Cytokines and radiation-induced pulmonary injuries

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ABSTRACT

Radiation therapy is one of the most common treatment strategies for thorax malignancies. One of the considerable limitations of this therapy is its toxicity to normal tissue. The lung is the major dose-limiting organ for radiotherapy. That is because ionizing radiation produces reactive oxygen species that induce lesions, and not only is tumor tissue damaged, but overwhelming inflammatory lung damage can occur in the alveolar epithelium and capillary endothelium. This damage may result in radiation-induced pneumonitis and/or fibrosis. While describing the lung response to irradiation generally, the main focus of this review is on cytokines and their roles and functions within the individual stages. We discuss the relationship between radiation and cytokines and their direct and indirect effects on the formation and development of radiation injuries. Although this topic has been intensively studied and discussed for years, we still do not completely understand the roles of cytokines. Experimental data on cytokine involvement are fragmented across a large number of experimental studies; hence, the need for this review of the current knowledge. Cytokines are considered not only as molecular factors involved in the signaling network in pathological processes, but also for their diagnostic potential. A concentrated effort has been made to identify the significant immune system proteins showing positive correlation between serum levels and tissue damages. Elucidating the correlations between the extent and nature of radiation-induced pulmonary injuries and the levels of one or more key cytokines that initiate and control those damages may improve the efficacy of radiotherapy in cancer treatment and ultimately the well-being of patients.

Keywords: ionizing radiation; lung; cytokine; radiation pneumonitis; radiation fibrosis

INTRODUCTION: HOW DOES THE LUNG RESPOND TO IONIZING RADIATION?

The lung is an organ composed of more than 40 different types of cells. Individually, these cells would be considered relatively radioreistant, but the organ as a whole has little regenerative capacity, meaning it is intolerant to higher doses of radiation [1]. Alveolar epithelial cells (or pneumocytes) are irreplaceable in their important role in lung homeostasis. The ends of the respiratory tree, the lung alveoli (Fig. 1), are lined with two types of alveolar epithelial cells: type I (AECI), also known as pneumocytes type I; and alveolar epithelial cells type II (AECII), also known as pneumocytes type II. Type I pneumocytes cover 90–95% of the alveoli. AECI are squamous, extremely flattened epithelial cells that form a complete, thin lining. These flat cells are responsible for oxygen and carbon dioxide exchange between the airspace and the underlying capillaries and express protein transport that maintains fluid homeostasis. By contrast, AECII cover just 7% of the total alveolar surface. Pneumocytes type II are typical cuboidal granular cells. They contain lamellar bodies in their cytoplasm that synthesize and secrete such pulmonary surfactant and immunomodulatory molecules as products of complement system, cytokines, lysozymes, and surfactant proteins (SPs) SP-A and SP-D (C-type lectins) involved in host defense. AECII are responsible for epithelium repair. They serve as stem cells for type I cells and can repopulate type I after insult [2–5].
fig. 1. structural scheme of an alveolus in the lung under physiological conditions. the lung alveoli consist of an epithelial layer and an extracellular matrix surrounded by capillaries. the major cells in the alveolar wall comprise pneumocytes types i and ii, and alveolar macrophages. the gas-exchanging region, the alveolar–capillary barrier, is formed by type i alveolar epithelial cells and the endothelial cells of the capillaries, and consists of the basement membrane between these cells. the interstitium of the alveoli is occupied by resident fibroblasts and forms an extracellular matrix.

in addition to epithelial cells, the alveolar space is also occupied by alveolar macrophages. they constitute a long-lived resident population, established in the lung during development and constantly patrolling the lung microenvironment. these macrophages are essential for tissue homeostasis, early pathogen recognition, as well as initiation of the local immune response and resolution of inflammation. even in a homeostatic condition within the tissue, alveolar macrophages retain high phagocytic activity, working to clear particulate antigens and dead epithelial cells from the airways [6–8].

the functional subunit of the lung is the alveolar–capillary barrier (acb), which is also the subunit most sensitive to the effects of ionizing radiation [9]. this barrier is formed ultrastructurally by aecz and the endothelial cells of capillaries and their basement membranes. both endothelial and epithelial cells are flat and thus have large surface areas but minimal cytoplasm. they compose the acb, with a thickness of just 0.3 μm [6–10]. moreover, the formation of tight junctions between alveolar epithelial cells is mostly responsible for the acb’s resistance to the movement of proteins [11].

the most numerous cell populations in the alveolar interstitium are resident fibroblasts. the fibroblast population makes up >95% of the interstitial cells. resident fibroblasts descend from various precursors and position themselves beneath epithelial cells or are scattered through the interstitium between the epithelial and endothelial layers, but without directly contacting the vasculature. these cells are highly adaptable, continuously adjusting the support they provide to growth, injuries repair or regeneration, and capable of cytokine production. under certain pathological circumstances, resident fibroblasts may be activated and transform into myofibroblasts, which constitute the key effector cell type in tissue fibrosis [12–14].

as ionizing radiation passes through lung tissue, its energy has sufficient strength to directly cause double-strand breaks in dna molecules, as well as to hydrolyze water and other molecules. this hydrolysis resolves into the generation of reactive radicals [reactive oxygen (ros) and nitric oxide (nos) species] and may lead to secondary interaction with dna and other cellular components or the extracellular matrix [15]. although most dna damage is repaired, any incorrect reparation may lead to cellular defects and cell death over a much longer period of time and can initiate a robust immune response even before any significant tissue damages occur [16].

irradiation of normal lung by thoracic or whole-body irradiation damages resident cells in the tissue. epithelial and endothelial cell deaths after exposure to radiation can occur within hours through an apoptotic pathway. apoptosis has been identified experimentally in the lung parenchyma within hours after injury [17]. furthermore, dna damages involving radiation can induce dna repair system sensors, such as dna-dependent protein kinase (dna-pk), an ataxia telangiectasia-mutated gene (atm), and an ataxia telangiectasia-related gene (atr) [18]. activation of these sensors resolves by initiating specific transcriptional factors, such as nuclear factor kappa-light-chain-enhancer of activated b cells (nflyb) [19], p53, and sp-1 [20]. additionally, oxidative stress and free radicals can result from the actions of pro-inflammatory cytokines. moreover, the radiation itself also triggers a non-specific, acute ‘cytokine storm’ that resolves within 24 h. release of these cytokines and other protein products of activated transcription factors may play a major role in subsequent radiation-induced lung injuries [21].

radiation-induced pulmonary injury
ionizing radiation on lung tissue may constitute a continuous spectrum of sequential molecular events. radiation-induced pulmonary injury (ripi) may result when there are insufficient repair mechanisms in the irradiated pulmonary tissue, or low efficiency in repopulating to replace damaged and/or dead lung tissue. in 1968, rubin and casserat [22] published their original description of radiation pneumonitis in three main clinical phases. these are: (i) an early phase lasting up to the first month that is a latent period of pneumonitis, (ii) an acute (intermediate, exudative) period that occurs in the period between 1 and 6 months, and (iii) a late or chronic phase occurring after 6 months and termed ‘radiation fibrosis’. the most critical phase for preventing and possible treatment of ripi is the early, latent phase (fig. 2a), also known as radiation pneumonitis associated with early response. this occurs 2–4 weeks post-irradiation. although during this phase histological and physiological damages present in the lungs are not evident by light microscopy, the changes are obvious on the molecular and cellular levels. ultrastructural damages, meanwhile, are evident only by electron microscopy [23].

the first occurrences are observed on the pneumocytes types i and ii and endothelial cells. experimental data show that aecz react to ionizing radiation by swelling and necrosis, which resolve in basement membrane denudation. this effect has been observed in various experimental models (rat, mouse) and also in relation to different irradiation schedules (whole body and local thorax) [24]. within hours after exposure, aecii release surfactant into the
alveolar space [25], but the proportions of surfactant subtypes are altered by a combination of increased synthesis and decreased metabolism of the heavier subtypes, and surface activity of surfactant subtypes is impaired [26]. Ultrastructural change in AECII is characterized by a decreased number of lamellar bodies, while the remaining lamellar bodies become enlarged and irregular within 24 h after irradiation [27–29]. Also, an insult on endothelial cells occurs within days after irradiation. The cytoplasm of endothelial cells is hypertrophied and vacuolated. These changes resolve into increased microvascular permeability, and 1 week post-irradiation there becomes evident separation from the basement membrane, interstitial edema, swelling, and obstruction in numerous capillaries [30].

There is strong evidence from experimental data that hypoxia can be one of the driving forces in initializing and perpetuating RIPI. In rats, moderate hypoxia was revealed at 6 weeks and severe hypoxia at 6 months after hemithorax irradiation (Fisher-344 rats, right lobe, single-dose 28 Gy) [31], but in a later study using the same model the occurrence of temporal tissue hypoxia was observed just 3 days after irradiation [32]. These dramatic biochemical and ultrastructural events occurring a short time after irradiation are

Fig. 2. (A) Latent phase of radiation-induced pulmonary injury: radiation-induced changes at molecular and cellular levels. The subunit most sensitive to ionizing radiation is the alveolar–capillary barrier (ACB). The cytoplasm of endothelial cells is hypertrophied and vacuolated, resulting in increased microvascular permeability. Ultrastructural interstitial edema can be found in the ACB complex. AECII has decreased the number of lamellar bodies and releases impaired surfactant inside the alveoli. AECII reacts by swelling and necrosis, which results in basement membrane denudation. (B) Acute phase of radiation-induced pulmonary injury (radiation pneumonitis). This phase is characterized mainly by an inflammatory process triggered by damage to lung parenchyma, epithelial cells, vascular endothelial cells, and stroma. It involves the induction of proinflammatory cytokines and chemokines that recruit immune cells in the lung tissue. Recruited peripheral neutrophils, monocytes differentiated into macrophages and the cytokines produced by different cell types are most responsible for the acute inflammation.
subsequently amplified by the cellular and tissue system and immediately activate perpetual cascades of cytokines and various gene expressions that persist until the acute phase of radiation pneumonitis becomes apparent [33–35].

When a single dose was applied in a single fraction of <7.5 Gy, the incidence of RIPI development was close to 0%, but that incidence rose to 50% in humans when the single fraction was applied at a dose of 9.3 Gy [36]. Variability of lung sensitivity to ionizing radiation in rodents strongly depends on the genetic background of the individual experimental strains and the pulmonary damages exhibited after irradiation [37]. Doses range from 10 to 15 Gy for murine experimental models. Many factors affect the risk for developing pathologic syndromes, including, among others, the method of applying radiation, the volume of the irradiated lung, the total dosage and frequency of irradiation, associated chemotheraphy, the genetic and other background of the patient, age, presence of chronic obstructive pulmonary disease, and smoking history [38, 39]. When RIPI develops, two gradually distinct, well-defined syndromes result: at the beginning there is an acute syndrome that can be characterized as radiation pneumonitis, and later there occurs radiation fibrosis. As many as 43% of patients will display radiographic evidence of lung injury, and the RIPI incidence has been reported to range between 5% and 24% in patients that have undergone thoracic irradiation and/or chemoradiotherapy [39].

Although the mechanisms underlying radiation-induced pulmonary toxicity remain uncertain, recently the view regarding the mechanisms involved has shifted from the classical paradigm of the target cell kill hypothesis from the 1970s [40] to a theory of orchestrated response [33], starting with ROS generation, followed by various activations of signal transduction pathways inducing processes leading to replacement of damaged cells, influx of inflammatory cells from peripheral blood and cytokine production, and development of radiation complications [41]. Despite these improvements in understanding, RIPI causes significant morbidity and mortality in patients treated for tumors in the thoracic region. In the following section, we will summarize general information about radiation pneumonitis and radiation fibrosis, as well as provide an update of recently observed evidence from scientists bringing new insight into old paradigms and establishing a new viewpoint that brings us closer to successfully understanding the tangled skein of processes that this radiation induces.

RADIATION PNEUMONITIS

Radiation pneumonitis develops as an acute reaction within 4–12 weeks after irradiation of the thorax region. Characteristic manifestations of this phase include non-specific symptoms like fever, chest pain, dry cough, and mild dyspnea or even respiratory failure in severe cases [42, 43].

The acute pneumonitic phase (Fig. 2B) is characterized by recruitment of diverse immune cells into the alveolar space, thickening of the alveolar septa, and disruption of the integrity of pulmonary alveoli. Infiltration of myeloid and lymphoid cells initiates tissue reactions, resulting in lung inflammation and edema of alveolar interstitium and air spaces [43]. AECI undergo apoptosis or necrosis, thereby causing denudation of the basement membrane. Depletion of AECI is followed by hyperplasia of AECII. Accelerated proliferation of AECII also leads, however, to neglect of their primary function, which is to produce pulmonary surfactant. The lack of surfactant results in a loss of surface tension, followed by edema and atelectasis of lung tissue [44]. The edema increases vascular permeability, which causes leakage of plasma protein and fibrin-rich exudates into the alveolar spaces.

One of the reasons radiation pneumonitis remains a major dose-limiting complication for patients is that no specific treatment exists for it. The current treatment strategy is still non-specific and symptomatic and includes steroids and corticosteroids, diuretics, and such other substances as hormones, enzymes and antioxidants. A promising step forward is being made by supplementing soy products before and after irradiation as protection against radiation toxicity in tissue. The anticancer agents, soy isoflavones are non-toxic dietary plant estrogens extracted from soybeans. Their potential as a biological/nutritional intervention to protect against radiation-induced pneumonitis [45–47] improves the efficiency of radiotherapy and decreases its toxicity, as has been demonstrated in research and in a controlled clinical trial [48].

If these damages are so intensive that it is not possible to restore homeostasis by any repair mechanisms, then the integrity of the ACB breaks down to a point of no return and regeneration of parenchyma becomes inadequate. The subsequent processes in lung tissue will then lead to the promotion of radiation fibrosis.

RADIATION FIBROSIS

Radiation fibrosis occurs as the last, irreversible phase in excess of 6 months after irradiation. It stabilizes after 2 years. A characteristic manifestation of radiation fibrosis is an accumulation of fibroblasts and myofibroblasts, resolving in extensive production of collagen (mainly subtypes I and III), infiltration of inflammatory cells, and remodeling of the extracellular matrix followed by fibrosis of alveolar septa, which cause widespread obliteration of the residual alveoli.

Pulmonary fibrosis is characterized by an accumulation of extracellular matrix proteins that ultimately compromise the lung’s ability to exchange oxygen. These extracellular matrix proteins are secreted by scar-forming myofibroblasts, which express markers of smooth muscle cells. Myofibroblasts arise from undifferentiated resident lung fibroblasts in response to profibrotic stimuli, including ionizing radiation, with lung fibroblasts differentiating into myofibroblasts and secreting extracellular matrix proteins that can contribute to fibrogenesis. Therefore, inhibiting radiation-induced myofibroblast differentiation may be an important therapeutic approach in preventing radiation-induced extracellular matrix accumulation and fibrosis [49, 50]. Moreover, myofibroblasts constitute the main source of collagen production. They can derive from various cell types: resident stromal fibroblasts [51], fibrocytes from bone marrow [52], and even from ACEII [23]. Ionizing radiation activates fibroblasts/myofibroblasts, and that results in dysregulated and exaggerated repair processes leading to fibrosis [53]. Epithelial–mesenchymal transition (EMT) is one of the processes responsible for lung fibrosis. This process is activated in reaction to irradiation, but also in other pulmonary pathogenesis resolving with fibrosis; it is involved, too, in embryonic development [54] and tumorigenesis [55].

The key fibrogenic cytokine of the EMT program is transforming growth factor-β (TGF-β) along with its signaling pathway. The
crucial role of this cytokine in epithelial–mesenchymal transition will be described below. This process is initiated by transdifferentiation of epithelial cells to activated myofibroblasts [53]. A central role of these cells undergoing EMT is synthesis and deposition of extracellular matrix proteins. The extracellular matrix is a mixture of proteins (collagens and elastin), glycoproteins, proteoglycans (fibronectin, laminin and tenasin), glycosaminoglycans (heparin and chondroitin) and hyaluronic acid [56].

