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Targeting Tumor Necrosis Factor Alpha to Mitigate Lung Injury Induced by Mustard Vesicants and Radiation

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Systematic Review

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Abstract

Pulmonary injury induced by mustard vesicants and radiation is characterized by DNA damage, oxidative stress, and inflammation. This is associated with increases in levels of inflammatory mediators, including tumor necrosis factor (TNF)α in the lung and upregulation of its receptor TNFR1. Dysregulated production of TNFα and TNFα signaling has been implicated in lung injury, oxidative and nitrosative stress, apoptosis, and necrosis, which contribute to tissue damage, chronic inflammation, airway hyperresponsiveness, and tissue remodeling. These findings suggest that targeting production of TNFα or TNFα activity may represent an efficacious approach to mitigating lung toxicity induced by both mustards and radiation. This review summarizes current knowledge on the role of TNFα in pathologies associated with exposure to mustard vesicants and radiation, with a focus on the therapeutic potential of TNFα-targeting agents in reducing acute injury and chronic disease pathogenesis.

Mustard vesicants and ionizing radiation are cytotoxic to the lung, causing progressive injury at low to moderate doses and lethality at high doses.[1–3] Acute lung injury, pulmonary edema, respiratory epithelial necrosis and sloughing, and pneumonitis are noted within days to weeks of exposure, whereas chronic bronchitis, asthma, bronchiectasis, chronic obstructive pulmonary disease (COPD), pulmonary fibrosis, and/or cancer are observed 6–12 months post-radiation or 10–30 years after mustard exposure.[3,4] A common feature of acute injury and chronic disease induced by mustards and radiation is an accumulation of inflammatory cells within the lung and release of cytokines such as tumor necrosis factor (TNF)α.[5–7] TNFα is an early response cytokine important in initiating inflammatory responses; it also promotes cellular proliferation and tissue regeneration.[8] Excessive production of TNFα is associated with uncontrolled inflammation and disease pathogenesis. In this context, elevated levels of TNFα have been identified in a number of inflammatory diseases, including COPD, rheumatoid arthritis, psoriasis, and inflammatory bowel disease; moreover, the administration of biologics, which block TNFα, has demonstrated therapeutic efficacy against various pathologies and diseases.[9,10] In this review, we discuss the role of TNFα in mustard vesicant- and radiation-induced pulmonary disease pathogenesis, with a focus on the therapeutic potential of TNFα-targeting agents in mitigating toxicity.

TNFα Production, Receptors, and Biological Activity

TNFα is primarily produced by macrophages in response to tissue injury or infection.[11,12] Synthesized as a transmembrane homotrimer consisting of three 26 KDa subunits (mTNFα), it is cleaved by TNFα-converting enzyme (TACE) to soluble TNFα (sTNFα), a homotrimer consisting of three 17 KDa subunits.[13] The activity of both mTNFα and sTNFα is mediated by binding to cell surface receptors identified as type 1 (TNFR1) and type 2 (TNFR2) (Figure 1). TNFR1 is expressed on the surface of most cell types, whereas TNFR2 is largely restricted to immune cells and endothelial cells.[12] Both forms of TNFα bind to TNFR1 and TNFR2. However, TNFR2 binds to TNFα with lower affinity and may easily dissociate from the ligand.[12,14] Thus, it appears that the biological activity of TNFα mainly involves TNFR1 signaling.[10,15,16] Reports also suggest that signaling pathways activated by these 2 receptors overlap or they transduce signaling cooperatively as genetic deletion of either receptor blocks signaling initiated by TNFα.[15,17] Ligand binding to TNFR1 initiates signaling, resulting in the activation of mitogen activated protein (MAP) kinases and transcription factors, including AP-1 and nuclear factor-kappa B (NF-κB), which regulate genes involved in inflammation, cell proliferation, and differentiation.[10,15] TNFR1 also recruits TNFR1-associated death domain (TRADD) protein, which promotes cell death. TNFR2 recruits TNFR-associated factor (TRAF)-1 and TRAF-2

