Inhibition of the Tumor Necrosis Factor-α Pathway Is Radioprotective for the Lung

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Abstract

Purpose: Radiation-induced lung toxicity limits the delivery of high-dose radiation to thoracic tumors. Here, we investigated the potential of inhibiting the tumor necrosis factor-α (TNF-α) pathway as a novel radioprotection strategy.

Experimental Design: Mouse lungs were irradiated with various doses and assessed at varying times for TNF-α production. Lung toxicity was measured by apoptosis and pulmonary function testing. TNF receptor 1 (TNFRI) inhibition, achieved by genetic knockout or antisense oligonucleotide (ASO) silencing, was tested for selective lung protection in a mouse lung metastasis model of colon cancer.

Results: Lung radiation induced local production of TNF-α by macrophages in BALB/c mice 3 to 24 hours after radiation (15 Gy). A similar maximal induction was found 1 week after the start of radiation when 15 Gy was divided into five daily fractions. Cell apoptosis in the lung, measured by terminal deoxyribonucleotide transferase-mediated nick-end labeling staining (mostly epithelial cells) and Western blot for caspase-3, was induced by radiation in a dose- and time-dependent manner. Specific ASO inhibited lung TNFRI expression and reduced radiation-induced apoptosis. Radiation decreased lung function in BALB/c and C57BL mice 4 to 8 weeks after completion of fractionated radiation (40 Gy). Inhibition of TNFRI by genetic deficiency (C57BL mice) or therapeutic silencing with ASO (BALB/c mice) tended to preserve lung function without compromising lung tumor sensitivity to radiation.

Conclusion: Radiation-induced lung TNF-α production correlates with early cell apoptosis and latent lung function damage. Inhibition of lung TNFRI is selectively radioprotective for the lung without compromising tumor response. These findings support the development of a novel radio-protection strategy using inhibition of the TNF-α pathway.

The risk of lung damage in response to radiation becomes a major limitation for delivering high-dose radiation to a tumor site even with the use of advanced conformal and hyper-fractionated techniques (1–3). One limitation to the delivery of higher doses is that there are no effective normal lung radioprotectors. Amifostine (WR-2721), the only Food and Drug Administration–approved radioprotector, can protect the parotid gland (4, 5) but has not been convincingly shown to decrease esophagus or lung toxicity (6, 7). Although clearly needed, development of new radioprotectors has been hampered by the complexity of mechanisms related to normal tissue response to radiation.

Radiation-induced lung toxicity results from a sequence of biological changes, including early cell apoptosis that is mediated by direct radiation ionization, intermediate inflammation that is characterized by pneumonitis, and latent fibrosis (8, 9) that ultimately causes pulmonary function failure. It has been generally believed that a series of biochemical events triggers these changes. At the cellular level, radiation activates free radical production, triggering DNA damage, apoptosis, cell cycle changes, and reduced cell survival. In addition, interactions among different cell types, in particular, the activation of macrophages as a result of clearing apoptotic cells or as a direct response to radiation, may cause cytokine production, leading to tissue damage. This cytokine production contributes to lung toxicity through stimulation of apoptosis, inflammation, and fibrosis (10, 11). In particular, transforming growth factor-β1 (TGF-β1) production is elevated after radiation in various organs, including lung (12, 13), contributing at least partially to lung cell apoptosis (14, 15). The role of TGF-β1 has been well established in mediating radiation-induced lung fibrosis (16) by activating its receptor (TβR) and subsequent signaling pathway mediated by Smad3, leading to increased collagen gene expression and ultimately lung fibrosis (17–20). Agents that can block TGF-β1 activity, such as soluble TβRII protein fragments, decorin, tranilast, neutralizing antibodies, threonine kinase inhibitors, and specific antisense oligonucleotide (ASO), reduce fibrogenesis in many organs, including...
lung, under various pathologic conditions (21 – 26). However, blocking TGF-β1 activity in the lung confers only partial protection in these animal studies. Tumor necrosis factor-α (TNF-α), a major proinflammatory and apoptotic cytokine, has also been shown to be significantly elevated in the lung after radiation (27, 28). Aberrant production of TNF-α is associated with pathologic changes in response to stress conditions such as radiation by activating the TNF receptor 1 (TNFR1) signaling pathway, leading to pneumonitis, apoptosis, lung fibrosis, and a subsequent decrease in lung function (29 – 32). On the other hand, specific inhibition of TNF-α by etanercept (Enbrel) is effective in dramatically improving lung function in patients with idiopathic pulmonary syndrome after allogeneic hematopoietic stem cell transplantation (33). Furthermore, TNFR1 knockout mice fail to develop fibroproliferative lesions in lung after asbestos exposure (34), suggesting that TNF-α activation may play a pivotal role in lung fibrosis.