A recent paper by Balli et al. provides new insight into the molecular mechanism of radiation-induced pulmonary fibrosis. It was discovered that a transcriptional factor, Forkhead box M1 (Foxm1), critical for the EMT process is induced through direct activation of Snai1 transcription and increase in inflammatory mediator expressions. Based upon two experimental models, lung samples from patients with idiopathic pulmonary fibrosis and transgenic epiFoxm1 KO mice were confirmed to have increased activity of Foxm1 in AECII-enhanced, radiation-induced pulmonary fibrosis [57].

A later study by Xiong et al. identified that regulatory T lymphocyte (Treg) depletion in C57Bl/6 mice decreased alveolar septal thickening and collagen deposition in lung tissues of mice exposed to irradiation, and significantly increased nuclear or cytoplasmic β-catenin expression in alveolar epithelium, and Treg depletion by anti-CD25 antibody led to high Pro-SPC expression (epithelial marker) and low N-cadherin expression (mesenchymal marker) [58]. Evidence of an important role of β-catenin during the EMT process has been confirmed also in bleomycin-induced lung fibrosis [59], and primary fibroblast cultures have been derived from lung tissue from a patient with idiopathic pulmonary fibrosis [60].

Pan et al. offer a new and interesting outlook in their study showing that ABT-263 could selectively kill senescent cells and could reverse pulmonary fibrosis even when it already had become persistent in mice after thoracic irradiation. This finding suggests that ABT-263, a specific Bcl-2/xl inhibitor and a newly identified drug, has the potential to be an effective treatment for pulmonary fibrosis [61].

CYTOKINES

Cytokines are small molecules of proteins, peptides or glycoproteins, produced as soluble factors by a variety of distinct cells, including all cells of the immune system, as well as endothelial, epithelial and stromal cells [62]. Cytokines are involved in nearly every response to immunity, inflammation and infection stimuli. Their function is triggered in such processes as proliferation, chemotaxis, mediation of inflammatory responses, homeostasis, differentiation, elimination of pathogens, and cell death [63]. During the past three decades, identification and classification of cytokines has undergone multiple developments. Initially, cytokines were identified based on their biological activities and termed lymphokines, monokines, interleukins and chemokines and numbered by order of discovery [64]. Since then, cloning and recombinant strategies have led to better understanding of cytokine biology and identified individual cytokines’ expression patterns. Most recently, a successful and complete sequencing of the human genome has discovered a huge library of previously unrecognized proteins, and this also affected cytokine identification [65]. New cytokine classification has been assigned, and cytokines are now grouped into (super)families based on comparing sequences of known genes with sequences of discovered genes while assuming that homologous proteins should have related functions. In addition to this genomic classification, cytokines may be grouped in accordance with their functional properties or by the cells that produce them. Most cytokines have more than one prominent property, however, and are produced by different cell sources. Cytokines nomenclature is comprehensively summarized in an overview written by Brocker et al. [66].

Despite some 50 years of studies to identify cellular and molecular mechanisms of radiation-induced damages, the exact mechanisms have not been recognized and not all signaling molecules have been discovered. It nevertheless is known that cytokines play a very important role. There have been many findings describing the propensity for a variety of cytokines to affect particular processes of RIPI [67]. For that reason, cytokines are intensively studied as signaling molecules relevant to the RIPI mechanism and as possible biomarkers to identify risk of RIPI development. Newer cytokine-based therapies and intervention in their signaling pathways comprise one of the fields of research. The goal of this review is to interpret the most important cytokines that are involved during RIPI, the relative mechanisms of action and consequences for irradiated tissue, as well as new approaches in treatment strategies or therapies.

CYTOKINES RESPONSES TO RADIATION

The production of each cytokine is time-dependent in response to ionizing radiation. Although each cytokine has its own, characteristic profile, within 4–24 h after irradiation there often occurs a non-specific acute reaction (so-called ‘cytokine storming’), and that is followed by decrease to baseline levels within a period of 24 h to a few days [68]. Based on all available data, there are two major investigative approaches for RIPI. The first relates mainly to patients having had clinically relevant high doses of thoracic radiation. After partial irradiation of the thoracic region, persistent waves of increased cytokine expression have been described in the lung tissue. Animal models (mainly murine) were established in order to better approximate the patient’s situation. Murine models have been subjected to a localized high dose in a single exposure, and it is widely accepted that a dose threshold of 12 Gy needs to be exceeded for pneumonitis to occur [69]. The second approach involves identifying risk of RIPI after total body irradiation (TBI). This approach is based on historical nuclear accidents, such as those at Chernobyl [70] or Tokai-mura [71], where people were undesirably exposed to ionizing radiation, as well as possible incidents involving dirty bombs or similar devices. TBI irradiation is also a commonplace procedure indicated for patients with bone marrow transplantation for hematologic malignancy [72]. Even when exposing the lungs to a dose within the ‘sub-threshold’ range, there have been reported cases of patients suffering RIPI [73]. The roles of cytokines in TBI and lung damages caused by irradiation.

Hundreds of studies are available today that may provide great insight into a wide spectrum of cytokine profile and peaking after whole- and partial-body irradiation in patients and animal models. Herein, murine models of whole-thorax or hemithorax irradiation nicely reproduce the pathogenesis of the human disease with respect to its time course and clinical symptoms. The use of preclinical...
in vivo models has helped substantially to advance our understanding of the molecular mechanisms and signaling molecules that participate in the pathogenesis of radiation-induced adverse late effects in the lung.

In a study by Zhang et al. [74], for example, plasma concentrations of 32 cytokines were determined after TBI with dose 9 Gy in identical murine models. This publication reports five temporal patterns of circulating cytokine expression. Furthermore, Johnson et al. [75] assessed whether a TBI dose in the range 0.5–10 Gy is necessary for the development of lung pathological changes and reported the responses of 22 cytokines in early and late time intervals. Unfortunately, this study showed no correlation between the acute cytokine responses to radiation and the late elevations in cytokine expression and/or pathophysiologic changes, but mice in later time intervals did manifest an increased and prolonged response to microbial lipopolysaccharide. After whole-lung irradiation with a dose of 12 Gy, Ao et al. [76] profiled 22 cytokines from different sources (serum, lung tissue, and bronchoalveolar lavage) in various time intervals and for two types of radiation-sensitive murine strains (C57Bl/6 and C3H). An interesting aspect of this study is that it endeavored to find a positive correlation between serum and tissue levels. Its findings clearly demonstrate that thoracic radiation induced significant strain-dependent early expressions of certain cytokines, as well as the strength of the correlations between their levels in tissue and blood.

All studies dealing with this matter have been conducted while attempting to identify the potential value of cytokines, chemokines, and/or their combinations as biomarkers for the individual stages of RIPI. We must bear in mind that for a cytokine to be useful as a biomarker its level in plasma should have a positive correlation with RIPI. We must bear in mind that for a cytokine to be useful as a biomarker its level in plasma should have a positive correlation with those of IL-6 (P = 0.001) [83]. Therefore, the profibrotic cytokines and chemokines were not reliably predictive of radiation pneumonitis [39, 83]. Similarly, in a study by Arpin et al., correlation was reported between the occurrence of radiation pneumonitis and serum levels of IL-6 and IL-10 in patients during radiotherapy [84]. A not very positive result of IL-6 research was found in patients with advanced non–small-cell lung carcinoma. In this case, correlations were also observed between tumor response and the plasma concentration of IL-6 and TGF-β1, but this study concluded that this cytokine was being produced by a tumor and thus that it may impair the prospective identification of patients at risk [85].

INTERLEUKIN-1

Interleukin-1 (IL-1) is a member of the IL-1 superfamly of cytokines that are important regulators of innate and adaptive immunity, playing key roles in host defense against infection, inflammation, injury and stress [86]. IL-1 includes two subtypes: interleukin-1α (IL-1α) and interleukin-1β (IL-1β). Both are agonists and are expressed in multiple cell lines throughout the body, including in monocytes, macrophages, neutrophils, hepatocytes, and tissue macrophages. IL-1α is expressed in cytoplasm as a 31 kDa precursor form (pro-IL-1α) that is biologically active and capable of binding to IL-1R and activating cells [87]. Moreover, IL-1α and IL-1β are functionally antagonized by the IL-1 receptor antagonist (IL-1RA), a soluble factor that binds non-productively to IL-1 receptors. IL-1β is an inducible cytokine and is not generally expressed in healthy cells or tissue. This type, however, is rapidly induced in cells by activation of such pattern recognition receptors as toll-like receptors and by pathogen products or factors released by damaged cells, thus leading to intracellular accumulation of the protein [88, 89].

Damages to the epithelium of the lung as a result of exposure to ionizing radiation lead to release of IL-1α from stressed and/or necrotic cells into the extracellular space. The release of IL-1α from necrotic cells is unique and it can be considered to function as a damage-associated molecular pattern (DAMP) under these circumstances or as an alarm by binding to IL-1R1 and rapidly initiating the production of chemokines and inflammatory cytokines [90, 91].

Because the two forms of IL-1 almost completely overlap in function and both are recognized by the same receptor, their
| Evaluated cytokines | Models | Types of RT | Sources/doses | Time intervals | Methods | Materials | Main results/trends in irradiated groups only | Authors |
|---------------------|--------|-------------|---------------|---------------|---------|-----------|---------------------------------------------|---------|
| IL-2, IL-6, IL-10, TGF-β | C57BL/6j | TBI | X-ray/3 × 5 Gy | 16 d | Miliplex kit Serum | Increased level of IL-2 (×1.6) and IL-10 (×2.25); decreased level of IL-6 (×2); no change for IL-5, IL-12 and TGF-β1. | Zhang et al. [133] |
| IL-6, TNF-α | BALB/c | TBI | NM/6.4 Gy | 15 d | ELISA Plasma | TBI caused an increase in circulating IL-6 and TNF-α in surviving mice. | Wang et al. [134] |
| IL-4, IL-5 | C57Bl/6 | TBI | 137Cs/0.3 and 1 Gy continuously for 24 d | 26 d | ELISA BALF | Continuous exposure to low-dose-rate radiation significantly reduced these cytokines. | Kim et al. [135] |
| IL-1β, TNF-α, TGF-β1 | C57Bl/6 | TBI | 137Cs/6 × 0.25 Gy per 2 w | 2 w | ELISA, RT-PCR BAFL | Significant induction of mRNA TGF-β1 in mice exposed to hypoxia and/or radiation. Levels of active TGF-β1 were significantly elevated in all cohorts in the BALF of mice. Exposure to challenge conditions led to significant increases in the levels of IL-18 and TNF-α in mice exposed to each of O2, IR and O2 + IR. | Petrofesa et al. [136] |
| IL-6, IL-1β, IFNγ, MCP-1, TNF-α, MIP-1α | C57BL/6 | TBI | X-ray/2, 4, 6 and 8 Gy | 16 h (doses); 16, 48 h, 7, 9 and 20 d (8 Gy) | Lumines 6-plex Serum | Serum IFN-γ was significantly increased 16 h after exposure to 8 Gy (non-specific effect of ionizing radiation). | Kao et al. [137] |
| G-CSF, GM-CSF, SCF, IL-3, VEGF, TSLP, IL-β, IL-2, IL-4, IL-5, IL-10, IL-17, 1L-2p70; KC, MCP-1, MIP-1α, MIP-1γ, CXCL13, CXCL16, eotaxin, TCA-3, TARC, MDC, TNF-α, CD30, CD40, Fasl, sTNF-R1 | C3H/HeJ, C57BL/6j | TBI | 137Cs/9 Gy | 6 h, 1, 2, 4 and 10 d | Antibodies R&D Systems + microspheres Lumines | Growth factors: G-CSF 10 d;†; GM-CSF 16 h and 1 d, then the level subsequently decreased and ↑ Day 10; IL-3 16 h (similar with GM-CSF); SCF ↑ 1 and 4 d (C57); Interleukins: IL-1β ↑ 10 h (diff. response between strains); IL-4, IL-6 and IL-10 ↑ rapidly at 6 h, decreased, ↑10 d (C57BL), IL12p70 16 h until 10 d. Chemokines: CC chemokines and CXC responded similarly in the two strains MIP-1α, eotaxin, and MDC(4–10 d;↓), CXCL 13, CXCL 16 and KC ↑10 d; TNF-16. Five temporal patterns of expression: (1) Two peaks: ↑6 h, subsequently decreased and ↑ Day 10 (G-CSF, GM-CSF, IL-3, SCF, IL-4, IL-6, IL-10, IL-12p70, TNF-α, KC), (2) Increased over time (CXCL-16). (4) No immediate change, subsequent ↑ 2–10 (IL-1β, MIP-1γ, MCP-1, TCA-3, CXCL13, sTNF-R1). (4) ↓ (MIP-1α, eotaxin, MDC). (5) No response: VEGF, IL-2, IL-5, IL-17. | Zhang et al. [74] |
| IL-1α, IL-18, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 (p40), IL-12(p70), IL-13, GM-CSF, IFNγ, KC, MCP1, MIP-1β, RANTES, TNFα, VEGF | C57BL/6j | TBI | 137Cs/0–10 Gy | 1, 4, 24 h, 1, 3, 6, 9, 12 and 18 mo | Millipore beads Serum | A significant, dose-dependent increase in expression of IL-6 and KC was seen at early (1–4 h) time points, followed by a return to baseline levels by 1 d. At 9 mo after RT, there appeared to be a dose-dependent increase in KC expression. | Johnston et al. [75] |
| Evaluated cytokines | Models | Types of RT | Sources/doses | Time intervals | Methods | Materials | Main results/trends in irradiated groups only are listed: Authors |
|---------------------|--------|-------------|---------------|----------------|---------|-----------|---------------------------------------------------------------|
| IL-1α/β, IL-6, TNF-α, TGF-β | BALB/c | TBI | X-ray/20 Gy | 24 h | RT-PCR | Lungs, intestine, liver | Irradiation induced a significant increase in the mRNA levels of all detected cytokines (IL-1α/β, IL-6, TNF-α, TGF-β) in the lung and intestinal tissues. Wu et al. [138] |
| IL-6, TGF-β, IL-1α, IL-6, IL-10, TNF-α, CTGF | BALB/c | TBI | 60Co/6 Gy; 2 × 2.5 Gy | 4, 24 h and 3 w | ELISA, RT-PCR | Serum, lung tissue, liver, intestine | TBI after 4 h did not provoke in the lung significant changes in the mRNA expression of pro-inflammatory or pro-fibrotic cytokines. The lung seemed to be more prone to subchronic after 2 × 2.5 Gy radiation, due to upregulation of the mRNA levels of TGF-β and IL-6 at 3 w. Ostrau et al. [139] |
| KC, IL-6 | CS7BL/6J | TBI | 60Co/9 Gy 137Cs/10 Gy | 10–18 d | ELISA | Plasma | KC and IL-6 were considerably increased in the plasma of irradiated mice by 9 Gy from Days 11 to 18. Radiation-induced increased KC (×4.5) in the plasma with 137Cs at 14 d. Van der Meeren et al. [140] |
| GM-CSF, IL-6, TNF-α | ICR | TBI | 60Co/3–20 Gy | 1, 4, 8, 18, 24 h, 3, 7, and 9 d | ELISA | Serum, lung tissue | The concentration of GM-CSF decreased significantly as early as 1 h after irradiation and increased at 3 and 7 days after RT in lung tissue. The level of TNF-α significantly increased 5 days after irradiation and reached a maximum at 9 days. No differences were detected in the production of IL-6. Significantly increased TNF-α in sera was observed on Day 5, and IL-6 production on Days 5, 7, and 9, compared with non-irradiated controls. Fedoroczk et al. [35] |
| IL-1β, IL-6, TNF-α, GRO1, RANTES | CS7BL/6J | TBI | 60Co/8 Gy | 1 and 24 h | ELISA | Plasma, lung tissue | IL-6 and GRO1 were increased in plasma, with a peak attained at 6 h; sustained production was observed at least up to 24 h. RANTES remained unchanged after radiation exposure. IL-1β and TNF-α remained undetectable in the plasma of irradiated animals. In the lung, TNF-α remained undetectable; IL-1β, IL-6 and GRO1 were produced in the lung, and radiation significantly elevated IL-1β and GRO1 levels. Van der Meeren et al. [141] |
| TGF-β1 | CS7BL/6, C3H/HeJ | TBI | 60Co/7 Gy | 9 and 56 d | RT-PCR | Lung tissue | A very moderate positivity was found in the C3H, and obvious positivity in the CS7, in the lung tissue on Day 9 after irradiation. Olejár et al. [142] |
| TGF-β1, HIF-1α | CS7BL/6 | TBI and thorax | 137Cs/5 Gy TBI + 10 Gy thorax | 26 w | RT PCR | Lung tissue | Significant increase in HIF-1, TGFβ1. Judge et al. [49] |
| IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12, IL-12(p40), IL-12(p70), IL-15, GM-CSF, IFNγ, KC, MCP1, MIP1β, RANTES, TNF-α, VEGF, IP-10, IL-18, CCR1, CXC2, IL18, IL12, IL10, IL1A, IL1β, IL1Ra, TNFRp75, TNFRp55, CCR5, CCR2, CXC3 | CS7BL/6 | TBI and thorax | 137Cs/0–10 Gy | 1, 6, 24 and 48 h | Millipore beads, RNA assay | Plasma, lung tissue | Plasma: IL6 and KC changes after both whole-lung irradiation and TBI, with a dose-responsive increase. Expression ↑ 1 h, a maximum at 6 h after irradiation, and return to baseline at 24 h. Robust response in TBI. Lung: IL-1β all doses ↑1 h (×3–4). Similar responses with TBI (×3–9). At 6 h ↑ after irradiation, only the doses 5 and 10 Gy to the whole lung, in both, the levels Johnston et al. [17] |
returned to baseline by 24 h. IL1R2: all doses ↑ at 1 h. At 6 h, ↑ only for 10 Gy (lung); returned to baseline at 24 h. More robust response in TBI (1 h). CCR1 and CXCR2 receptors all doses ↑ 1 h after whole-lung irradiation (×2.5–5). At 6 h only the higher-dose (2.5 Gy) ↑ CXCR2; returned to baseline at 24 h.