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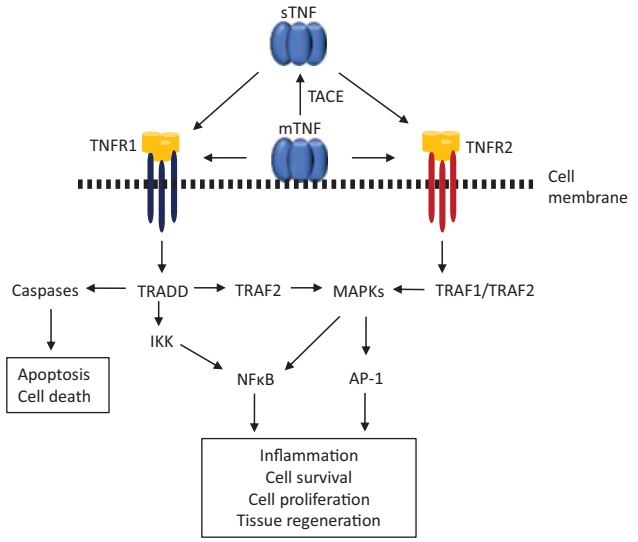

Figure 1. Tumor necrosis factor (TNF)α signaling. Binding of soluble or membrane bound TNFα to TNFR1 and TNFR2 initiates signaling events associated with apoptosis or activation of transcription factors, NFκB and AP-1. TNFα binding to TNFR1 and/or TNFR2 can also activate protein kinase B/Akt, which leads to prolonged NF-κB activation. Together, these responses contribute to inflammation, leukocyte trafficking, cell death, cell proliferation, and tissue remodeling.
AP-1, activator protein-1; IKK, IκB kinase; MAPKs, mitogen-associated protein kinases; mTNF, membrane-bound TNFα; NFκB, nuclear factor kappa B; sTNF, soluble TNFα; TACE, TNFα-converting enzyme; TNFR1, TNFα receptor 1; TNFR2, TNFα receptor 2; TRADD, TNFR associated death domain; TRAF1, TNFR associated factor 1; TRAF2, TNFR associated factor 2.

proteins, resulting in the activation of MAP kinases, NF-κB, and protein kinase B.[10,15] Functionally, TNFR1 activation is associated with the induction of cytotoxic and proinflammatory responses of TNFα, whereas TNFR2 mediates homeostatic bioactivities, including tissue regeneration, cell proliferation, and cell survival.[15,18]

TNFα is a master regulator of inflammation generated early after injury or infection in response to bacterially derived lipopolysaccharide, as well as interleukin (IL)-1, interferon-γ, granulocyte macrophage colony stimulating factor, platelet derived growth factor, and TNFα itself.[19–21] The biological actions of TNFα are varied and summarized in Table 1. TNFα promotes inflammation by upregulating adhesion molecules important in leukocyte trafficking to inflammatory sites, including intracellular leukocyte adhesion molecule, endothelial leukocyte adhesion molecule-1, and vascular cell adhesion molecule-1, and by stimulating the release of macrophage and neutrophil chemokines, such as CXCL8 (IL-8), CCL2 (MCP)-1, interferon-inducible protein 10 (IP-10) (CXCL10), as well as bioactive lipids (eg, eicosanoids and platelet activating factor), which promote vasodilatation, leukocyte adhesion, and chemotaxis.[12,22–25] TNFα also stimulates phagocytic cells to release proinflammatory cytokines (eg, IL-1, IL-6, IL-12, IL-15, IL-23, and TNFα) and reactive oxygen and nitrogen species. In the lung, these cytotoxic/proinflammatory mediators cause alveolar epithelial cell injury, denudation of the basement membrane, hyalin membrane formation, impaired surfactant activity, and altered pulmonary functioning.[19,26,27]

TNFα is known to cause oxidative and nitrosative stress.[28,29] It also depletes intracellular glutathione, which contributes to its pro-oxidant actions.[28,30] Oxidative stress is associated with activation of redox sensitive transcription factors, including NF-κB and AP-1 that upregulate proinflammatory genes, further contributing to inflammation and tissue injury.[11] TNFα is also a potent mitogen, stimulating epithelial cell proliferation.[31] This is thought to be due in part to activation of AP-1 and upregulation of cyclin-D1, a cell cycle regulatory protein.[28,32] TNFα-induced proliferation leads to epithelial thickening and pulmonary fibrosis.[33,34] TNFα also promotes fibrosis by inducing focal accumulation of fibroblasts and collagen deposition and by stimulating the production of matrix metalloproteinases (MMPs) and transforming growth factor (TGF)β.[34–37] In humans, circulating levels of MMP-9 and TGFβ correlate with the extent of fibrosis.[38] Collectively, these data suggest that blocking TNFα may be efficacious in mitigating mustard or radiation-induced acute lung injury and inflammation, as well as their long-term pulmonary complications.

