is usual return to baseline levels of the pulmonary flora or pulmonary microbiome. Some changes in microbiome have been noted to occur, particularly in those patients, who have comorbid pulmonary disease. In clinical radiotherapy, those patients, who complete a course of radiotherapy are advised to cease the cigarette smoking and remain at rest for some time to allow healing of the lungs, including ability to normal exercise tolerance. The risk of chronic infection is low, but has been reported in some clinical series.

Interaction of Inflammatory Cells with Pulmonary Function

In bone marrow transplantation patients, graft vs. host disease is rarely seen in the lung, but can occur. Association with pulmonary GVH and lung fibrosis has been discussed (12). Lung transplant patients have also been reported to demonstrate pulmonary fibrosis under conditions of both immunosuppressive therapy, and in conditions of haploidentical lung transplant, where the immune response is decreased.

Chronic Radiation Damage (Radiation Pulmonary Fibrosis)

The pathophysiology of pulmonary fibrosis has been well described in both animal models and in the clinic. In humans, lung volume, total dose, and radiation fraction size lead to pulmonary fibrosis originating in the irradiated field, but noted to spread outside the irradiated field. For example, in some patients treated to one hemithorax for lung cancer, fibrosis is shown to be first detected in the irradiated area, but can migrate to the opposite side usually following a similar migration of the acute radiation pulmonary surgery, radiation pneumonitis (12).

Radiation Fibrosis in Heavy Dose Irradiated Areas.

While there is clear genetic variation, and differences between patients of different rates of comorbid disease, radiation fibrosis is usually detected six months to two years after a course of clinical radiotherapy and usually in areas that receive doses in excess of 60 Gy. In Hodgkin's disease, patients, who receive 40 – 44 Gy to large fields (mantle irradiation), radiation fibrosis is often detected in the apex of the lung, where 44 Gy over 4 ½ weeks was delivered.

Hodgkin's Disease Patient's X-rays on Follow-Up.

A common late finding in Hodgkin's disease patients who received mantle irradiation is the presence of fibrosis.

Pulmonary Fibrosis in Lung Cancer Patients.

There is increasing evidence that combination chemotherapy and radical radiotherapy can produce five-year survival of 20 to 30% in patients with unresectable stage IIIA and IIIB non-small cell carcinoma. These patients are at risk for pulmonary fibrosis. For this reason, lung radiotherapy treatment planning takes into account the V50, V30, and V70 doses in which the tumor dose (60 -66 Gy) is considered the V100 (volume that receives 100% of the dose). Minimizing volume of lung that receives a 30% - 70% of the tumor 100% dose is utilized in all 3-dimensional treatment planning techniques, and may usually be the deciding factor in deciding whether to use Intensity Modulated Radiotherapy to minimize this chance of high dose region and thus chance of fibrosis.

Interaction of the Pathophysiology of Radiation Pulmonary Fibrosis with Idiopathic Pulmonary Fibrosis, Lung Toxicity from Inhaled Carcinogens, Coal – Miner's Lung, Mesothelioma.

There are many causes of pulmonary damage in the general medical patients. Many patients, who present with need for radiotherapy of lung cancer, esophagus cancer, or other tumors in the chest (thymoma, soft tissue sarcoma, lymphoma) may have comorbid pulmonary disease. For this reason, it is critical to assess the patient's full medical history and minimize radiotherapy dose to the lung. The greater volume of lung that receives doses in the range of 70 – 100% of the tumor dose, the greater the risk of pulmonary fibrosis. In patients, who have comorbid disease including those listed above, there is greater risk for pulmonary compromise, primarily the acute effects. Acute radiation pneumonitis is associated with many of the histopathologic changes seen in acute inflammatory reactions of the lung. Thus, while not caused by the same mechanism, pathology and pathophysiologic patterns can overlap and produce increased symptomatology.

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