### KC, IL-6, TNF-α

| CS7BL/6J | TBI, total abdominal | 60Co/15 Gy | 3 and 6 d | RT-PCR | Lung tissue, plasma | IL-6 and KC were significantly increased in the plasma 3 d after radiation exposure; the increase in KC was significantly higher for TBI than for abdominal irradiation. Six days after irradiation, IL-6 and KC remained significantly elevated in the plasma. TNF-α remained undetectable in the plasma or lung. KC was significantly elevated at 3 d, and IL-6 levels were slightly increased 6 d after exposure with both configurations of irradiation. | Van der Meeren et al. [143] |

### TGF-β1, IFN-γ

| CS7BL/6J | Thorax | 60Co/20 Gy | 120 d | ELISA | Serum | TGF-β1 levels significantly increased; IFN-γ level not changed. | Wei et al. [144] |

### IL-1β, IL-6, TNF-α, TGF-β1, IL-1β, IL-6, TNF-α

| CS7BL/6 | Thorax | X-ray/16 Gy | 7, 14 and 28 d | RT-PCR, liquichip | Lung tissue, plasma | Plasma: significant increase in IL-1β, IL-6 and TNF-α plasma levels. Tissue: a slight increase in the levels of IL-1β and IL-6 on Days 7, 14 and 28. TGF-β1 was significantly increased in all time intervals. | Tang et al. [145] |

### IL-1α, IL-1β, TNF-α, TGF-β, TIMP-1

| CS7BL/6 | Right lung | X-ray/17 Gy | 8, 16 and 23 w | RT-PCR | Lung tissue | Increased expression of TNF-α, IL-1α, IL-1β at 8 w and TGF-β1 at 16 w. At 23 w, expression of IL-1α, IL-1β and TIMP-1 was increased | Fan et al. [61] |

### IL-1β, IL-6, TNF-α, TGF-β1

| CS7BL/6 | Thorax | X-ray/16 Gy | 30 and 120 d | Luminex + RT-PCR | Plasma, lung tissue | Early (30 d) increased mRNAs and protein levels for IL-1β, IL-6, TNF-α and TGF-β1 in the lungs and plasma. At 120 d relative increase in IL-1β, IL-6, TNF-α and active TGF-β1. | Li et al. [146] |

### GM-CSF, IP-10, FGF-β1, IFN-γ, KC, VEGF, IL-1α, MCP-1, IL-1β, MIG, IL-2, MIP-1α, IL-4, IL-5, IL-6, IL-10, IL-12 (p40/p70), IL-13, IL-17, TNF-α

| CS7BL/6 | Thorax | X-ray/13.5 Gy | 2, 4 and 18 w | Invitrogen 20-Plex, BALF, plasma | Plasma FGF-β and IL-2 showed a significant increase at 18 w. | Christofidou et al. [147] |

### TGF-β1

| CS7BL/6 | Thorax | X-ray/12 Gy | 2 and 17 d | ELISA, RT-PCR | Lung tissue | TGF-β1 concentrations were significantly higher at Day 2 (×1.4) and Day 17 (×1.7). | Chen et al. [148] |

### IL-1, IL-6, IL-10, TNF-α

| Diabetic/SCID | Thorax | 60Co/13 Gy | 30 d | ELISA | Plasma | Radiation increased plasma levels of IL-1β, IL-6, TNFα and IL-10. | Chen et al. [149] |

### IL-1β, IL-6, TNF-α, GM-CSF, M-CSF, TGF-β1

| CS7BL/6 | Thorax | X-ray/16 Gy | 4 and 9 w | MBA, WB | Plasma, lung tissue | More than ×2 concentrations of IL-1β, IL-6 and GM-CSF were induced at 4 w after radiation. WB analysis of TGF-β1 detected a significantly different level between plasma and lung tissue. | Chen et al. [150] |

### IL-1β, IL-6, TNF-α, IFN-γ, IL-4, IL-10, IL-13, IL-15

| BALB/c | Thorax | X-ray/10 Gy | 4, 24 h, 1, 4 and 12 w | ELISA | Lung tissue | IL-6 significantly increased at 4 h until 4 w after radiation. TNF-α level in the lungs increased early at 4 h then remained low. IL-1β increased at time points of 4 and 12 w. IL-10 peaked at 24 h and remained until an interval of 4 w. IL-15 maximal concentration at 1 w after radiation. IFN-γ, IL-4, IL-13 levels had no significant changes. | Abertnathy et al. [151] |

### TGF-β1, TNF-α, IL-1β, IL-6

| CS7BL/6 | Thorax | 60Co/16 Gy | 1, 2, 4, 8, 16 w | ELISA, RT-PCR | Serum, lung tissue | TNF-α, IL-1β, IL-6 and TGF-β were increased at 2, 4, 8 and 16 w in both tissues. Gene expression in lung tissue showed an | Zhao et al. [152] |

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Continued
Table 1. Continued

| Evaluated cytokines | Models | Types of RT | Sources/doses | Time intervals | Methods | Materials | Main results/trends in irradiated groups only are listed: | Authors |
|---------------------|--------|-------------|---------------|----------------|---------|-----------|----------------------------------------------------------|---------|
| IFN-γ, IL-12, IL-5, IL-13 | C57Bl/6 | Thorax | X-ray/12 Gy | 16 w | CBA | Serum | IFN-γ and IL-12 levels were lower in the RT group. IL-5 and IL-13 levels from mice in the RT group were higher than the serum levels. | Chen et al. [153] |
| IL-4, IL-13, IFN-γ, IL-1β | C57Bl/6 | Left lung | X-ray/75 Gy | 3 w | RT-PCR | Lung tissue | High gene expression of all cytokines in irradiated group. | Shin et al. [154] |
| TGF-β, IL-1β, IL-6, TNF-α | C57Bl/6 | Thorax | X-ray/5 × 6 Gy | 2, 16 w | ELISA | Lung tissue, BALF | IL-4 and IL-13 was significantly increased as early as 2 w after irradiation; IL-13 was increased until 16 w. IFN-γ and IL-1β were not elevated in BAL fluid. IL-13 is a critical factor promoting radiation-induced pulmonary fibrosis. | Chung et al. [99] |
| TGF-β, IL-1β, IL-6, IGF-1 | C57Bl/6 | Thorax | X-ray/5 × 6 Gy | 0, 2, 4, 8, 16 w | RT-PCR | Lung tissue | Several of these pro-inflammatory cytokines (IL-6, IL-1β) and TGF-β were increased in the irradiated lung. The expression level of IGF-1 was also increased due to the irradiation. | Chung et al. [155] |
| IFN-γ, IL-12, IL-4, IL-5 | C57Bl/6 | Thorax | 60Co/20 Gy | 3, 14 d, 1, 3 and 6 mo | RT-PCR | Lung tissue | Thoracic irradiation led to a marked increase in the levels of IFN-γ and IL-12 in irradiated mice from Day 14 to 3 mo post-irradiation. The IL-4 and IL-5 cytokines examined showed a significant increase from Day 14 to 6 mo post-thoracic irradiation. | Xiong et al. [157] |
| IL-1α, IL-1β, IL-6, TGF-β1, IL-13, MIP-2, MIP-1, CXCL16, IL-12p30, Ltn, RANTES, IL-5, sTNFR-1 | C57Bl/6 | Thorax | 137Cs/15 Gy | 5 mo | ELISA | Lung tissue | Increased levels of TGF-β1, IL-18, IL-13, IL-1α, MIP-2, MIP-1, CXCL16, IL-12p30, Ltn, RANTES, IL-5, sTNFR-1 were observed in the radiation/vehicle group. Increased levels were also observed in the following cytokines: MIP-1, IL-5, IL-12 (p40), CXCL16, lymphotoxin, RANTES and sTNFR-1 levels did not change. | Yang et al. [158] |
| IL-6, IL-10, TGF-β | C57Bl/6 | Thorax | X-ray/RP: 12.5 Gy, RF: 22.5 Gy | 3 w | ELISA | Lung tissue | IL-6 and IL-10 levels in the lung homogenates were increased after irradiation. The TGF-β concentration increased significantly (>4×) compared with the control group. | Wang et al. [159] |
| TNF-α, IL-17A, IL-6, IFN-γ | C57Bl/6 | Thorax | X-ray/15 Gy | 1 w | ELISA | BALF, BALF | TNF-α, IL-17A and IL-6 were elevated in BALF of irradiated mice, while IFN-γ was reduced. | Wang et al. [160] |
| IL-4, IL-5, IL-6, IL-13, IL-1α, IL-1β | C57Bl/6 | Left lung | X-ray/75 Gy | 2, 3 w | ELISA, RT-PCR | BALF, lung tissue | The levels of IL-4, IL-5, IL-6 and IL-13 were slightly increased in BALF by RT, compared the levels in the control group. Expression of IL6, IL-1α and IL-1β mRNA in lung tissue was significantly higher 3 w after irradiation. | Sohn et al. [161] |
| Cytokine/Chemokine | Strain | Exposure | Time Points | Assays | Tissue Sample | Findings |
|--------------------|--------|----------|-------------|--------|--------------|----------|
| IL-6, IL-4, IL-1β, IL-10, IL-13, IL-17, IFN-γ, TNF-α | AKR/J, C3H/HeJ, A/J, C57BL/6J, 129S1/SvImJ, KK/HJ | Thorax X-ray/18 Gy | 6 h, 1 and 7 d | Bio-ead, 8-plex | BALF, serum | Radiation exposure produced minimal changes in the cytokine profiles of these strains. Strain affects the reactions and cytokine background (the highest response in strain A/J). IL-1β and IL-10 levels, measured at the 7-day time point in BALF, were inversely correlated with fibrosis score. In serum, IL-6, IL-17 and TNF-α levels were affected by radiation in four of the six strains. |
| TGF-β1 | C57Bl/6 | Thorax X-ray/12 Gy | 2, 15, 28 d, 8, 12, 16, 20 and 24 w | ELISA, RT-PCR | Serum BALF, lung tissue | Levels of TGF-β1 in the serum of irradiated mice increased with time, significantly by 4 w, and peaked 8 w after radiation, compared with the control. Changes in the BALF were similar to those in the serum. Expression of TGF-β1 was significantly upregulated at all time intervals (maximally at time point 12 w). |
| Eotaxin, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-6, IL-9, IL-10, IL-12(p70), IL-12(p40), IL-13, IL-17, IFN-γ, G-CSF, GM-CSF, TNF-α, KC, MCP-1, MIP-1α, MIP-1β, RANTES, TGF-β1 | C3H/HeJ | Left lung X-ray/90 Gy, 2 and 3.5 mm collimators | 0, 1, 2, 6, 12 w | Bio-Plex Pro 23-plex kit + ELISA (TGF-β1) | Serum Cytokine levels in the serum after irradiation using a 3.5-mm collimator: IL-12 (p40) and G-CSF were increased at 2 w, while all other cytokine levels (IL-2, IL-6, IL-1b-1b, IL-13, TNF-α, MIP-1α, GM-SCF, IL-17, IFN-γ, MCP-1, eotaxin, MIP-1b, IL-4, IL-12(p70), p40, IL-10, G-CSF and RANTES) were increased at 1 w compared with the control. IL-3 was increased at the late time point 12 w. IL-1α and IL-9 decreased at 12 w. IL-5, KC and TGF-β1 did not show significant changes. Cytokine levels in the serum after irradiation using a 2-mm collimator: G-CSF was increased at 2 w; IFN-γ and IL-2 levels were increased at 1 w; TGF-β1 and IL-9 increased at 6 w; the remainder of the cytokines did not show significant changes between any time points. |
| TNF-α, TGF-β1 | C57Bl/6 | Thorax X-ray/15 Gy | 2 and 10 d | ELISA, RT-PCR | Lung tissue mRNA expression and protein levels of TNF-α and TGF-β1 at 10 d after irradiation were significantly increased compared with control group. |
| IL-1β, IL-4, TNF-α, EGF, TGF-β, IL-6 TGF-α | C57Bl/6, C57BL/6TNF−/− | Thorax X-ray/5 × 6 Gy | 2, 4, 8 and 20 w | ELISA | Lung tissue IL-1β, IL-6 and TNF-α concentration increased from baseline as early as 2 w. No variations in IL-4 or EGF at any time after irradiation were noted. There was a small but significant increase in the level of TGF-β in the lung tissue of TGF-α−/− mice compared with WT mice. TGF-β levels increased in the lung tissue of both WT and TGF-α−/− mice at 2 w. |
| TGF-β1, IL-4, IL-13, IFN-γ | C57Bl/6 | Thorax 60Co/15 Gy | 1 d, 1, 4, 8 and 16 w | ELISA, RT-PCR | Serum, lung tissue Pulmonary irradiation led to a remarkable increase in TGF-β1 waves of increase occurred at each of 1 d and 8 w (increase in mRNA was not detectable). There were two waves of increase in IFN-γ (same trend as for TGF-β1). IL-4 and IL-13 levels of mRNA in the lungs remained lower in the early time points, but stepped up progressively over 4 w. |
| IL-4, IFN-γ, IL-12 | C57Bl/6 | Thorax 60Co/20 Gy | 1, 3 and 6 mo | Invitrogen Bioplex | BALF The fibrotic cytokine IL-4 level in BALF from mice in the irradiation group increased significantly, the IL-12 level in BALF increased 1 mo after irradiation, and the IFN-γ level in BALF decreased significantly at intervals 3 and 6 mo. |