Role of TNFα in Mustard-Induced Lung Injury

Mustard vesicants, including sulfur mustard (SM) and nitrogen mustard (NM), are cytotoxic alkylating agents that cause incapacitating injury to the respiratory tract.[39] Toxicity is largely due to its lipophilic nature that allows it to rapidly penetrate tissues and cells, and alkylate and cross-link cellular macromolecules, including nucleic acids, lipids, and proteins.[40] Both conducting and respiratory airways are affected by mustards.[1,41] Early symptoms include cough, hoarseness, sore throat, mucus discharge, loss of smell and taste, and irritation of the nasal mucosa.[1,42–44] Pulmonary edema and damage to the pharynx induced by acute mustard inhalation result in an inability to speak, moist rales, tachypnea, and tachycardia.[45,46] At high doses, necrosis of the respiratory epithelium, epithelial sloughing, pseudo-membrane formation, lung lobe collapse, and death occur.[41,43,47,48]

Chronic clinical and pathological manifestations of mustard exposure have been observed in survivors of chemical attacks and in manufacturing plant workers. The most common symptoms in long-term survivors of mustard gas exposure are chronic cough, dyspnea, increases in sputum and hemoptysis (airway bleeding), progressive airway deterioration, hyperreactivity, and stenosis of the conducting airways.[1,40,49,50] Common pathologies in victims of the Iran–Iraq war include asthma, bronchitis, bronchiectasis, airway narrowing, COPD, and pulmonary fibrosis.[40,50] Emphysema, bronchiectasis, centrilobular nodules, bronchial wall thickening, reticular opacity, ground glass opacity, consolidation, honeycombing, and other respiratory pathologies have similarly been reported in survivors of mustard gas exposure in a manufacturing factory.[40,51–53]

Pulmonary injury from mustard exposure is associated with an accumulation of large numbers of inflammatory cells, including macrophages, neutrophils, and eosinophils at sites of injury in the lung, as well as oxidative stress and production of cytotoxic/proinflammatory cytokines, including TNFα.[4,40,54,55] Levels of TNFR1 are also upregulated, suggesting a role of TNFα signaling through this receptor in the pathogenic response to mustards.[56] Accumulating evidence described below provides support for this activity.

Rodent Models of Mustard Lung Injury Used to Investigate the Impact of Targeting TNFα

In initial mechanistic studies, 2-chloroethyl ethyl sulfide (CEES), a monofunctional analog of SM and NM, was used as a model for mustard lung toxicity. In rodents, CEES causes injury to the alveolar epithelial barrier as measured by increases in cells, protein, and IgM in bronchoalveolar lavage fluid (BAL); fibrinogen and prothrombin levels also increase, a response associated with

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Table 1. Biological activities of tumor necrosis factor (TNF)α in mustard or radiation-induced lung injury

Activity	Mustards	Radiation	References
Oxidative stress	SOD, HO-1, Ym-1, lipocalin-2, 8-hydroxy-2-deoxyguanosine, malondialdehyde, 4-hydroxynonenal, nitrite/nitrates	HO-1, lipocalin-2, Ym-1, superoxide anions, hydroxyl radicals, H_2O_2, NO	60,62,66,67,76,78,82, 84,85,88,97,103,104, 114,125
Inflammatory proteins	iNOS, COX-2, sRAGE, HMGB-1, prostaglandins	COX-2, iNOS, PGI2, TXA2	56,60,62,67,73,76,78, 82,86,88,97,111,114, 125,126
Cytokines/ chemokines	IL-1, IL-2, IL-8, IL-6, IL-12, IL-10, TNFα, IFNγ, KC/GRO (CXCL1), CCR2, CCR5, CCL2, CCL3, CCL5, CCL11, CX3CR1, fractalkine	IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-13, KC/GRO (CXCL1), TNFα, CCL2, CCL3, CXCL13, CCR1, CXCR2	6,61,62,67,78,79,81, 82,84,104,109,110, 114,126
Cell injury/ DNA damage/ apoptosis/ autophagy	Caspase-3, 6, 8, 9, Fas L, Fas R, PARP-1, LC3BI, LC3BII, γH2A.X, fibrinogen, fibrin	Caspase-3, PARP-1, p53, γH2A.X	67,76,77,104,113,114, 126,127
Cell proliferation	PCNA, cyclin D1,	Cyclin D1	59,67,78,87,88,104,128
Tissue remodeling	MMP-9, MMP-10, TGFβ, MR, Gal-3, Arg, CTGF, α-SMA	TGFβ, PDGF, CTGF, FGF, Arg1, TIMP-1	67,76,79,81,84,97,104, 109,110,125,126