Our previous study showed that radiation induces liver damage through TNF-α/TNFR1 activation and that inhibition of TNFR1 by ASO is radioprotective in liver (35). In this study, we examined the role of TNF-α in radiation-induced liver toxicity. After we found an elevation of liver TNF-α in response to single or fractionated radiation, we determined cell apoptosis as an early indication of TNF-α – mediated liver damage. We then assessed the potential of blocking TNFR1 for radioprotection in TNFR1 knockout mice and the effectiveness and selectivity of TNFR1 ASO in improving lung function in a mouse lung metastases model of colon cancer.

Materials and Methods

Animal model, lung tumor xenografts, and bioluminescence imaging analysis. Male BALB/c and C57BL mice (wild type and TNFR1−/−), 6 to 8 wk old, were purchased from Charles River Laboratories. Mouse colon luminal luciferin reaction was generated on an Argus Image Processor and a pseudocolor image representing light intensity from the luciferase-collection and integration for 4 min in light-tight specimen chamber. (160 mg/kg, i.p., 15 min before anesthetization) followed by photon experimental period when mice were first injected with luciferin (The same assay was used to monitor tumor growth during the tail vein of the mouse. The presence of lung tumors was verified analysis.

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lungs, multiple fractions (2 Gy/d during a 2-wk period) for pulmonary function test and tumor suppression study. Control mice were subjected to exposed bronchus and embedded immediately in optimum perfusion was done through inferior vena cava using 20 mL of PBS to remove blood from portal vein. The left lung was collected after ligation with suture in the left hilum and immediately placed on dry ice. The right lung was filled with the optimum cutting temperature compound through exposed bronchus and embedded immediately in optimum cutting temperature. Cryosections were prepared (10-μm thickness) for apoptosis or immunohistochemical staining.

ELISA for lung TNF-α. Samples from homogenized lung tissue were applied onto 96-well plates precoated with antibody specific for mouse TNF-α from commercial kits (R&D Systems) followed by serial incubation and washing according to the manufacturer’s recommendations. Each sample was measured in triplicate, and TNF-α concentration was calculated based on a standard curve and the total amount of protein in the lysate.

Real-time reverse transcription-PCR quantification of mRNA for TNF-α and TNFR1. Fifty nanograms of total RNAs isolated from lung tissue by Trizol reagent (Promega) were applied in a real-time reverse transcription-PCR assay system (Opticon, MJ Research, Inc.) using a QuantiTect SYBR Green RT-PCR kit (Qiagen, Inc.) according to the manufacturer’s suggested conditions. Specific primers used were as follows: TNF-α 5’-AGCGCATGTAGCTCAAGAC; TNF-α 3’-GTGG-GTGAAGGACGACTGAT; TNFR1 5’-GCCCTCGCCGATAAGCCACCC; TNFR1 3’-CTTGGCCAGTCCTCAAGCGCAG; and α-actin 5’-CAGTTCCCAAAATCAAG; α-actin 3’-TGGTGCATCAGCTCCTCCTCA.

The levels of mRNA for TNF-α and TNFR1 were normalized to each α-actin level and were expressed as fold increases in comparison with nonirradiated controls.

Terminal deoxynucleobronucleotide transerase – mediated nick-end labeling and immunohistochemical staining of lung tissue. Lung cryosections were assessed for DNA strand breaks using an In situ Cell Death Detection Kit (Roche Applied Science) based on terminal deoxynucleobronucleotide transerase – mediated nick-end labeling (TUNEL) staining or immunohistochemical staining using specific antibodies against mouse TNF-α, TNFR1 (R&D Systems), or macrophages (F4/80, Abcam). Briefly, tissue slides were fixed in 4% paraformaldehyde for 10 min followed by washing in PBS and blocking in 3% H2O2 methanol for 10 min. Tissue sections were then permeabilized in solution containing 0.1% Triton X-100 and 0.1% sodium citrate. For TUNEL, sections were labeled with 25 μL of TUNEL reaction mixture containing 1:2 dilution of enzyme for 2 h at 37°C in a humidified chamber. After extensive washing, fluorescent signals positive for TUNEL were counted from five randomly selected areas under a microscope (×20). For immunohistochemical staining, sections were incubated with antigen retrieval
buffer [10 mmol/L sodium citrate, 0.05% Tween 20 (pH 6.0)] at 95°C for 20 min before washing in PBS followed by incubation with antibodies against TNFR-α (1:25), TNFR1 (1:50), or macrophages (1:50) overnight at 4°C. Signals were developed by substrate reaction with horseradish peroxidase conjugated to the second antibody before counterstaining with methyl green or visualized directly by using FITC-conjugated second antibody.