Continued
| Evaluated cytokines                  | Models | Types of RT | Sources/doses | Time intervals | Methods          | Materials | Main results/trends in irradiated groups only are listed: | Authors          |
|------------------------------------|--------|-------------|---------------|----------------|------------------|-----------|----------------------------------------------------------|------------------|
| TNF-α, IL-17A, TGF-β1, IL-6        | C57Bl/6| Thorax      | X-ray/15 Gy   | 1, 4, 8 and 16 w | ELISA, BALF      |           | The levels of TNF-α, IL-6 and TGF-β1 were significantly higher after irradiation at the 4 w time point; IL-17A reached a maximal concentration at 4 w, then slightly decreased. | Wang et al. [169]|
| IL-17A, TGF-β1, IL-6               | C57Bl/6| Thorax      | X-ray/15 Gy   | 8 h, 1, 4, 8 and 16 w | ELISA, BALF      |           | IL-17A, TGF-β1 and IL-6 peaked at 4 w post-irradiation, and decreased thereafter. | Wang et al. [170]|
| TGF-β1, TNF-α, IL-1β, IL-6         | C57Bl/6| Thorax      | X-ray/12 Gy   | 1 d and 2 w       | RT-PCR, ELISA (TGF-β1) | Lung tissue | The mRNA expression and protein level of TGF-β1 in lung tissue was decreased at Day 1 and increased at 2 w after thoracic irradiation. There were increased mRNA expressions of proinflammatory cytokines IL-6 and TGF-α at 2 w after irradiation. | Jang et al. [171]|
| G-CSF                              | C3H/HeJ| Thorax      | X-ray/18 Gy   | 8 and 12 w        | ELISA, BALF      |           | G-CSF levels were significantly increased in the BALF of irradiated mice at 12 w after irradiation in comparison with levels in sham-irradiated mice. The level of G-CSF decreased at time interval 8 w. | Kunwar et al. [172]|
| MIF                                | C57Bl/6, C57Bl/6/wt−/− | Thorax | NM/20 Gy       | 8 w and 16 m     | ELISA, BALF, plasma |           | Protective regulatory role of MIF in response to ionizing radiation. | Mathew et al. [173]|
| FGF, GM-CSF, IFN-γ, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12 (p40/p70), IL-13, IL-17, IP-10, KC, MCP-1, MIG, MIP-1α, TNF-α, VEGF | C57Bl/6| Thorax      | X-ray/13.5 Gy  | 16 w           | Invitrogen 20-Plex | BALF      | Thoracic radiation significantly increased levels of key cytokines FGF, IL-5, IL-6, IL-12 (p40/p70), KC, MCP-1 and VEGF in BAL fluid when compared with non-irradiated controls. | Pietrofesa et al. [174]|
| CXCR4, CXCL12                      | C57Bl/6| Thorax, right lung | X-ray/20 Gy      | 0, 1, 3, 7, 14 and 28 d | ELISA, RT-PCR, lung tissue | BALF serum, lung tissue | Both serum and BALF CXCL12 concentrations showed fluctuating but increasing trends over the 28 d. Lung CXCR4 and CXCL12 mRNA levels also fluctuated, but showed a general increasing trend, with the peak at Day 28 post-irradiation. | Shu et al. [175]|
| SDF-1α, IL-1β, TNF-α, IL-6, IL-10, TGF-β1 | C57Bl/6| Thorax       | 60Co/14 Gy    | 7, 30 and 120 d  | ELISA, BALF      | BALF, plasma | Two peaks in TGF-β1 production in the BALF: at 7–14 and 120 d after the irradiation. Plasma TGF-β1 also displayed two peaks. The irradiation induced increased levels of IL-1β, TNF-α, IL-10 and IL-6 in plasma at 30 d. | Xue et al. [176]|
| IL-1α, IL-1β, IL-6, TNFα, TGF-β    | C57Bl/6| Thorax, TNFα−/−, TNFR1−/−, TNFR2−/− | X-ray/10 Gy   | 12 and 24 w      | RT-PCR, Lung tissue |           | IL-6 had higher levels of mRNA expression in the C57-WT mice relative to the other three groups of mice. There was increased mRNA expression at 12 and 24 wks after irradiation in the C57-WT mice. The TNFα−/− mice showed higher IL-6 protein levels than in the controls, and a smaller relative increase following irradiation. TNF-α mRNA expression in the C57-WT, TNFR1−/−, and TNFR2−/− mice increased following irradiation. TGF-β mRNA expression in the lung tissue showed an increase | Zaidi et al. [177]|
following irradiation at all the time points for all the groups of mice.

The most outstanding changes in expression of inflammatory factors in WT animals were due to radiation inductions of RNA expressing IL-6 ($\times 10^{-45}$), MCP-1 ($\times 7$) and KC ($\times 10$). Increases were most dramatic at 4 w, and generally subsided by 6 w. In contrast, the induced expressions of IL-6, MCP-1 and KC were ameliorated in irradiated Myd88$^{-/-}$ animals. The mRNA level of TGF-$\beta$ was modestly induced in irradiated WT mice at 10 d and 4 w. The expression of IL-1$\beta$ was elevated primarily by 7–10 d after irradiation in Myd88 mice, but not in WT mice. Serum levels of IL-5, IL-10 and IL-17 were more abundant in Myd88 compared with WT mice.

A significant decrease in the levels of IL-1$\beta$, IL-2, IL-4, MIG and MIP-1$\alpha$ was detected in irradiated animals. Only IL-6, IL-12 (p40/p70) and VEGF were evaluated at time point 4 mo.

Concentrations of IL-1$\beta$, TNF-$\alpha$ and TGF-$\beta$1 were elevated in the plasma after irradiation. Radiation induced mRNA expression of TGF-$\beta$1 and CTGF in the lung tissue by a significant amount.

The responses of A/J and C3H mice were more similar to each other than to B6 mice; 4042 genes (65% of the C3H response, 54% of A/J) were differentially expressed in irradiated mice.
| Evaluated cytokines | Models | Types of RT | Sources/doses | Time intervals | Methods | Materials | Main results/trends in irradiated groups only are listed: | Authors |
|---------------------|--------|-------------|---------------|----------------|---------|-----------|------------------------------------------------|---------|
| IL-6                | BALB/cAnNCrj | Thorax | X-ray/21 Gy | 50, 100, and 150 d | ELISA | Plasma | A significant increase in IL-6 was observed at 50 and 100 d after irradiation, compared with the control (non-irradiated) mice. | Ogata et al. [187] |
| IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17, IFN-γ, IP-10, G-CSF, GM-CSF, TNF-α, KC, MCP-1, MIP-1α, RANTES | C3H, C57BL/6 | Thorax | X-ray/12Gy | 3, 6, 12, 24 h and 1 w | Lincoplex kit | Lung lysates: GM-CSF, G-CSF, IL-6, IL-10(C57), IL-9, IP-10, KC, MCP-1, MIP-1α and RANTES. In most cases, cytokine levels peaked 3–6 h earlier in C57BL/6 mice. BALF: G-CSF, IL-6 and KC cytokines in both strains. The peak levels were similar for G-CSF—max ↑ 6 h in C57BL/6 mice and at 12 h in C3H. IL-6 was greatly increased in the C57 mice, while there was a minimal ↑ in the C3H mice. KC levels were higher in C3H mice. Serum: G-CSF, GM-CSF, IP-10, KC, IL-6, MCP-1, IL-1α, IL-17, IL-13, MIP-1α and IL-12(p70). Correlation in cytokine KC between species and materials. | Ao et al. [76] |
| TGF-β1              | WT, Alb/TGF-β1 | Thorax | X-ray/12 Gy | 4 and 8 w | ELISA, WB | Plasma, lung tissue | The mean TGF-β1 plasma level of the transgenic mice was twice as much as that in WT mice. | Yang et al. [188] |
| TGF-β1              | C57BL/6, C3H/J | Thorax | 60Co/15 Gy | 9 d | RT-PCR | Lung tissue | In the fibrosing strain C57Bl/6 after 9 d, the mRNA TGF-β1 levels in irradiated lungs increased (×2.4); in the non-fibrosing murine strain C3H/J, the mRNA TGF-β1 levels slightly increased (×1.4) compared with non-irradiated controls. | Matej et al. [189] |
| TGF-β1              | WT, Alb/TGF-β1 | Thorax | X-ray/12 Gy | 8 w | WB | Lung tissue | TGF-β1 level was increased (×3) in lung protein homogenates from transgenic mice as compared with control mice. Protein expression of TGF-β1 in lung homogenates from TG mice was ~3 times that from WT mice. Radiation alone induced only a minor induction of protein expression in the lung tissue at the analyzed time points. | Yang et al. [190] |
| IL-6, TGF-β1        | C57BL/6 | Thorax | γ-ray/20 Gy | 16 w | RT-PCR | Lung tissue | There were elevated mRNA levels of IL-6 (×15) and TGF-β1 (×6) in lung tissues at 16 w after 20 Gy irradiation, compared with controls. | Tabata et al. [191] |
| IL-6                | C57BL/6 | Thorax | γ-ray/20 Gy | 2 mo | RT-PCR | Lung tissue | At 2 mo after irradiation, IL-6 mRNA was increased (×20) in the whole lung of mice compared with controls. | Tabata et al. [192] |
| Cytokine | Mouse Strain | Organ | Exposure | Time Points | Assay | Tissue | Notes |
|----------|--------------|-------|----------|-------------|-------|--------|-------|
| TGF-β | C57BL/6 | Thorax | X-ray/13.5 Gy | 24, 48, 72 h, 1, 2 and 3 w | RT-PCR | Lung tissue | The TGF-β level showed an early increase at 48 h post-irradiation and remained elevated until 1 w. Machtay et al. [193] |
| TNF-α, IL-1α, IL-6 | C57BL/6 | Thorax | X-ray/12 Gy | 0, 5, 1, 3, 6, 12, 24, 48, 72 h, 1, 2, 4, 8, 16 and 24 w | RT-PCR, IHC (protein levels) | Lung tissue | TNF-α, IL-1α and IL-6 was detectable in the lung tissue within the first hours after thoracic irradiation, and there were significant upregulations of TNF-α at 1 h (mRNA) and at 6 h (protein), and of IL-1α and IL-6 at 6 h (mRNA) and at 12 h (protein). During the stage of acute pneumonitis, there was production of TNF-α (the maximal value at 4 w), and of IL-1α and IL-6 (both of which peaked at 8 w). Rübe et al. [194] |
| IL-1α, IL-1β, TNF-α, IL-2, IL-3, IL-4, IL-5, IL-6, IFN-γ | C3H/HeJ, C57BL/6J | Thorax | X-ray/12, 20 Gy | 1, 2, 3, 4, 5 and 6 mo | RNA assay | Lung tissue, BALF | In lung tissue after 20 Gy radiation, expression of mRNA for IL-1α, IL-1β and TNF-α increased in a bimodal fashion with time, peaking at 1–2 and 5–6 mo. In BALF, only IL-1α and IL-1β were increased. Similar, but less marked, changes were seen in both lung tissues and BAL cells after 12 Gy irradiation. Chiang et al. [195] |
| TGF-β, TNF-α, IL-1α/β, IL-6 | BALB/c | Thorax | X-ray/20 Gy | 6 and 24 h | RT-PCR, RNA assay | Lung tissue | Irradiation (20 Gy) induced a significant increase in TNF-α, IL-6, IL-1α/β and TGF-β mRNA 6 h and 24 h after irradiation. Chen et al. [196] |
| TNF-α, IL-1α, IL-6 | C57BL/6J | Thorax | X-ray/12 Gy | 1 h, 1, 3 d, 1, 2 and 4 w | PCR | Lung tissue | TNF-α was elevated as early as 1 h, subsequently returning to baseline by 1 d. TNF-α expression reached significant values at 3 d, 1, 2 and 4 w, with the highest value being observed at 2 w. There was increased IL-1α mRNA expression in the lung tissue at 1 h and 1 d, with the higher value at 1 d. IL-6 release in the lung tissue was appreciable within the first day. Rübe et al. [197] |
| TNF-α, IL-1α, IL-6 | C57BL/6J | Thorax | X-ray/12 Gy | 0.5, 1, 3, 6, 12, 24, 48, 72 h, 1, 2, 4, 8, 16 and 24 w | RT-PCR | Lung tissue | Initial increases were at 1 h for TNF-α and at 6 h for IL-1α and IL-6 post irradiation, then expression of these pro-inflammatory cytokines returned to basal levels (48 h–2 w). During the pneumonic phase, TNF-α, IL-1α and IL-6 were significantly elevated and revealed their maximum at 8 w. Rübe et al. [198] |
| IL-1α, IL-1β, TNF-α, TNF-β, IL-2, IL-3, IL-4, IL-5, IL-6, IFN-γ | C3H/HeN | Thorax | X-ray/6, 12, 20 Gy | 30, 60, 90 and 120 d | RNA assay | Lung tissue, BALF | The main cytokine genes expressed in response to radiation were IL-1β, IL-1α and TNF-α, in decreasing order. The maximum expression in lung tissue appeared at 3–4 mo, while the maximum expression in BALF appeared at 1–2 mo. Sublethal doses of irradiation (6 and 12 Gy) showed similar, but less marked, changes. Hong et al. [199] |
| TNF-α | C57BL/6J | Thorax | X-ray/12 Gy | 1, 24, 72 h, 1, 2, 4, 8, 16 and 24 w | RT-PCR | Lung tissue | Radiation-induced TNF-α release in the lung tissue within the first hour, and this was subsequently decreased to basal levels during the latent period (24 h–1 w). During the pneumonic phase, TNF-α release was significantly increased and reached maximal values at 8 w. Rübe et al. [200] |
| Chemokine and their receptors gene profiling | C57BL/6, C3H/HeJ | Thorax | 137Cs/12.5 Gy | 182 d (26 w) | Microarray, RNA assay | Lung tissue | Chemokines from the CC family: C57BL/6: ↑ MCP-1, RANTES, C10, MCP-3, MIP-β; C3H/HeJ: none. Chemokines from the CX family: C57BL/6: ↑ IF10, BLC; C3H/HeJ: only ↑ SDF-1. Receptors: C57BL/6: ↑ Ccr1, Ccr2, Ccr5, Ccr6, C3H/HeJ: only ↑ Ccr1. Johnston et al. [301] |
| Evaluated cytokines | Models | Types of RT | Sources/doses | Time intervals | Methods | Materials | Main results/trends in irradiated groups only are listed: | Authors |
|---------------------|--------|-------------|---------------|---------------|---------|-----------|-------------------------------------------------|--------|
| TNF-α, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IFNγ | C3H/HeJ | Thorax | X-ray/20 Gy | 6, 12, 16, 24, 36, 48, 72 h, 7, and 14 d | RNA assay | Lung tissue | The level of IL-1β mRNA and, to a lesser extent TNF-α were significantly elevated at 6–12 h. Resurgence of IL-1β after 48 h, which persisted from 1 to 2 w. TNF-α was barely increased over the first week but was elevated at 2 w. IL-2, IL-3, IL-4, IL-5, IL-6 and IFN-γ were barely detectable. | Hong et al. [202] |
| TGF-β | C57BL/6J | Thorax | X-ray/6, 12 Gy | 1, 3, 6, 12, 24, 48, 72 h, 1, 2, 4, 8, 16, and 24 w | PCR | Lung tissue | Thoracic irradiation with the dose of 12 Gy induced TGF-β release in lung tissue within the first hours (1–6 h) and reached a significant increase after 12 h, followed by a decline to basal levels. During the pneumonic phase, TGF-β release reached maximal values at 2–4 w, followed by declined expression at the next time interval. After a radiation dose of 6 Gy, the lung tissue revealed only a minor radiation-mediated TGF-β mRNA response, with modest upregulation at 24–48 h. | Rübe et al. [203] |
| TNF-α, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IFNγ | C3H/HeJ, C57BL/6J | Thorax | X-ray/0, 2, 4, 8, 16 and 20 Gy | 1, 2, 4, 8, 16, 24 h + 6 h (dose-dep. expr.) | RNA assay | Lung tissue | Level of IL-1β in C3H/HeJ mice increased 1 h after 1 Gy irradiation (×2.6) and 8–20 Gy (×2–2.5), but not after 4 Gy—bimodal response. In C57BL/6J mice there was a greater response (×6.5–8.3) 1 h after 20 Gy. TNF-α and IL-1γ were also changed. All three cytokines increased as early as 1 h after 20 Gy, an increase that continued up to 16 h and subsided at 24 h. | Hong et al. [204] |
| IL-1, TNF-α, TGF-β1, TGF-β2 and TGF-β3 | C57BL/6J | Thorax | X-ray/20 Gy | 1, 4, 7, 14, 80, 100 and 120 d | RNA assay | Lung tissue | Significant increase in mRNA levels for IL-1 at 1 d, further elevated at 7 d after irradiation, followed by decrease after day without rising again. Irradiated mice demonstrated an increase in TNF-α mRNA levels detectable at Day 4 and significantly increasing at Days 80 and 100. Significant increase in TGF-β at 1 d after irradiation followed by a decrease to levels similar to that prior to irradiation. A late increase in total TGFβ levels at 120 d (elevation of TGF-β1 and TGF-β2). | Epperly et al. [205] |
| Ltn, RANTES, eotaxin, MIP-1α, MIP-1β, MIP-2, IP-10, MCP-1 | C3H/HeJ, C57BL/6J | Thorax | 137Cs/5 and 12.5 Gy | 8 and 26 w | RNA assay | Lung tissue | Increased levels of eotaxin, MIP-1α, MIP-1β and MIP-2 (×2), RANTES and Ltn (×2.5–3.5) were detected in both strains at the 8 w time point. By 26 w post-irradiation, all cytokines had returned to control levels in C3H/HeJ mice. In C57BL/6 mice, RANTES and Ltn remained elevated, and IP-10 and MCP-1 were increased (×4.5). | Johnston et al. [122] |
| IL-1, TGF-β, TNF-α | C57BL/6J | Thorax | X-ray/18–20 Gy | 1, 4, 14 and 28 d | RT-PCR | Lung tissue | There were increased levels of mRNA for the inflammatory mediators IL-1, TGF-β and TNF-α in the lungs at 7 and 14 d after irradiation. | Epperly et al. [206] |
| TNF-α | BALB/c | Thorax | X-ray/25 Gy | 6, 8 and 10 d | RNA assay | Lung tissue | 25 Gy of thoracic irradiation was a potent stimulator of TNF-α mRNA expression at all three time points. | Redlich et al. [207] |
| IL-1α, IL-1β, TNF-α | C3H/HeJ, C57BL/6J | Thorax | $^{137}$Cs/S and 12.5 Gy | RNA assay | Lung tissue | In the C57BL/6 mice, after 5 Gy, TNF-α levels were increased on Days 1 ($\times 2.7$), 7 ($\times 4.8$) and 14 ($\times 3.8$), but were significantly decreased by 112 d post-irradiation. After a dose of 12.5 Gy, mRNA levels were increased on Day 14 ($\times 2.8$), then decreased at 112 d (similar in C3H). IL-1α mRNA in the C57 mice was increased on Days 112 and 182 d after 5 Gy and after 12.5 Gy increases after 56 d (similar in C3H). IL-1β levels increased at Day 7 ($\times 3$), but decreased on Days 1 and 112. | Johnston et al. [92] |
| --- | --- | --- | --- | --- | --- | --- | --- |
| TGF-β1, -β3 | C3H/HeJ, C57BL/6J | Thorax | $^{137}$Cs/S and 12.5 Gy | RNA assay | Lung tissue | In the C57Bl/6 mice, TGF-β1 increased ($\times 2$) for both doses at 8 w. The mRNA of TGF-β3 was altered slightly after 12.5 Gy at this time point. Levels of TGF-β1 and -β3 were decreased in both strains at 16 w after irradiation, and only in C57 mice treated with 12.5 Gy at 26 w. | Johnston et al. [208] |
| IL-1α, IL-1β, TGF-β1, -β3 PDGF | C57BL/6 | Thorax | $^{137}$Cs/S and 12.5 Gy | RNA assay | Lung tissue | IL-1α was elevated ($\times 2$) at 2 w, returned to the normal baseline, then increased at 8–26 w. An immediate fall in TGF-β1, TGF-β3 directly after irradiation was followed by increases in both seen at 2 w (larger increase in TGF-β3). This was followed by a general decline in TGF-β3, while the TGF-β1 level was continuing to increase ($\times 2$) at 8 w. Both returned to baseline at 16 w, but increased at 26 w. | Rubin et al. [33] |
| TGF-β1, 2, 3 | C57Bl/6 | Thorax | $^{137}$Cs/S and 12.5 Gy | RNA assay | Lung tissue | TGF-β1, TGF-β2 and TGF-β3 were altered after irradiation, even at 5 Gy. Dramatic alterations in mRNA occurred as a function of dose, but there was no evident dose-response correlation. | Finkelstein et al. [209] |