Arg, arginase; CCL, C-C chemokine ligand; CCR, C-C chemokine receptor; COX, cyclooxygenase; CTGF, connective tissue growth factor; CXCL, C-X-C chemokine ligand; CXCR, C-X-C chemokine receptor; Fas L, Fas ligand; Fas R, Fas receptor; FGF, fibroblast growth factor; γH2A.X, histone variant H2A.X; HMGB, high mobility group box; HO, heme oxygenase; H_2O_2, hydrogen peroxide; IFN, interferon; IL, interleukin; iNOS, inducible nitric oxide synthase; KC/GRO, keratinocyte chemoattractant/human growth-regulated oncogenes; LC3B, light chain 3B; MMP, matrix metalloproteinase; MR, mannose receptor; Gal, galactin; NO, nitric oxide; PARP, poly (ADP-ribose) polymerase; PCNA, proliferating cell nuclear antigen; PDGF, platelet-derived growth factor; PGI_2, prostacyclin; SMA, smooth muscle actin; SOD, superoxide dismutase; sRAGE, soluble receptor for advanced glycation end product; TGF, tumor growth factor; TIMP, tissue inhibitors of metalloproteinases; TNF, tumor necrosis factor; TXA, thromboxane A.

impairment of fibrin-degrading activity in the lung.[56,57] Proliferating cell nuclear antigen (PCNA), a marker of cellular proliferation in response to injury,[58] is upregulated after CEES administration to rodents, along with cyclin D1.[59] CEES also causes pulmonary oxidative stress, characterized by increases in superoxide dismutase (SOD), Ym-1, and lipid peroxidation end products and decreases in intracellular glutathione levels.[60–62] Inflammatory proteins, including inducible nitric oxide synthase (iNOS), cyclooxygenase (COX)-2, TNFα, TNFR1, and CCR2 are also increased in the lung after CEES.[56,61,62] These responses are linked to functional alterations, including decreases in lung compliance and increases in elastance.[56,62] Additionally, methacholine-induced alterations in total lung resistance and central airway resistance are dampened following CEES exposure.

More recently, rodent models of lung injury and chronic disease using NM and SM have been developed as they more closely reflect pulmonary responses in humans.[4,63] In general, injury, oxidative stress, and inflammatory responses are similar to CEES, appearing early (1–3 days) after exposure; however, they are more severe and prolonged. Additionally, pulmonary fibrosis is observed, typically within 28 days of exposure. The increased pathologic response to SM and NM when compared to CEES is likely due to the fact that they are bifunctional alkylating agents, allowing them to induce DNA intrastrand and interstrand cross-links, as well as DNA-protein cross-links.[64] These cross-links can alter the structure of DNA and interfere with replication and transcription. In contrast, monofunctional alkylation of DNA and proteins caused by CEES can more readily be repaired.[65]

Owing to high reactivity of SM and NM with mucosal surfaces of the upper respiratory track, rodent models of lung injury involve intratracheal exposure to ensure delivery to the lower lungs, where most damage occurs in humans.[66–69] A single exposure of rats to SM or NM causes dose and time-related histopathological changes in the lungs, including multifocal lesions comprising perivascular and peribronchial edema, blood vessel hemorrhage, patchy mild thickening of alveolar septa, increased numbers of alveolar macrophages and neutrophils, and luminal accumulation of cellular debris and fibrin.[66,67,70–73] Bronchiolization of alveolar walls, indicating type I epithelial cell damage and repair by type II epithelial cells, hyperplasia, and hypertrophy of the bronchial epithelium leading to piling of bronchiolar epithelial cells have also been noted. SM also causes severe ulceration of the proximal bronchioles and deposits of fibrillar membranes in bronchiolar lumen, suggesting apoptosis and necrosis.[67,74] Consistent with early SM-induced histopathologic evidence of acute lung injury and bronchiolar epithelial denudation, proteins involved in cell apoptosis and autophagy, including caspase-3, caspase-6, caspase-8, caspase-9, poly (ADP-ribose) polymerase (PARP)-1 and LC3BI and LC3BII, are upregulated in the lung; terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) positive cells are also present.[75–77]