**Western blotting for caspase-3 activation.** Apoptosis was determined, by caspase-3 activation (cleavage), using Western blotting. Tissues were homogenized in PBS containing protease inhibitors. Twenty microliters of lysates were fractionated on 10% acrylamide gel followed by protein transfer onto polyvinylidene difluoride membrane (Millipore Corp.). Cleaved caspase-3 was detected by a rabbit anti-mouse caspase-3 antibody (R&D Systems), whereas h-actin expression (Sigma) was used as a loading standard.

**Hydroxyproline assay.** The hydroxyproline content in the lung tissue was determined by a colorimetric assay to assess collagen production in response to radiation. Briefly, 20 mg of lung specimen were lyophilized and then hydrolyzed in 600 μL of 6 mol/L HCl at 100°C overnight. Samples were dried completely by speed vacuum to remove the acid solution and then redissolved in 200 μL of H2O followed by centrifugation. Fifty microliters of the supernatant were incubated with 500 μL of chloramine-T solution (Fisher Scientific) for 10 min at room temperature followed by a 15-min incubation at 65°C in 500 μL of fresh Ehrlich’s perchorlic acid solution containing 1 mol/L p-dimethylamino-benzaldehyde (Sigma), 62% n-propyl alcohol (Fisher Scientific), and 15.6% perchloric acid (Sigma). Sample absorbances were assessed at 560 nm by a microtiter reader, and resulting values were compared to a hydroxyproline standard (Sigma) curve. The hydroxyproline contents were corrected by the dry weight of the initial tissue. Each sample was assayed in triplicate.

**Data analysis and statistics.** Mouse experiments were carried out in triplicate for each condition. The ability of measuring pulmonary function noninvasively in the same animal at various time points allows us to use a smaller number of animals to achieve statistical power. Scoring for TUNEL staining was done by two individuals. Values

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**Fig. 1.** Radiation increased lung TNF-α production. BALB/c mice, 6 to 8 wk old, were irradiated in the lung with a single (15 Gy) or fractionated dose (3 Gy/d × 5). Lung tissues were collected at various time points (0 h, 3 h, 24 h, and 7 d) and assessed for TNF-α protein (per milligram of lung lysate) by ELISA (A, C, and D) and for mRNA (set as 1 for nonirradiated lung) by real-time RT-PCR (B). Cells expressing TNF-α at 3 h after radiation were detected by immunohistochemical staining using goat anti-mouse TNF-α and second antibody conjugated with horseradish peroxidase (brown) and counterstained with methyl green (E). The section of irradiated lung (3 h) was double stained with antibodies for TNF-α (green by white arrow) and macrophages (F4/80; brown by black arrow) before methyl green counterstaining (F). *, significantly increased in comparison with nonirradiated lung, P < 0.05, n = 3.
are expressed as means ± SE and were compared by ANOVA analysis. Data were considered significantly different between control group and comparable treatment group (± radiation or ± ASO treatment) when $P < 0.05$.