IL-α = interleukin-α, TGF-β1, 2 and 3 = transforming growth factor β1, 2 and 3, PDGF = platelet-derived growth factor, TNF-α = tumor necrosis factor alpha, Ltn = lymphotaxin (XCL1), IFN-γ = interferon γ, IP-10 = interferon gamma–induced protein-10 (CXCL10), G-CSF = granulocyte colony-stimulating factor, GM-CSF = granulocyte-macrophage colony-stimulating factor, CTGF = connective tissue growth factor, SCF = stem cell factor, TGF-β = basic fibroblast growth factor, VEGF = vascular endothelial growth factor, MIF = macrophage migration inhibitory factor, MIG = monokine induced by gamma interferon (CXCL8), SDF-1α = stromal cell-derived factor 1, GRO1 (CXCL1) = growth-regulated oncogene-1, TSLP = thymic stromal lymphopoietin, TCA-3 (CCL1) = TARC thymus and activation-regulated chemokine (CCL17), MDC = macrophage-derived chemokine (CCL22), BALF = bronchoalveolar lavage fluid, ELISA = enzyme-linked immunosorbent assay, IHC = immunohistochemistry, WB = western blotting, (RT-) PCR = (reverse transcription)-polymerase chain reaction, Gy = gray, d = days, w = weeks, mo = months, y = years.
Table 2. Summary of published studies analyzing radiation-induced cytokine expressions from ‘rat models’ after total body or thorax irradiation, including types of ionizing radiation sources, time intervals, and methods and materials used for cytokine quantification after irradiation

| Evaluated cytokines | Models | Types of RT | Sources/doses | Time intervals | Methods | Materials | Main results/trends in irradiated groups only are listed: | Authors |
|---------------------|--------|-------------|---------------|----------------|---------|-----------|----------------------------------------------------------|---------|
| TNF-α, IL-1β        | Wistar | TBI         | X-ray/6 Gy    | 6 and 72 h     | ELISA   | Serum     | Animals in the irradiated group had significantly increased serum TNF-α and IL-1β at both 6 and 72 h post-irradiation compared with control animals. | Bakkal et al. [210] |
| IL-6, TGF-β1, TNF-α | Wistar-Hannover | TBI | 60Co/3,5 Gy | 1 and 7 d | WB | Lung tissue | For all cytokines studied, no significant difference occurred between the control and radiation-treated groups in the first day post-irradiation. The expression of TNF-α, IL-6 and TGF-β1 showed significant elevation at 7 post-irradiation. | Bianchi et al. [211] |
| TNF-α               | SD     | TBI         | X-ray/8 Gy    | 6 and 72 h     | IRMA    | Serum     | TNF-α levels were significantly increased at both intervals compared with those in the control group. | Sener et al. [212, 213] |
| HGF                 | Wistar | TBI         | X-ray/6 Gy    | 6, 12, 24, 48 h, 1, 2 w and 1 mo | ELISA, RNA assay | Liver, kidney, lung tissue | Although slight elevation of HGF protein levels was found 1 d after TBI, maximal concentration peaks were observed 2 w after TBI in all tested tissues. | Yamazaki et al. [214] |
| IL-6, IL-1β, TNF-α  | Wistar | Thorax      | X-ray/25 Gy   | 1, 3, 6, 12, 24 and 48 h | RT-PCR | Lung tissue | IL-6 mRNA expression was significantly elevated at 3 h (×60). IL-1β was significantly elevated at 1 h, with maximum level at 3 h (×12). TNF-α gene expression reached a maximum at 1 h (×30) until 3 h, and a subsequent decrease. | Sultan et al. [215] |
| TGF-β1, TNF-α, IL-6 | SD     | Right lung  | X-ray/15 Gy   | 4, 8, 12 and 16 w | ELISA | BALF | Significant increase of all cytokines (TGF-β1, TNF-α, IL-6) at all time intervals. Maximal concentration peaks were observed 4 w after irradiation. | Yu et al. [216] |
| IL-1β, IL-2, IL-6, IL-10, IL-17A, IL-18, IP-10, GRO/KC, RANTES | Wistar | Right lung  | X-ray/6 Gy × 5 | 7, 15 and 30 d | Human Miliplex | Remarkable elevation of IL-1β at 7 and 15 d post-irradiation. | Zhang et al. [217] |
| TGF-β1, IL-6, TNF-α | SD     | Thorax      | 60Co/22 Gy    | 4 mo           | ELISA   | Serum     | Radiation induced the production of TGF-β1 (×2,5), IL-6 (×2) and TNF-α (×6) compared with the control group. | Zhou et al. [218] |
| Cytokine(s) | Strain | Treatment | Time Points | Assay | Tissue | References |
|------------|--------|-----------|-------------|-------|--------|------------|
| IL-1α, IL-1β | SD     | Right lung | X-ray/15 Gy [tomotherapy and CCRT (static/helical)] | 1, 2 and 3 w | RT-PCR Plasma | No significant differences in the levels of expression of IL-1α or IL-1β were found between the groups at Weeks 1 or 2. There were, however, significant differences in the expression of IL-1α and IL-1β between the tomotherapy and CCRT groups at 3 w. | Zhang et al. [219] |
| HGF, TGF-β1, IL-10, TNF-α | SD     | Right lung | X-ray/15 Gy | 3 d, 1, 2, 4, 12 and 24 w | ELISA Serum, BALF | The secretion of TGF-β1 in serum exhibited two peaks, with the highest concentrations at 24 w post-irradiation. TNF-α in serum was increased at intervals of 1 w and 4 w after irradiation. Serum levels of HGF in the irradiated group reached a maximal concentration at 3 d, and then decreased. Concentrations of cytokines in BALF at intervals of 4 and 24 w showed decreasing levels of HGF and TNF-α; TGF-β1 was increased; IL-10 levels did not significantly differ between the groups. | Dong et al. [220] |
| IL-1, IL-6, IL-10, TNF-α, TGF-β1, CTGF, HGF | SD     | Right lung | X-ray/15 Gy | 1, 3, 7, 14 and 28 d | ELISA, RT-PCR Serum, lung tissue | Thorax irradiation resulted in the production of IL-1, IL-6 and TNF-α on Day 14 in the serum, and TGF-β1 had increased production from Days 7 to 14. IL-10 concentration decreased at 3 d. mRNA expression of TGF-β1 was significantly upregulated from Day 7 onwards in the irradiated group. | Jiang et al. [221] |
| TGF-β1, IL-6, IL-10, TNF-α | SD     | Thorax | 60Co/22 Gy | 10, 20, 40 or 80 d | ELISA + CBA Serum | Radiation caused a significant increase in serum levels of IL-6, IL-10, TNF-α and TGF-β1 at all time intervals after irradiation. | Liu et al. [222] |
| TGF-β1, IL-6, IL-10, TNF-α | SD     | Thorax | 60Co/22 Gy | 15, 30, 60 and 120 d | CBA + ELISA (TGF-b1) Serum | Measured serum levels of TGF-β1, IL-6, IL-10 and TNF-α were significantly higher in radiation-treated animals at all time intervals. | You et al. [223] |
| IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, IFN-γ, TNF-α | WAG/RijCmcr | Thorax | X-ray/10, 15 Gy | 8 w | Bio-Rad plex Plasma | After irradiation with 10 Gy, the levels of all the cytokines did not differ from those of controls. With 15 Gy, each of the cytokines except for TNF-α showed a decreasing trend compared with controls; however, none of these differences were significant. | Szabo et al. [224] |
| TGF-β | SD     | Right lung | X-ray/20 Gy | 3, 7, 14, 28 and 56 d | ELISA BALF | TGF-β production was significantly higher in the irradiated right lung compared with the non-irradiated left lung and the control group at 56 d after irradiation; however, TGF-β levels in BALF from | Park et al. [225] |
| Evaluated cytokines | Models | Types of RT | Sources/doses | Time intervals | Methods | Materials | Main results/trends in irradiated groups only are listed: | Authors |
|---------------------|--------|-------------|---------------|---------------|---------|-----------|-------------------------------------------------|---------|
| TGF-β1, TNF-α, IL-6 | SD     | Thorax      | X-ray/20 Gy   | 2 h, 4, 8, 16 and 24 w | ELISA, WB | BALF, lung tissue | Irradiated animals had significantly increased serum TGF-β1 and IL-6, and BALF TGF-β1 levels, at 2 h. These levels peaked at 4 w in all groups. Similar results were obtained for TNF-α by WB after irradiation. | Bao et al. [226] |
| IL-10               | Fischer | Right lung  | X-ray/20 Gy   | 4–12 w         | WB      | Lung tissue | IL-10 was induced after irradiation of the rat lung with 20 Gy at all time intervals. | Haase et al. [227] |
| IL-1α, IL-1β, IL-6, TNF-α, TGF-β | SD | Lower lung | 60Co/10 Gy | 2, 3, 5, 7, 12, 18, 24, 48 h, 2, 5, 7, 9, 12 d, 2, 5, 8 and 16 w | RT-PCR | Lung tissue | Following 10 Gy of lower-lung irradiation, there was similar (increased) expression of these cytokines in both the upper (unirradiated) and lower (irradiated) lobes. IL-1α and TNF-α levels did not differ significantly between the lobes, but TNF-α greatly increased at 4–16 w after irradiation. IL-1β showed the greatest changes in expression, especially during the first 48 h, between regions and lobes. Expression of IL-6 varied in lobes at the control time point, 2 h, 12 h, 7 d and 16 w. TGF-β levels were significantly higher in the lower, irradiated region, with significant differences in expression at 2 h, 12 h, 18 h and 48 h and between quadrants at 5 h, 12 h and 18 h. After 48 h, there was no difference between lobes, and a peak was observed in both regions at 7 d. | Carveley et al. [228] |
| TGF-β1              | Fischer-344 | Right lung  | X-ray/30 Gy   | 4 w            | ELISA   | Lung tissue | Quantification of the lung tissue level of TGF-β1 revealed that the ratio of active: total TGF-β1 was significantly higher for the radiation-alone group. | Rabbani et al. [229] |
| IL-6                | Fischer | Right lung  | X-ray/20 Gy   | 3 mo           | RT-PCR  | Lung tissue | It was found that IL-6 mRNA is induced (x12) by radiation after 3 mo. | Haase et al. [230] |
| TGF-β               | Fischer-344 | Right lung  | X-ray/28 Gy   | 2 w–6 mo       | ELISA   | Plasma      | Elevation in the plasma TGF-β level, starting 2 w after irradiation with the peak at 12 w. | Vujaskovic et al. |
| **HGF** | Wistar  | Thorax  | X-ray/12 Gy | 1, 2, 3 and 6 mo | ELISA   | Lung tissue, plasma | In lung tissue, a decrease in the HGF level was observed 1 mo after irradiation, but an increase was observed 2 and 3 mo after irradiation. HGF remained below detected levels throughout the examined period of examination in the plasma. |
|---------|---------|---------|-------------|----------------|---------|---------------------|---------------------------------------------------------------|
| **TGF-β** | Wistar Hsd/cpb | Right lung | X-ray/18 Gy | 1, 4, 7, 10 d and 2–34 w | PAI-1 bioassay | Plasma | No significant changes in levels of TGF-β were observed at Days 1, 4, 7, 10 or 14 after irradiation. The first increase was observed at 16 w, with a maximum at 20 w. |
| **IL-4** | Fischer-344 | Right lung | X-ray/20 Gy | 1, 7, 14, 21, 28, 56 and 84 d | RNA assay + WB | Lung tissue | IL-4 mRNA was upregulated in the irradiated lung tissue. The amount of IL-4 protein varied at different time points after irradiation. The highest protein concentration was found in the lung at Day 84. IL-4 mRNA levels and the IL-4 protein levels did not closely correlate with the late stages of the development of pulmonary fibrosis. |
| **TGF-β** | SD | Thorax, right lung | X-ray/15 or 30 Gy | 1–16 w | ELISA, BALF, RNA assay | Lung tissue | Analysis of the BALF showed an increase in TGF-β between 3 and 8 w, peaking at 3-6 w for hemithorax irradiation by dose 30 Gy. The kinetics for irradiation15 Gy–whole thorax was similar kinetics but to a lesser extent (ELISA). The upregulation of TGF-β expression was dissemble at interval 1–4 w after whole-lung irradiation. |