The chronic phase of respiratory injury caused by SM and NM in rats (beginning 28 days post-exposure) is characterized by a predominance of fibroblasts, neutrophils, lymphocytes, and enlarged foamy macrophages in the alveoli and/or alveolar septal walls, multifocal fibrotic lesions, and collagen deposition.[40,78–80] Fibroplasia, squamous metaplasia of the bronchial wall, honeycombing, and emphysema-like changes in alveolar regions of the lung are also evident.[40,67,68,81] Hyperplasia and squamous metaplasia in ulcerated proximal bronchiolar epithelium are also observed after SM exposure, indicative of aberrant wound repair.[67] These structural changes are correlated with impaired pulmonary functioning.[80,82] As observed with CEES, NM- and SM-induced lung injury and apoptosis/necrosis of epithelial cells are associated with increases in BAL cells, protein, IgM, fibrinogen, and total phospholipids.[66,67,82] Levels of fibrinogen/fibrin and surfactant protein (SP)-D are also increased in the lung and/or BAL. This is evident early after exposure and remains elevated up to 28 days.[67,82,83]

Lung injury induced by SM and NM is associated with oxidative stress characterized by increases in 8-hydroxy-2-deoxyguanosine (8OHdG), 2-deoxyguanosine, malondialdehyde and 4-hydroxy-nonenal, and decreases in glutathione levels.[66,84,85] Nitrates and

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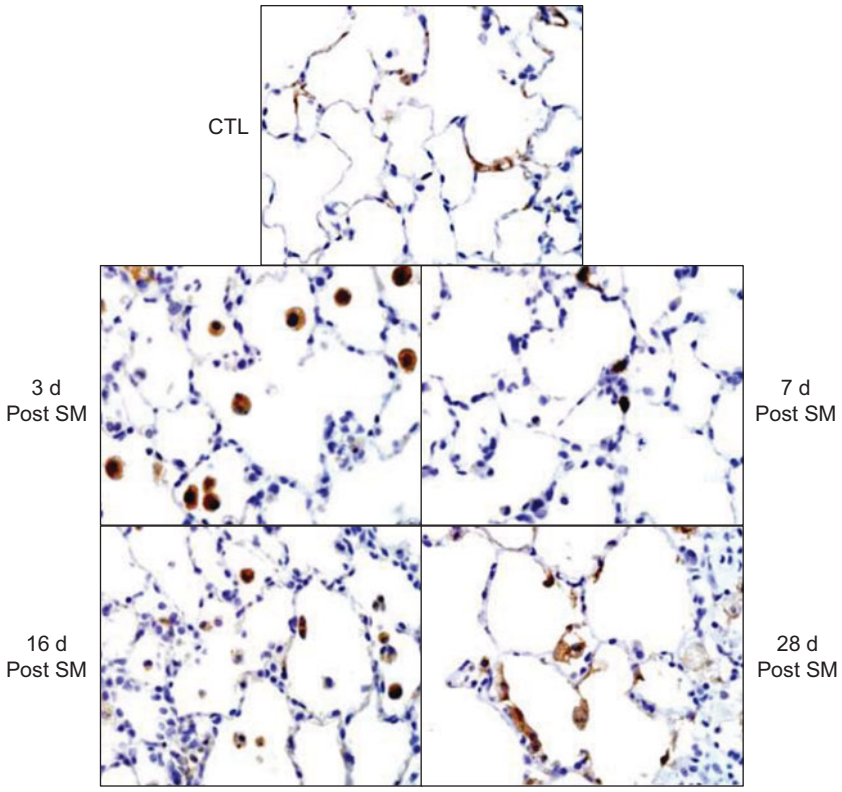