**Results**

**Lung radiation increases local production of TNF-α.** Similar to our previous findings in the liver (35), radiation caused an elevation of TNF-α in the lungs of BALB/c mice in a dose-dependent manner. An increase in TNF-α of ~68% (1,259 ± 89 pg/mg) was observed as early as 3 hours after 15 Gy compared with the levels immediately after radiation (746 ± 51 pg/mg) or in nonirradiated control mice (±RT; 754 ± 43 pg/mg; Fig. 1A). This radiation-induced TNF-α remained moderately increased 24 hours after radiation (975 ± 68 pg/mg) and returned by day 7 to the level observed in nonirradiated mice. Consistent with these changes, the mRNA levels for TNF-α in the lung measured by real-time RT-PCR were also increased by ~1.9- and ~1.8-fold at 3 and 24 hours after irradiation with 15 Gy before returning to control levels by day 7 (Fig. 1B). These results suggested that radiation transiently induced lung TNF-α expression. These inductions seemed to be radiation dose dependent in which no or moderate induction was observed at 5 or 10 Gy and significant or maximal induction was observed at 15 or 25 Gy (Fig. 1C). To explore the effect of radiation scheduling, mice were treated with 15 Gy of fractionated radiation (3 Gy/d × 5). We found that one fraction (3 Gy) increased lung TNF-α at 3 hours after radiation by only ~23% (943 ± 68 pg/mg) compared with the increase of ~68% by a single 15 Gy dose (Fig. 1D). However, fractionated treatment significantly increased lung TNF-α (1,312 ± 41 pg/mg) by day 7 (2 days after completion of treatment), suggesting that radiation-induced TNF-α accumulates during a course of treatment. Moreover, the majority of TNF-α seemed to be produced by lung macrophages as indicated by immunohistochemical staining of lung tissue where most TNF-α–positive cells (brown) were nonepithelial large cells located in the junctions of alveolar spaces (Fig. 1E) or by the double staining where TNF-α–producing cells (green) colocalized with cells positive for macrophage-specific staining (brown; Fig. 1F).

**Radiation-induced lung cell apoptosis.** To assess the potential biological changes relevant to TNF-α–mediated lung toxicity, apoptosis was measured in lung tissue by TUNEL staining and Western blot for caspase-3 activation. We found a significant increase in the number of apoptotic (TUNEL-positive) cells (green) 3 hours after irradiation with 15 Gy compared with...
nonirradiated lung (-RT; Fig. 2A). Apoptosis seemed to occur in both lung epithelial cells (black arrow) and macrophages (white arrow) based on morphology, although it was not clear whether this apoptosis also occurred in lung endothelial cells (Fig. 2A, histology). We found that TUNEL-positive cells were increased 8-fold 3 hours after treatment with 5 Gy (25 ± 2 cells) and 12-fold after 15 Gy (38 ± 5 cells) compared with nonirradiated lung (3 ± 1; Fig. 2B, bottom). Apoptosis-linked DNA breaks increased detectably 0.5 to 1 hour after radiation and maximally at 2 to 3 hours after radiation with 15 Gy (31 ± 4 to 38 ± 5) compared with basal level in nonirradiated lung or moderate increase in irradiated lung for 0.5 to 1 hour (12 ± 1 to 16 ± 1). Breaks were reduced ~70% to 80% at 8 to 24 hours after radiation (Fig. 2B, top), indicating rapid clearing of apoptotic cells. The similar time course change of caspase-3 cleavage by Western blot (Fig. 2C) further supports the notion that these DNA breaks were mediated by cell apoptosis. These results showed that radiation increases apoptosis in a dose- and time-dependent manner.

Specific inhibition of lung TNFR1 reduces radiation-induced apoptosis. To determine whether inhibition of TNF-α action could reduce radiation-induced apoptosis, a specific ASO was used to block lung TNFR1. In control mice pretreated with saline, irradiation with 15 Gy increased TNFR1 mRNA level by ~30% at 3 hours, similar to the induction in mice pretreated with an irrelevant ASO (Ctrl-ASO; Fig. 3A). In contrast,
pretreatment with ASO for TNFR1 (TNFR1-ASO) reduced lung TNFR1 mRNA level by ~50% compared with nonirradiated control lungs (both saline and Ctrl-ASO treated). More importantly, this inhibition was sustained when the lung pretreated with TNFR1-ASO was irradiated (Fig. 3A) up to 48 hours after radiation (data not shown). The inhibition of TNFR1 was further assessed by immunohistochemical staining, which confirmed the reduction of cells expressing TNFR1 (brown) in the lung pretreated with TNFR1-ASO compared with the nonirradiated (-RT) or irradiated (15 Gy) lung (Fig. 3B). As we anticipated, apoptosis measured by TUNEL staining was also significantly reduced in the lung pretreated with TNFR1-ASO at 3 and 24 hours after radiation (6 ± 2 and 4 ± 1 cells, respectively) compared with saline-treated lung (38 ± 5 and 10.0 ± 1 cells, respectively) or Ctrl-ASO–treated lung (32 ± 2 and 13 ± 3 cells, respectively; Fig. 4A). This decrease in TUNEL staining by TNFR1 inhibition was correlated with a reduction of caspase-3 cleavage, in contrast to the elevation of caspase-3 cleavage observed in saline- or Ctrl-ASO–treated lung (Fig. 4B). These results suggest that specific inhibition of lung TNFR1 by ASO protected normal cells from radiation-induced apoptosis.