IL-α = interleukin-α, TGF-β1 = transforming growth factor β1, PDGF = platelet-derived growth factor, TNF-α = tumor necrosis factor-α, HGF = hepatocyte growth factor, CTGF = connective tissue growth factor, IFN-γ = interferon γ, IP-10 = interferon γ-induced protein 10 (CXCL10), KC = keratinocyte chemoattractant (CXCL1), MCP-1 = monocyte chemoattractant protein-1 (CCL2), RANTES = regulated on activation normal T cell expressed and secreted (CCL5), GRO1 (CXCL1) = growth-regulated oncogene-1, CCRT = computer controlled radiation therapy, SD = Sprague–Dawley rat, BALF = bronchoalveolar lavage fluid, ELISA = enzyme-linked immune sorbent assay, IHC = immunohistochemistry, WB = western blotting, (RT-) PCR = (reverse transcription)–polymerase chain reaction, Gy = gray, d = days, w = weeks, mo = months, y = years.
Table 3. Summary of published studies analyzing cytokine expressions and in cancer patients undergoing radiotherapy of thoracic region, including type of radiotherapy, total received dose, time intervals, methods and materials used for cytokine quantification after irradiation.

| Evaluated cytokines | Patient descriptions | Types of RT | Total dose (range) | Time intervals | Methods | Materials | Main results/trends | Authors |
|---------------------|----------------------|-------------|--------------------|----------------|---------|-----------|---------------------|---------|
| EGF, eotaxin, fractalkine, G-CSF, GM-CSF, IFN-γ, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17, IL-1RA, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IP-10, MCP-1, MIP-1α, MIP-1β, sCD40L, TGF-α, TNFα, VEGF, TGF-β1 | 142 NSCLC patients | 3D-CRT | 70 Gy (44–87.9) | Pre RT, 2 w, 4 w during RT | Milliplex, ELISA | Plasma | Lower pre-treatment level of IL-8 and higher 2 w : pre RT ratio of TGF-β1 were associated with higher risk of RILT2. | Wang et al. [130] |
| CCL18 | 67 patients with RT for thoracic malign. | IMRT, 3D-CRT | 53 Gy (30–76) | Pre RT, during and end RT, 1, 3 mo after RT | ELISA | Plasma | An association between CCL18 level and the development of RILT was not demonstrated. | Gkkika et al. [236] |
| EGF, eotaxin, fractalkine, G-CSF, GM-CSF, IFN-γ, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17, IL-1RA, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IP-10, MCP-1, MIP-1α, MIP-1β, sCD40L, TGF-α, TNFα, VEGF, TGF-β1 | 125 NSCLC patients | 3D-CRT, 4 × IMRT | 34–87.9 Gy (40.8–115.5) | Pre RT, weekly during RT, 1 mo after, then every 3 mo/1 y and then 6 mo/y | Milliplex, ELISA | Plasma | High levels of 13 cytokines (IL-10, IL-18, IL-1β, IL-5, IL-7, IL-12p40, IL-12p70, IL15, TGF-α, G-CSF, MIP-1β, IL1RA and fractalkine) were associated with a decreased effect of dose on risk; 4 (MCP-1, SCD40L, IL-8 and IL-1α) were associated with an increased effect of dose on risk. | Hawkins et al. [123] |
| EGF, eotaxin, fractalkine, G-CSF, GM-CSF, IFN-γ, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17, IL-1RA, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IP-10, MCP-1, MIP-1α, MIP-1β, sCD40L, TGF-α, TNFα, VEGF, TGF-β1 | 141 NSCLC patients | SBRT/ChRT/CFRT | 60–74 Gy | Pre RT, and during RT | Milliplex, ELISA | Plasma | Variations in cytokine levels at baseline and during RT: fractalkine, GM-CSF, IL-10, IL-12p40, IFN-γ, IP-10, MIP-1β, sCD40L and VEGF. | Ellsworth et al. [237] |
| Eotaxin, IFNγ, IL-6, IL-10, IL-11, IL-22, IL-3, IL-33, IP-10, MCP-1, MCP-3, MDC, MIP-1α, MIP-1β, MIP-3α, MIP-3β, TGF-β1, TGF-β2, TGF-β3, TNF-α, VEGF | 16 NSCLC patients | 3D-CRT | 60 Gy | Pre RT, at 1 h, 24 h and 4 w during RT and 12 w after RT | ELISA, FC beads array | Plasma | The plasma levels of eotaxin, IL6, IP10, MCP1, MCP3, MDC, MIP1α, MIP1β and VEGF varied significantly during treatment. Chemoradiotherapy induced changes in 8 cytokines, and radiotherapy alone induced changes in 4 cytokines within this time window. | Siva et al. [238] |
| IL-6, TNF-α | 26 patients NSCLC | 3D-CRT | 54–74 Gy | Pre RT, 2 and 4 w | ELISA Serum | The IL-6 and TNF-α baseline levels were significantly higher in NSCLC patients. No significant differences in the levels before and during RT were observed. | Chalubinska-Fendler et al. [239] |
| IL-1, IL-1Ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17A, EGF, FGF-2, IFN-γ, MIP-1α, MIP-1β, TGF-α, TNF-α, VEGF | 15 early-stage NSCLC/13 advanced NSCLC | SBRT/IMRT Early: 52 Gy/advanced: 60 Gy | SBRT: first and last day of RT, and 45 d after RT. IMRT: 1 d, 2 w and 4 w during RT, last day of RT, and 45 d after RT | Miliplex Serum | NSCLC patients had elevated levels of IL-1Ra, IL-12, IL-17, IFN-γ and FGF-2, and significantly lower EGF, MIP-1β, TGF-α, TNF-α and VEGF. No difference in baseline levels with respect to radiation approaches (except for MIP-1α). For SBRT patients, a mean reduction of the IL-10 and IL-17 plasma level was documented during treatment. IMRT patients had significant plasma level reduction between 4 w and the last day of RT for cytokines IL-1, IL-1Ra, IL-2, IL-12, FGF-2, MIP-1α, MIP-1β and TGF-α, TNF-α and VEGF. | Trovo et al. [240] |
| TGF-β1 | 112 patients undergoing trimodality treatment | IMRT 40 Gy | Before and after CCRT | ELISA Serum | The pre-CCRT and post-CCRT level of TGF-β1 decline was 27.4%. A lowered level of TGF-β1 showed a borderline association with a pathologic response being encountered as a postoperative complication. | Lu et al. [165] |
| IL-6, TNF-α | 120 lung cancer patients | CRT 60–66 Gy | Before and after treatment | ELISA NM | Reduction in both cytokines after treatment. | Ma et al. [241] |
| TGF-β1, IL-1β | 63 patients with esophageal cancer | 3DCRT 50–70 Gy | Before RT, during RT and at 1 d, 1 mo and 3 mo after RT | ELISA Plasma | TGF-β1 levels were elevated and became significant after 40 Gy irradiation in the patients that had RP, compared with levels in the patients who did not have RP. The plasma IL-1β levels were not changed. | Li et al. [242] |
| Eotaxin, IFNγ, IL-6, IL-10, IL-11, IL-22, IL-3, IL-33, IP-10, MCP-1, MCP-3, MDC, MIP-1α, MIP-1β, MIP-3α, MIP-3β, TGF-β1, TGF-β2, TGF-β3, TNF-α, VEGF | 12 NSCLC patients | 3D-CRT 60 Gy | 1 h and 24 h after 1st RT, 4 w during RT and 12 after RT | ELISA Plasma | Early changes in levels of IP-10, MCP-1, eotaxin, IL-6 and TIMP-1 were associated with higher grade toxicity. Levels of eotaxin, IL-33, IL-6, MDC, MIP-1α and VEGF in those patients receiving chemoRT differed from the levels of those receiving RT alone. Concentrations of IP-10, MCP-1 (1 h:↓), MCP-3, MIP-1β, TIMP-1 and | Siva et al. [21] |

Continued
| Evaluated cytokines | Patient descriptions | Types of RT | Total dose (range) | Time intervals | Methods | Materials | Main results/trends | Authors |
|---------------------|----------------------|-------------|-------------------|----------------|---------|----------|-------------------|---------|
| VEGF, TGF-β1        | 58 patients with unresectable NSCL | 3D-CRT      | 60 – 68 Gy        | Before and after RT | ELISA   | Serum    | TNF-α were not dependent upon the treatment group and varied differently across the time points. | Fu et al. [243] |
| IL-2, IFN-γ         | 63 patients with esophageal carcinoma | 3D-CRT      | 60-66 Gy          | Once per week during RT | ELISA   | Serum    | No significant differences in ratio pre-RT/ during RT IL-2 or IFN-γ levels were found. In the effective response group, serum concentrations increased with the number of radiotherapy fractions, reaching a maximum after ~2–3 w. IL-2 and IFN-γ levels are associated with an increased probability of acute hematologic toxicity, further, changes in IFN-γ concentrations are associated with an increased probability of acute organ toxicity of the esophagus, lung or skin. | Ma et al. [244] |
| TGF-β1              | 84 patients with RT for Stage III disease | 3D-CRT      | 60–72 Gy          | Before, every 2 w during and at 4 w after RT | ELISA   | Plasma   | Early variations in TGF-β1 levels during 3D-CRT were significantly associated with the risk of RP. Variations in circulating TGF-β1 may serve as independent predictive factors for RP. | Liu et al. [245] |
| TGF-β1              | 76 NSCLC patients     | 3D-CRT      | 66 Gy             | Weekly during RT, and then 1, 3, 6, 12 and 24 mo after RT | ELISA   | Plasma   | The pre-RT TGF-β1 level was 10.7 ± 2.3 ng/ml and the mean during-RT TGFβ1 level was 6.0 ± 0.7 ng/ml. Differences in TGFβ1 levels were found at pre-RT and during RT in patients according to genotypes for TGFβ1, tPA, ACE DD, II and ID. | Yuan et al. [246] |
| IL-1β, IL-6, IL-8, TNF-α, TGF-β1 | 58 patients | 3D-CRT | 64.2–70 Gy | Prior to and at Weeks 2 and 4 during RT | Luminex kit | Plasma | Lower pre-treatment IL-8 levels were significantly correlated with development of RIPI. Radiation-induced elevations of TGF-β1 were weakly correlated with development of RIPI. Combining IL-8, TGF-β1 and mean lung dose into a single model yielded an improved predictive ability. None of the remaining cytokines or any clinical or dosimetric parameters were correlated with development of RIPI. |
|---------------------------------|------------|--------|------------|---------------------------------------|------------|--------|-------------------------------------------------|
| TGF-β1, IL-6                    | 57 NSCLC patients | 3D-CRT, IMRT | 59.6–68 Gy | Pre-RT and at a time point of 40 Gy (4 w) | ELISA | Serum | The serum IL-6 and TGF-β1 ratios (serum levels at the time 4 w after commencement of RT/pre-RT) differed significantly, and a borderline significant difference was found between RP and no-RP groups. IL-6 and TGF-β1 serum levels after delivery of 40 Gy increased more strikingly for patients who experienced RP. |
| IL-6, IL-8                      | 106 NSCLC inoperable patients | Various types of RT | Various doses | Before RT | ELISA | Serum | The performance of the prognostic model for survival improved markedly by combining two blood biomarkers: CEA and IL-6. |
| TGF-β1                           | 65 NSCLC patients diagnosed Stage IIIA or IIIB | 3D-CRT | 60 Gy (45–70) | 1 w before RT and at Week 4 during RT | ELISA | Plasma | The mean TGF-β1 level was significantly higher than in normal controls. At Week 4 of RT, the level of plasma TGF-β1 did not differ significantly from the pre-RT level. In univariate analysis and multivariate analysis, performance status, weight loss, radiation dose, and TGF-β1 ratio (during-RT/pre-RT level) were all significantly correlated with overall survival. |
| IL-6, IL-8, IL-10, IL-12(p40/p70), IL-1RA, TNF-α, sTNF-R1 | 62 NSCLC patients | CXRT | 64 Gy (50–70) | Before RT and then weekly for 8 w during RT | ELISA | Serum | There was a significant weekly increase in IL-6 (average of 4.4% each week). IL-6 was the only cytokine, whose increase was associated with an increase in the mean severity of the five most severe symptoms (pain, fatigue, disturbed sleep, lack of appetite, sore throat). There were also significant weekly increases in sTNF-R1 and IL-10. Not detectable: IL-1RA, IL-8, IL-12 (p40/p70) and TNF-α. |

Continued
| Evaluated cytokines | Patient descriptions | Types of RT | Total dose (range) | Time intervals | Methods | Materials | Main results/trends | Authors |
|---------------------|----------------------|-------------|--------------------|----------------|---------|-----------|---------------------|---------|
| TGF-β1              | 23 NSCLC patients    | 3D-CRT, IMRT | ≥50 Gy            | Before and post RT | ELISA   | Serum, sputum | There was an increased serum TGF-β1 level at the end of radiation therapy in the serum. There was an increasing trend compared with values before the radiotherapy (no significance). There was a significant increase in the TGF-β1 level expression in sputum at the end of radiotherapy. | Wang et al. [251] |
| TGF-β1, TNF-α       | 120 advanced lung cancer patients | CRT, 3D-CRT | 60 Gy (45–75) | Before and post RT | ELISA   | Plasma   | Levels of TGF-β1 and TNF-α were markedly increased after radiotherapy. | Xia et al. [252] |
| IL-6, IL-10, TNF-α, TGF-β1 | 96 NSCLC patients | 3D-CRT | 66 Gy (46–72) | Before, every 2 w until 6 w and then at 6 mo after RT | ELISA   | Serum     | None of the baseline cytokine levels were significantly associated with the occurrence of radiation fibrosis. Chronological changes in serum IL-6 levels were found in patients with fibrosis during the first 2 w of 3D-CRT (not significant). | Mazeron et al. [253] |
| TNF-α, IL-1β, IL-6, IL-8, VEGF, IL-12, IL-18 | 36 lung cancer patients | CRT, 3D-CRT | 46.9 Gy (30–60) | Before, during RT (2 w and 3 mo) | ELISA   | Serum, BALF | Patients had significantly higher levels of serum IL-6, IL-8, IL-18 and VEGF than controls. IL-1β, IL-6, IL-8 and IL-18 levels were significantly higher in the BALF from patients than in BALF from controls. IL-1β levels were significantly lower among the patients, VEGF was not significantly different. | Crohns et al. [254] |
| TGF-β1              | 165 NSCLC patients  | 3D-CRT | 59.6 Gy (50.9–74.3) | Pre-RT and at Week 4 of treatment | ELISA   | Plasma     | The pre-RT TGF-β1 level was marginally lower in patients with RILT at the University of Michigan Medical Center (UM), but not at Peking Union Medical College Cancer Hospital (PU). However, the during-RT TGF-β1 level was significantly higher in patients with RILT at PU, but not at UM. The pre-RT TGF-β1 level was not significantly different in patients with RILT compared with those without RILT, but the during-RT TGF-β1 level was | Zhao et al. [255] |
| Cytokines | Number of Patients | Radiation Technique | Radiation Dose | Time Points | Assay | Sample Type | Note |
|----------|--------------------|---------------------|----------------|-------------|-------|-------------|------|
| IL-1α, IL-6, IL-10, TGF-β1 | 34 lung cancer patients | 3D-CRT | ≥45 Gy | Beginning, at the end of RT and at 2 and 4 w after RT | ELISA | Plasma | Significantly higher in patients with RILT than in those without RILT. Kim et al. [256] |
| TGF-β1 | 52 NSCLC patients | 3D-CRT | 60–70 Gy | Before and at 3, 6 and 12 w of RT | ELISA | Plasma | The patients who developed pneumonitis showed a higher level of pre-treatment TGF-β1 (but this was not significant). Liu et al. [257] |
| TNF-α, IL-1β, IL-6, TGF-β1 | 52 NSCLC patients | 3D-CRT | 66 Gy, palliative 32 Gy | Pre RT, weekly during RT and 1, 3, 6 and 9 mo post-RT | ELISA | Plasma | There were increased levels of IL-6 and TGF-β1 in patients suffering moderate and severe lung toxicities (no clear correlation). Rübe et al. [85] |
| TNF-α, IL-1β, TGF-β, PDGF | 20 Hodgkin's disease patients | Mediastinal RT | 30 Gy (25–30.6) | Before, after RT and during the follow-up | ELISA | Serum | The IL-1β serum concentration was significantly increased after the completion of treatment, particularly Villani et al. [258] |