**Inhibition of TNFR1 is radioprotective for the lung.** We wished to determine if TNFR1 inhibition also improved lung function after radiation. We assessed lung function in TNFR1-deficient mice (TNFR1−/−) in the background of C57BL, a strain that is sensitive to radiation for lung damage, receiving fractionated lung radiation (2 Gy, twice a day × 10 days during a 2-week period, total of 40 Gy) compared with wild-type mice given the same treatment. We found that airway resistance (measured using an unrestrained whole-body plethysmography as described in Materials and Methods) in wild-type mice gradually increased by 2.0 ± 0.2– and 2.4 ± 0.3–fold at 2 and 6 weeks, respectively, after the completion of radiation treatment compared with that before radiation (Fig. 5A). This increase in airway resistance was blunted in TNFR1−/− mice (1.5 ± 0.2– and 1.4 ± 0.2–fold), suggesting that radiation-induced lung function damage could be partially prevented by the deficiency of TNFR1. A reduction of TNFR1 in BALB/c mice was also achieved by systemic administration of TNFR1-ASO in conjunction with each radiation fraction (25 mg/kg, i.p., 36 hours before the first radiation fraction and 2 hours before each fraction afterward). Radiation substantially increased airway resistance in BALB/c mice by 1.8 ± 0.1– and 2.1 ± 0.1–fold at the same time points, comparable with the induction in C57BL-WT mice. As was the case in the TNFR1−/− mice, inhibition of TNFR1 produced by administration of TNFR1-ASO decreased the effect of radiation on airway resistance (elevation of only 1.4 ± 0.1– and 1.6 ± 0.1–fold at these time points). These data show that inhibition of TNFR1, either in genetically engineered animals or by silencing through ASO, can partially protect lung function after radiation treatment, thus providing a strong rationale for a radioprotection strategy based on specific blocking of TNFR1.

To determine whether the improvement of lung function by TNFR1-ASO correlates with a decrease in lung fibrosis, lung collagen was measured by hydroxyproline content from BALB/c mice receiving radiation and ASO treatments described above. We found that hydroxyproline was significantly increased by ~60% 8 weeks after fractionated radiation treatment (RT 8 w) compared with nonirradiated lung (No RT; Fig. 5B). With concurrent treatment of TNFR1-ASO (+ASO), only ~20%
increase in lung hydroxyproline was observed at the same time point (Fig. 5B), suggesting that inhibition of TNFR1, which blocked early apoptosis, was also effective in blocking radiation-induced lung collagen production at later time point.

**ASO inhibition of TNFR1 did not compromise radiation-mediated tumor killing.** We then needed to determine if inhibiting TNFR1 protected tumors. BALB/c mice bearing lung tumor metastases were established by i.v. injecting colon CT26 carcinoma cells stably expressing the luciferase gene. Tumor volumes were quantified by bioluminescence imaging before, during, and after fractionated lung radiation (2 Gy, daily × 10 days during a 2-week period, total of 20 Gy) with concurrent TNFR1-ASO treatment as described above. Under the same conditions that produced normal lung protection, TNFR1-ASO (RT+ASO) did not decrease the effectiveness of radiation in controlling tumor growth (Fig. 6). This was in contrast to the rapid tumor progression in control mice in which tumor volume increased by 4-, 16-, and 29-fold at 4, 5, and 6 weeks, respectively (Fig. 6B). These findings suggest that TNF-α inhibition has the potential to protect normal tissues from radiation injury without protecting tumors.

**Discussion**

Our previous studies showed that radiation induces liver damage through TNF-α–mediated apoptosis, and specific inhibition of TNFR1 is radioprotective for the liver. Here, we found that single fraction or fractionated radiation also increased lung TNF-α expression, which correlated with apoptosis of normal cells in the lung. TNFR1 knockout mice and normal mice administered ASO against TNFR1 were more resistant to radiation-induced lung function damage. Taken together, our findings show that inhibition of TNFR1 can reduce radiation-induced apoptosis and lung function damage without compromising tumor control.