Continued
### Table 3. Continued

| Evaluated cytokines | Patient descriptions | Types of RT | Total dose (range) | Time intervals | Methods | Materials | Main results/trends | Authors |
|---------------------|----------------------|-------------|-------------------|----------------|---------|-----------|---------------------|---------|
| IL-6, TGF-β1        | 80 lung cancer patients | 3D-CRT      | 66.16 Gy          | Before, then every 2 w during, and at 6 w after RT | ELISA   | Plasma    | No statistically significant difference at baseline was found between the two groups (RT/RT + rhubarb). During treatment, the levels of TGF-β1 were increasing in both groups, and all reached a peak at 6 w. The levels of TGF-β1 at 2, 4, 6 and 12 w in the RT group were all significantly higher. Compared with the baseline, IL-6 levels in the control group were significantly higher during the treatment and reached a peak at 2 w. TGF-β1 and IL-6 levels in the RT + rhubarb group were significantly lower than those in the RT group. | Yu et al. [259] |
| TGF-β1              | 26 NSCLC patients     | 3D-CRT      | 65.7 Gy (64.2–70.1) | Weekly during, and then 1, 3, 6, 12 and 24 mo after RT | ELISA   | Plasma    | The TGF-β1 levels in patients with lung cancer pre-RT were significantly higher than those of the normal controls. No significant difference pre-RT, at 2 and 4 w during RT, or at the end of RT was found between patients with and without RILI. The mean TGF-β1 ratios increased in patients with RILI and decreased slightly in patients without RILI during the course of RT. | Zhao et al. [260] |
| IL-1, IL-6, TGF-β1  | 134 NSCLC patients    | RTOG protocol | 60–66 Gy          | Before, weekly (by 10 Gy), follow-ups every 3 mo/y and then annually | ELISA   | Serum     | The TNF-α level was elevated at the baseline in 24% of patients, IL-6 in 35% and IL-1 in 10%. It was found that 32% of patients with an initial 0 level of TNF-α became elevated for at least one reading during the RT. TNF-α in 50%, IL-6 in 59% and IL-1 in 80% of patients remained at 0 throughout treatment. IL-6 at 10 Gy was the only factor to | Hartsell et al. [261] |
| Cytokine | Patients | Modality | Dose | Timing | Assay | Sample | Results |
|----------|----------|----------|------|--------|-------|--------|---------|
| **TGF-β1** | 251 lung cancer patients | 3D-CRT | 66 Gy (36–86.4) | Pre-RT, regularly during and after RT | ELISA | Plasma | A total of 32 patients developed Grade 1 or higher RP. Patients with a V30 higher than 30% and a ratio of end-RT/ baseline TGF-β1 level higher than 1 had a significantly higher incidence of RP. Grade 2 or higher RP was developed in 27 patients. Patients with a TGF-β1 concentration during RT lower than baseline had a significantly higher incidence of RP than did patients with a mid-RT TGF-β1 levels that were higher normal. Evans et al. [262] |
| **IL-1α, IL-6** | 31 lung cancer patients | CXRT, RT | 63 Gy | Pre RT, weekly during RT, and 2 w, 4 w, 12 w and 3 mo post-RT | ELISA | Plasma | Temporal changes in the circulating IL-1 and IL-6 trend toward a decrease in IL-1α during RT and increase in IL-6 after RT. There was a statistically significant correlation at 4 w after RT, 8 w after RT, and 6 mo after RT (clinically symptomatic radiation pneumonitis was manifested). Chen et al. [83] |
| **TGF-β1, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, IL-17, GM-CSF, IFN-γ, MCP1, MIP-1β, TNF-α, G-CSF** | 55 lung cancer patients | 3D-CRT | 66–72 Gy | Pre RT, during RT, post-RT | BioRad—beads, ELISA | Plasma | There was a significant difference in the levels of IL-8 between the patients who did or did not develop RILI after treatment. Patients who developed RILI had IL-8 levels that were significantly elevated as compared with normal control subjects. Patients with elevated treatment TGF-β1 had higher pretreatment IL-8 levels. In this study, TGFβ1 alone provided no statistically significant predictive value. Hart et al. [129] |
| **IL-6, IL-10, TNF-α** | 96 NSCLC patients | 3D-CRT | 66 Gy (46–72) | Before and every 2 w during RT | ELISA | Serum | None of the IL-6, IL-10 or TNF-α baseline levels were significantly associated with the occurrence of RP. The occurrence of RP was significantly correlated with the variation in IL-6 levels during 3D-CRT. The changes in IL-10 levels showed a marked (but not statistically significant) increase in IL-10 levels in patients without RP during the first 2 w of 3D-CRT. Arpin et al. [84] |

Continued
| Evaluated cytokines | Patient descriptions | Types of RT | Total dose (range) | Time intervals | Methods | Materials | Main results/trends | Authors |
|---------------------|---------------------|-------------|-------------------|---------------|---------|----------|---------------------|---------|
| TGF-β1              | 38 NSCLC patients   | CRT         | 60 Gy             | Before and weekly during RT | PAI-1 bioassay | Plasma | The TGF-β level varied between the groups of patients not developing or developing pneumonitis. There were no significant differences between the absolute TGF-β plasma levels from Weeks 0 to 6. TGF-β concentrations in patients subsequently developing RP tended to rise above the pre-RT value during the first 5 w of the treatment, while patients not developing pneumonitis had much narrower spread of the ratio, values fluctuating around or just below 1. | Novakova—Jiresova et al. [263] |
| TGF-β1              | 68 NSCLC patients   | 3D-CRT      | 76 Gy (60.8–94.5) | Before, at 4, 6, 18 w after the start of RT and at 3 mo follow-up | PAI-1 bioassay | Plasma | The proportion of patients who developed symptomatic RP was not significantly different between patients with elevated vs normal (21 μg/ml) pre-RT TGF-β1 levels. TGF-β1 concentration decreased to normal values by the end of RT and remained normal at 18 w after RT. Patients who developed symptomatic RP of Grade 2 or worse were not significantly different with respect to normal vs elevated pre-RT TGF-β1 levels. | De Jaeger et al. [264] |
| IL-6, TGF-β1        | 11 lung cancer patients | 3D-CRT | 60 Gy, 48 Gy, 54 Gy | Before, during, and 1, 3, and 6 mo after RT | ELISA BALF | The TGF-β1 and IL-6 concentrations in the BALF were significantly increased by thoracic RT. The increase in TGF-β1 levels tended to be greater in the group of patients who developed severe pneumonitis. In the BALF from the non-irradiated areas, the TGF-β1 and IL-6 concentrations remained unchanged. | Barthelemy—Brichant et al. [265] |
| TGF-β1              | 38 NSCLC patients   | 3D-CRT      | 73.6 Gy, 80 Gy, 86.4 Gy | 1 m after RT, then every 3 mo for first y, every 4 mo for second y, and then every 6 mo | ELISA | Plasma | TGF-β1 concentration escalation was found to be a marker of the maximal tolerated dose in patients and the incidence of late complications. | Anscher et al. [108] |
| IL-1α, IL-6, MCP-1, bFGF, TGF-β1 | 24 lung cancer patients | CXRT or RT | 60–64 Gy | Pre RT, weekly during RT, and 2 w, 4 w, 12 w and 3 mo post-RT | ELISA | Plasma | Only IL-6 and IL-1α were correlated with the risk of pneumonitis (↑ pretreatment levels in patients with developed RP); TGF-1 and bFGF (pro-fibrotic)—no | Chen et al. [82] |
**TGF-β1**

| Patients | Treatment | Follow-up | ELISA | Correlation |
|----------|-----------|-----------|-------|-------------|
| 103 lung cancer patients | 3D-CRT | 66 Gy (45–80) | every 3 mo/2 y, then every 6 mo | Plasma | MCP-1 declined compared with pretreatment and no differences were observed between pneumonitis and no pneumonitis groups. |
| 41 untreated NSCLC patients | CXRT, RT | 60 Gy | Months | ELISA | An elevated plasma TGF-β1 level at the end of RT is an independent risk factor for RILI. The combination of plasma TGF-β1 level and $V_{30}$ appears to facilitate stratification of patients into low-, intermediate- and high-risk groups. Fu et al. [266] |

**VEGF**

| Patients | Treatment | Months | ELISA | Correlation |
|----------|-----------|-------|-------|-------------|
| 24 patients with RP | CXRT, RT | 60–64 Gy | Pre RT, weekly during RT, and 2 w, 4 w, 12 w and 3 mo post-RT | Plasma | Patients were divided into groups based on VEGF concentrations: high VEGF (>312 pg/ml) or low VEGF (≤312 pg/ml), using the median value as a cut-off. There were no significant associations between serum VEGF levels and various clinical-pathological characteristics, including age, gender, histologic type, stage and treatment. After treatment, a decreasing tendency of VEGF levels was observed. Choi et al. [267] |

**IL-6, TNFα**

| Patients | Treatment | ELISA | Correlation |
|----------|-----------|-------|-------------|
| 27 patients with Stage III NSCLC | CRT | 60 Gy | Plasma | The difference in plasma TGF-β1 levels between the patients with and without radiation-induced pneumonitis were significant 4 w after the beginning of RT treatment. It was shown that patients who had responded to radiation had | Vujaskovic et al. [269] |

Continued
| Evaluated cytokines | Patient descriptions | Types of RT | Total dose (range) | Time intervals | Methods | Materials | Main results/trends                                                                 | Authors |
|---------------------|----------------------|-------------|-------------------|----------------|---------|----------|-------------------------------------------------------------------------------------|---------|
| VEGF                | 94 lung cancer patients | CRT         |                   | Before, during and after RT | ELISA   | BALF     | lower plasma TGF-β levels than patients who failed to respond.                       | Beinert et al. [270, 271] |
| TGF-β1              | 59 newly diagnosed lung cancer patients | CRT         |                   | Before, after and at each follow-up after RT | ELISA   | Plasma   | No significant difference was found in TGFβ1 levels between the different histologic types of lung cancers and disease stages. Patients were divided into two groups according to status at the time of last follow-up: no evidence of disease (NED) and alive with disease (WD). Retrospectively, the plasma TGFβ1 level before radiotherapy was significantly higher in the WD group. | Kong et al. [272] |
| IL-6, IL-8, TNFα, sTNFαR | 20 NSCLC patients | Any treatment (RT or chemo) | Newly detected | ELISA, BALF | Mediators in the plasma of lung cancer patients (compared with controls) were as follows: elevated levels of sTNF-R. IL-6, IL-8 were only present low concentrations. Concentration of cytokines in BALF were found for sTNFαR, IL-6 and IL-8 in patients with NSCLC and in controls. TNF-α was not detectable in any of the BALF samples. | Staal-van der Brekel et al. [273] |
| TGF-β1              | 73 lung cancer patients | 3D-CRT      | 64 Gy (34–73.6)   | Before, weekly during RT and at each follow-up after RT | ELISA   | Plasma   | The median pretreatment TGF-β1 concentration was higher in the patients who did not develop pneumonitis than in those who did. | Anscher et al. [107] |
| Cytokine  | Study Participants | Cytokine or Treatment | Before and After RT | ELISA Type | Patient Response |
|----------|--------------------|-----------------------|---------------------|------------|-----------------|
| IL-6     | 61 lung cancer CRT 60 Gy | Before and 3, 6, 9 and 12 mo after RT | ELISA Serum | IL-6 was found to be strongly elevated in lung cancer patients—there was a decrease in IL-6 serum level in responders, but not in non-responders. Wojciechowska-Lacka et al. [274] |
| TGF-β1   | 36 cancer patients 3D-CRT ≥30 Gy | Before, weekly during, and at each follow-up after RT | ELISA Plasma | The patients who developed symptomatic pneumonitis differed from those who did not with respect to the pattern of change in their plasma TGF-β1 concentration over the course of radiotherapy. Anscher et al. [275] |
| IL-6, IL-10 | 31 lung cancer patients CRT 60 Gy | Before and 3, 6, 9 and 12 mo after RT | ELISA Serum | All cancer patients showed significant increases in serum levels of IL-6 and IL-10. As a result of the treatment, IL-6 significantly decreased and IL-10 slightly decreased in responders (compared with almost normal ranges in non-responders). No correlation between serum IL-10 and IL-6 levels was found. Wojciechowska-Lacka et al. [276] |
| TGF-β1   | 120 lung cancer patients CRT NM | Before, weekly during, and at each follow-up after RT | ELISA Plasma | The concentration of TGF-β1 was significantly higher in patients than in controls. The plasma TGF-β1 level at last follow-up correlated with the disease status in those patients with an elevated level at diagnosis and follow-up greater than 6 mo. Kong et al. [277] |
| IL-6     | 75 lung cancer patients Any treatment (RT or chemo) | Newly detected | ELISA Serum | Of 75 patients with lung cancer, 29 had detectable serum IL-6 levels. Yanagawa et al. [278] |
| TGF-β1   | 8 patients with lung cancer 3D-CRT ≥30 Gy | Before, weekly during, and at each follow-up after RT | ELISA Plasma | No correlation between the pretreatment TGF-β1 values and either the incidence or severity of pneumonitis. The plasma level during treatment was useful in separating those who would develop pneumonitis from those who would not. Anscher et al. [109] |

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IL-6 = interleukin-6, EGF = epidermal growth factor, G-CSF = granulocyte-colony stimulating factor, GM-CSF = granulocyte-macrophage colony-stimulating factor, FGF = fibroblast growth factor, VEGF = vascular endothelial growth factor, PDGF = platelet-derived growth factor, IFNγ = interferon γ, IP-10 = interferon gamma-induced protein 10 (CXC10), MCP-1 and -3 = monocyte chemoattractant proteins 1 and 3 (CCL2 and CCL7), MDC = macrophage-derived chemokine (CCL22), TGF-β1 and -β2 = tumor necrosis factors alpha and beta, RT = radiotherapy, 3D-CRT = 3D conformal radiation therapy, IMRT = intensity-modulated radiotherapy, SBRT = stereotactic body radiotherapy, ChR = chemoradiotherapy, CRT = conventional radiotherapy, CXRT = concurrent chemoradiation therapy, RILI = radiation-induced lung injury, RTOG = radiation therapy oncology group protocol, CCRT = concurrent chemoradiation therapy, RIPI = radiation-induced pulmonary injuries, RP = radiation pneumonitis, RILT = radiation-induced pulmonary toxicity, NSCLC = non-small-cell lung cancer, ELISA = enzyme-linked immune sorbent assay, BALF = bronchoalveolar lavage fluid, Gy = gray, NM = not mentioned, d = days, w = weeks, mo = months, y = years.
functions have been investigated jointly in connection with RIPI. The kinetics of gene expressions were detected after 5 Gy and 12.5 Gy thoracic irradiation on two murine models: the fibrosis-sensitive strain C57BL/6 and the resistant C3H/HeJ. Gene expression of IL-1α and IL-1β demonstrated time- and dose-dependent kinetics and strain differences. IL-1α expression was upregulated from Day 56 post-irradiation in C57Bl/6. In contrast, IL-1β was increased in C3H/HeJ only at Days 56 and 182. The authors of this study, Johnston et al. [92], point out that an alternation of mRNA levels of IL-1α during the ‘latent’ phase may contribute to fibrosis in the fibrosis-prone strain. Because IL-1β was not observed to make such a contribution, it appeared that this protein may have a protective function. Moreover, as mentioned previously, a positive correlation between IL-1α and IL-6 has been shown in patients with higher risk of radiation pneumonia [83].