Our findings offer a novel strategy for selective radioprotection of the lung. Amifostine decreases radiation toxicity by scavenging free radicals produced immediately after radiation to the parotid gland (4, 5). Its use is limited by its toxicity (36) and the requirement that it be administered at the time of radiation. The recent development of manganese superoxide dismutase (MnSOD) gene therapy resulted in significant improvement in the radioprotection of various organs, including the lung (37), esophagus (38), and oral cavity (39), by stabilizing the mitochondrial membrane (40); however, its effectiveness relies on the efficiency and specificity of gene expression in target cells. Another relevant approach is the use of D-methionine (MRX-1074), a dextro isomer of the amino acid l-methionine that can be used orally to increase intracellular production of key antioxidants and reduced glutathione (41) based on the concept that normal and tumor cells have functionally distinct and inherent differences in the mitochondria biology (42). It has not yet been assessed in a lung model. One of the most important advances in lung radioprotection is the finding that radiation increases TGF-β1 and its receptor TβR activation, leading to lung fibrosis and functional damage. Inhibition of this pathway has been shown to significantly reduce lung fibrosis and improve lung function after radiation. However, treatment has not been completely effective. In addition, expression of a kinase-deficient TβR2 in transgenic mice leads to elevated pulmonary fibrosis regardless of radiation (17), suggesting that other mechanisms are involved in lung fibrogenesis in addition to the TGF-β1 pathway.

Our data show that intervening in the early tissue response to radiation, such as TNF-α–mediated apoptosis in the normal lung, may result in functional improvement at the later stage of radiation treatment. This delayed protective effect can be explained by various potential mechanisms. First, a single dose or fraction of radiation that can induce transient production of cytokines, in particular, TNF-α and TGF-β1, causes delayed sequential elevation of TNF-α and TGF-β1 in mice months after initial radiation (43). Sustained inhibition of TNFR1 by ASO, which was observed for at least 3 days after a single injection
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(data not shown), could potentially interrupt the linkage between early and latent TNF-α production, and thus exert a prolonged protection effect. Second, activation of the TNF-α pathway contributes to various biological outcomes, independently or interactively with other cytokine activation. The most relevant mechanism in radiation-induced lung toxicity is the involvement of the TGF-β1 pathway in lung fibrosis. Both pathways may share the same signaling molecule, such as c-Jun-NH₂-kinase, followed by an individual transduction pathway, that is, cytochrome c release from mitochondria (favoring TGF-β1 action) or caspase-3 activation (favoring TNF-α action; ref. 44), both leading to cell apoptosis. Also, both pathways can promote plasminogen activator inhibitor-1 gene expression in adipocytes through protein kinase C signaling (45). Therefore, it is reasonable to speculate that early TNF-α activation during a course of radiation may contribute to the latent activation of TGF-β1, where its role in lung fibrosis is well studied. Third, TNF-α itself most likely plays a role in lung fibrosis based on the fact that TNF-α can enhance expression of the collagen gene in human fibroblasts synergistically with TGF-β1 (46).

Understanding this role of TNF-α is particularly important during fractionated radiation where sustained TNF-α production may contribute substantially to lung fibrosis in addition to TGF-β1-mediated effects.

The role of TNF-α in radiation lung toxicity may be complicated by the presence of lung tumors that can produce additional cytokines, or their growth can be influenced by the change in TNF-α activation. In the current study, we showed that inhibition of TNFFR1 by ASO protects normal lung against radiation whereas radiosensitivity of tumor is not altered under the same treatment regimen. It is most likely that tumor killing is the direct outcome of radiation-induced cytotoxicity to the tumor cells independent of TNF-α-mediated apoptosis. Although we failed to detect tumor-derived TNF-α production after radiation in a separate study using s.c. colon cancer xenografts (data not shown), it should be noted that cancer cells of various types or their presence in the lung microenvironment may alter the response of tumor or normal lung to radiation. Thus, TNF-α-targeted radioprotection may show tumor specificity based on tumor cell production of TNF-α or TNFR1.

Radiation induces lung damage through a multiple-phase mechanism where various cytokines interact in a time-dependent fashion, leading to injury. In this study, we showed that radiation causes lung TNF-α production and that specific inhibition of TNFFR1 by ASO is radioprotective for the normal lung and not intrathoracic tumor. These findings provide a strong rationale for developing TNF-α-targeted radioprotection strategy for lung cancer patients. Specific inhibitors of TNF-α, such as Etanercept, are now used clinically for treating patients with arthritis, a disease that is mediated by elevation of local TNF-α (47, 48). Furthermore, development of radioprotectors based on TGF-β1 inhibition has generated promising results in preclinical studies. It is of particular interest to understand whether both cytokines mediate lung toxicity by distinct or shared mechanisms and whether combination treatment will be more effective than monotherapy.

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References


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