INTERLEUKIN-4 AND INTERLEUKIN-13

Both interleukin-4 (IL-4) and interleukin-13 (IL-13) are members of the IL-4 family. They share genetic and structural elements, biological functions, and receptor-binding properties. These two cytokines are associated with T helper type II (Th2) cell differentiation [93]. IL-4 is a multifunctional, pleiotropic cytokine and the crucial factor in Th2 response development. It is produced in cells of various types, including lymphocytes, macrophages and fibroblasts, as well as in epithelial and endothelial cells. Its actions are generally to promote establishment of the humoral response, it functions as a growth factor for mast cells, and it plays a major regulatory role in allergic responses [94]. In macrophages, IL-4 inhibits the secretion of inflammatory chemokines and cytokines TNF and IL-1β, impairs their ability to produce ROS and NOS intermediates, and blocks interferon-γ (IFN-γ)-stimulated expression of cellular adhesion molecules [95].

IL-13 is a pleiotropic cytokine that is also involved in stimulation of Th2 cells. IL-13 is suspected to be a more central mediator of allergic inflammation, as well as an important effector of gastrointestinal parasite expulsion, tumor cell growth, and intracellular parasitism. An important function of IL-13 was discovered within the process of tissue remodeling and fibrosis, but that function has not yet been fully defined [96]. IL-4 and IL-13 bind with high affinity to the receptor for IL-4 (IL-4R), which is widely expressed among various cell types, and both contribute to differentiation of lung fibroblasts into myofibroblasts, and collagen production [97].

Gene expression of IL-4 increases in a time-dependent manner after thoracic irradiation in animal models. The highest concentration has been found in lung tissue at Day 84 after hemithorax irradiation by a dose of 20 Gy. Cellular sources of this protein were identified in infiltrated cells from the peribronchial and perivascular areas, as well as in cells located in the alveolar lumen and in the alveolar septa. Further, macrophages showed substantial IL-4 production. These results suggest a correlation between local IL-4 protein expression and the development of RIPI. Another interesting finding from this study is that the IL-4 mRNA gene expression levels and the IL-4 protein levels do not closely correlate in the late stages [98].

The role of these cytokines in the development of radiation pneumonitis and radiation fibrosis is further elucidated by studies on deficient mice exposed to radiation. IL-13-deficient mice were shown to be resistant to the progression of radiation-induced fibrosis, and the plasma of wild-type animals contained a high concentration of soluble IL-13Rα2 decay receptor. Although this study clearly demonstrated IL-13 to be a critical mediator of radiation-induced lung injury, it also suggested that delivery of an IL-13 neutralizing agent may have therapeutic efficacy [99]. Also, evidence clearly suggests the therapeutic potential of IL-4. Exogenous administration to lethally irradiated mice (C57BL6/J) increased the 30-day survival fraction with a dose high as 8.5 Gy. The mechanism of action is not direct involvement in reconstruction of hematopoietic and/or gastrointestinal system damages, but the function may be as an effective agent with potential to limit radiation-induced inflammation [100].

IL-4 knockout mice have been shown to vary with respect to the effects seen in RIPI. It is noteworthy that development of radiation fibrosis is not prevented or delayed by loss of IL-4. Otherwise, IL-4 appears to be somewhat abrogated in the pneumonic phase. Studies have demonstrated the development and maintenance of pulmonary macrophage accumulation in the lung as occurring under the command of IL-4. An IL-4 mechanism during inflammation that has been identified involves blocking of granulocyte recruitment while instructing macrophages to downregulate production of pro-inflammatory chemotactic factors and to drive accumulation of tissue macrophages through self-renewal [101]. These cells are known as alternatively activated macrophages, or M2 cells. Due to increased IL-4 production, macrophages accumulate in sufficient numbers and proliferate at the site of injury, while tending toward the M2 phenotype. Mice lacking IL-4 show the enhanced pro-inflammatory macrophage phenotypes in the lung in response to irradiation, as well as higher occurrence of infiltrating monocytes and their maturation into alveolar macrophages [102].

TRANSFORMING GROWTH FACTOR B

TGF-β is the prototypical cytokine and first characterized member of the TGF-β family, comprising more than 60 proteins in multicellular organisms. Three distinct subtypes, TGFβ1–β3, are known in humans and show a high degree of homology between various species. These proteins regulate a wide range of processes, including a multiplicity factor mediating embryonic development and cellular processes including cell cycle control, cell differentiation, apoptosis, cellular homeostasis, and wound healing.

TGF-β isoforms are produced in excess in most organs and are stored in inactive form. They are synthesized as gene products that include both the active cytokine and a latency-associated peptide (LAP). LAP is an endogenous inhibitor of TGF-β activity. This product is cleaved in the endoplasmic reticulum by the endoprotease furin [103]. TGF-β activity is regulated by two proteins, LAP and TGF-β binding protein (LTBP), forming a larger complex in the cytoplasm called the ‘large latent complex’ (LLC). It can be activated by various physico-chemical treatments or by proteases [104]. TGF-β signals activate the Smad proteins, which act as both transcription proteins and transcription factors and are able to regulate gene expression of various targets, including procollagen I and III [105]. Ionizing radiation is one of the exogenous factors that have been shown to induce TGFβ activation within an hour, even at
Radiation pneumonitis and fibrosis and their cytokines profiles

Chemokines and Their Receptors

Chemokines comprise a large family of small proteins, mostly structurally related molecules. The initial discovery of the first chemokine (CXCL8, CCL2) led to the conclusion that the pivotal role of chemokines is to induce leukocyte migration during inflammation. Since then, with the identification of all chemokine genes (human and mouse) and as other chemokines have been discovered and their functions described, our understanding of chemokines and their roles in the immune system have changed. Chemokines are grouped into two main functional subfamilies: inflammatory and homeostatic chemokines. Inflammatory chemokines control the recruitment of leukocytes in inflammation and tissue injury. Homeostatic chemokines, meanwhile, carry out such housekeeping functions as navigating leukocytes to and within secondary lymphoid organs as well as in the bone marrow and the thymus during hematopoiesis. Many of the chemokines have critical roles in homeostasis or, depending on circumstances, overlap both functional areas in an organism [112], because some inflammatory chemokines may have homeostatic functions and some homeostatic chemokines may be upregulated under certain conditions of inflammation. These are known as dual-function chemokines. Homeostatic chemokines tend to be expressed in specific tissues or organs, whereas inflammatory chemokines can be produced by many cell types in multiple locations. In addition to affecting immune cell trafficking and homeostasis, they fulfill a wide range of functions in angiogenesis, wound healing, collagen production, and the proliferation of hematopoietic precursors [113, 114]. The system of nomenclature for chemokines is based on an arrangement of secondary structure between four conserved cysteine residues that form disulfide bonds on the N-terminus of the molecule. These groups are: (X)C, CC, CXC and CX3C. In zebra fish alone there exists a fifth group: C(X) [115].

CCL2 (MCP-1)

CCL2, also known as monocyte chemotactic protein-1 (MCP-1), is a member of the CC chemokine family and a potent chemoattractant factor for monocytes. The function of this chemoattractant protein involves the regulation of migration and infiltration of monocytes, memory T lymphocytes, and natural killer cells [116]. The major sources of CCL2 are monocytes and tissue macrophages in the lung, followed by endothelial and epithelial cells, fibroblasts, and alveolar macrophages [117, 118]. In addition, it is synthesized by smooth muscle, mesangial cells, astrocytes, and microglia. The production occurs constitutively, but higher concentrations appear after induction by oxidative stress, cytokines, or growth factors. The major sources of CCL2 are nevertheless monocytes and macrophages in tissue [119]. This chemokine is the most potent profibrotic chemokine, facilitating fibrosis both directly and indirectly through binding to its cell-surface receptor, CCR2, on hematopoietic cells (predominantly monocytes) and fibroblasts [120]. Data recently confirmed by Osterholzer et al. clarifies that the CCL2/CCR2 pathway is responsible for the accumulation of non-resident cells (monocytes and non-resident macrophages) and development of pulmonary fibrosis in response to diphtheria toxin-induced idiopathic pulmonary fibrosis [121].

Moreover, MCP-1 falls into a category of cytokines that are activated within minutes to hours after irradiation. The peak concentration occurs at 3 h after exposure to ionizing radiation, and heightened concentration remains even 182 days later [122].

Improvements have been made in estimating radiation-induced lung toxicity by developing a new model based on combinations of cytokine concentrations, dose volumes, and dosimetry parameters, as well as by considering variable patient factors. With the identification of additional factors that interact with the effect of dose on lung toxicities, it could be possible to distinguish those patients who demonstrate better tolerance for high-dose radiotherapy. Although this multivariable model unfortunately has limited clinical applicability, it has identified MCP-1 as a suitable predictive parameter. MCP-1 levels increased the dose-dependent effect on the risk of
RIPI formation, but the study did not describe the mechanisms by which this cytokine interacts with ionizing radiation. More interestingly, the prognostic usefulness of this cytokine was shown only from pretreatment plasma levels. Moreover, the same characteristics were demonstrated for cytokines IL-1α and IL-8 and for the soluble CD40 ligand. Surprisingly, this model did not identify TGF-β1 to be correlated with risk of RIPI, but the authors did suggest that this cytokine may be independent of the radiation dose–dependent mechanism [123]. A study by Siva et al. [21], meanwhile, recorded a temporal change in plasma concentration in these patients. Each patient sample was tested for a panel of 22 cytokines, from which just 12 cytokines were detected as responding to radiotherapy and only 6 of these were also dependent upon treatment. In patients with a higher grade of pulmonary toxicity, there was significant reduction in the levels of MCP-1 and CXCL10 [also known as interferon gamma-induced protein 10 (IP-10)] at 1 h. Also, significantly different concentration levels of eotaxin (within the CC group of chemokines), IL-6, and tissue inhibitor of metalloproteinases-1 (TIMP-1) occurred at 24 h in patients with sustained pulmonary toxicity. In addition, cytokine levels were strongly and linearly correlated with the dose applied to the irradiated normal lung tissue.

CXCL8 (IL-8)

CXCL8, previously known as interleukin-8, belongs to the CXC family. It was originally identified as a neutrophil chemoattractant factor and isolated from stimulated human mononuclear cells. It is produced by macrophages and other cell types, such as epithelial cells, airway smooth muscle cells, and endothelial cells in the lung. Moreover, CXCL8 production can be induced by inflammatory cytokines, such as IL-1 and TNF-α [124]. Functionally, it is one of the major mediators of inflammation, as well as a chemoattractant for trafficking of basophils, T lymphocytes, and neutrophils across the vascular wall. It has recently been demonstrated to have angiogenic activities, as it induces migration of endothelial cells, and also to be capable of inducing a loss of focal adhesion, followed by chemotaxis and chemokinetics of fibroblasts. CXCL8 also stimulates the endothelial permeability of endothelial cells, as well as adhesion and subsequent cell contraction, thereby facilitating leukocyte exit from the circulation [125]. Indeed, CXCL8 is an important mediator of pulmonary edema, a form of pathological permeability, after lung injury [126]. Surprisingly, even though the human and mouse genomes are remarkably similar, CXCL8 does not have a mouse counterpart. The chemokines Cxcl1 (KC), Cxcl2 (MIP-2) and Cxcl5 (LIX) are regarded as functional homologues of IL-8 in animal models. Cxcl15 (lungkine) is unique to mouse and does not exist in humans [127, 128]. Cxcl11, also termed keratinocyte chemoattractant (KC), has been analyzed as a functional homologue in animal models in relation to radiation exposure. Responding similarly after both whole-lung and whole-body irradiation, KC’s expression has been shown to be dose-responsive at 1 h, to peak at 6 h after irradiation, and to return to baseline at 24 h [17]. Nevertheless, important differences existing between the mouse and the human in the chemokine ligand CXC family must be taken into account. Thus, one must be cautious in attempting to extrapolate the mouse data into human disease biology.

Analysis of IL-8 plasma level has been confirmed to have prognostic value among cancer patients who do or do not develop radiation-induced lung injury. Patients with lower levels of plasma IL-8 before radiation therapy might be at increased risk of developing toxicity. This analysis demonstrated a significant difference in the IL-8 levels between those patients who did or did not develop RIPI after radiation treatment. Another finding was a positive correlation with elevated levels of TGF-β1 in combination with IL-8 but not with TGF-β1 alone [129]. A similar conclusion was validated in papers by Wang et al. [130] and Stenmark et al. [131]. All these studies point to the importance of cytokine concentrations reached within the first 2 weeks of treatment and thus that they may afford the opportunity to individualize therapy for patients.

CONCLUDING REMARKS

Tolerance of lung tissue to ionizing radiation and subsequent development of radiation pneumonitis and fibrosis remain the main limiting factors for efficient radiotherapy. Cytokines undeniably play various roles in the whole process. Research into immunological regulation of inflammation has discovered that there are many different types of cytokines mediating the inflammatory response of immune cells to radiation. These include chemokines, inflammatory cytokines, and fibrotic cytokines. The mechanisms of action for the majority of proteins have already been described in animal models and humans. These insights into radiation response have enabled many investigators to introduce and advance new ideas on how to interrupt this complex procedure. A major step forward for patients would be to identify useful biomarkers. Great efforts have been made using animal studies to identify cytokines showing positive correlation between samples of blood, bronchoalveolar fluid, and lung tissue, because it is not practical to obtain all those samples from patients. Even after many years of research, however, prognostic values have been proven only for a few cytokines, including IL-6 and TFG-β1. In those cases, higher pretreatment levels are correlated with subsequent development of radiation pneumonitis. Hopefully, these findings will provide opportunity for individualizing treatments to improve therapeutic outcomes.

While one viewpoint links increased cytokine levels to pathogenesis, another is directed to using specific cytokine inhibitors in certain situations. The cytokines and their signaling pathways are useful targets for intervention. Through its intensive work in this field of research, the scientific community has made it possible to identify certain critical mediators in the context of lung tissue’s response to radiation. IL-4 has been seen to be the most important cytokine in restoration and maintenance of pulmonary macrophage populations, which are critical mediators of chronic inflammation. On the other hand, the function of another cytokine from the same family, IL-13, appears to be important in the fibrotic phase. Neutralization of both cytokines by specific, monoclonal antibodies has resulted in improved RIPI. This approach may be among the most successful therapeutics in animal models, but it has not been approved for clinical use on patients. A humanized monoclonal antibody that selectively neutralized IL-13, tralokinumab, has already been tested with promising results for treating asthma and other inflammatory lung diseases [132]. Hopefully, further results will
bring us to fully understanding the role of cytokines in radiation-induced disorders. The next step may lead to the development of specific cytokine-based therapies that will alleviate the limitations presently holding back efficient radiotherapy treatment.

CONFLICT OF INTEREST
The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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