Figure 2. Effects of sulfur mustard on TNFα expression in the lung. Rats were exposed by intratracheal inhalation to air (CTL) or sulfur mustard (SM, 0.4 mg/kg) as previously described.[67] Lung sections were prepared 3, 7, 16, and 28 days later and immunostained with anti-TNFα antibody. Binding was visualized using a Vectastain kit. Original magnification, 600X. Representative images from 8–9 rats/group are shown.

nitrites also increase in BAL and urine.[84,85] Additionally, antioxidants such as heme oxygenase (HO)-1, lipocalin-2, Ym-1, and Mn-SOD are upregulated, a response that persists for at least 28 days post-SM or NM exposure.[66,67,86] A marker of DNA damage, γH2A.X, and PCNA are also detectable in the lungs.[67,87,88] Whereas γH2A.X increases 1–3 days after mustard exposure, PCNA is increased 3–28 days in bronchiolar epithelium, alveolar epithelial cells, interstitial cells, and in focal areas exhibiting honeycombing and/or fibrosis.

Inflammatory genes/proteins, including IL-1, IL-2, IL-6, TNFα, KC/GRO, CCR2, CCR5, CCL2, CCL3, CCL5, CCL11, CX3CR1, CX3CL1, high mobility group box (HMGB)1, and MMP-9, are evident in the lungs and/or BAL fluid from mustard-treated rodents within 1–3 days post-exposure.[67,79,81,84] Proinflammatory macrophages expressing TNFα (Figure 2), iNOS, MMP-9, HMGB1, or COX-2 are also present at this time.[67,82,89] Whereas NM-induced increases in expression of inflammatory markers are maximum at 3 days post-exposure, persisting at lower levels up to 28 days, the response to SM is biphasic.[67,88] Thus, SM exposure causes an early increase in inflammatory markers at 1–3 days, which is followed by a decrease at 7–16 days, and then a generally more robust increase at 28 days. Antiinflammatory/profibrotic genes (IL-10, pentraxin-2, connective tissue growth factor [CTGF], ApoE) have also been identified in the lungs; however, the timing of their appearance varies with the gene.[79] Antiinflammatory macrophages characterized by expression of CD206 (mannose receptor), CD68, CD163, galectin-3, and arginase-II are also present in histologic lung sections most prominently at later time points.[67,78,79] This is correlated with the upregulation of α-smooth muscle actin, TGFβ, platelet-derived growth factor (PDGF), PDGF receptor-α, and CTGF.[68,78,81]

Loss of TNFR1 Mitigates Half-Mustard-Induced Lung Injury

As indicated above, TNFR1 is the major receptor mediating the proinflammatory actions of TNFα. In initial studies, to assess the potential role of TNFα in mustard-induced lung injury, mice lacking TNFR1 were used.[56] In these studies, CEES was used as an experimental model vesicant. TNFR1$^{-/-}$ mice exhibited an attenuated response to CEES-induced lung injury, oxidative stress, and inflammation; thus, expression of oxidative stress markers and inflammatory proteins was reduced or delayed. Loss of TNFR1 also blunted aberrant functional responses to CEES. These findings provided initial evidence for a role of TNFα in mustard lung toxicity.[56]

Pharmacologic Inhibition of TNFα Mitigates Mustard-Induced Lung Injury, Inflammation, and Fibrosis

In further studies, the effects of pharmacologic inhibition of TNFα on mustard toxicity were assessed. Pentoxifylline (PTX) is a nonspecific phosphodiesterase inhibitor with anti-inflammatory activity, due largely to its ability to block TNFα synthesis.[90–92] PTX has been reported to blunt TNFα production by alveolar macrophages from patients with sarcoidosis.[93] PTX is also clinically efficacious in a number of inflammatory pathologies characterized by excessive TNFα production, including alcoholic liver disease and rheumatoid arthritis.[94–96] Treatment of rats with PTX (46.7 mg/kg, ip) daily for 3 days was found to reduce acute

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lung injury and inflammation induced by NM.[97] Thus, granulocyte infiltration into the lung is blunted, along with edema, fibrin deposition, and fibroplasia; BAL protein and cell numbers are also significantly reduced. PTX also reduces NM-induced oxidative stress and numbers of proinflammatory macrophages in the lung, while increasing numbers of antiinflammatory macrophages. This correlates with persistent upregulation of markers of wound repair, including pro-SP-C and PCNA. These data support the idea that targeting TNFα using more specific inhibitors represents a potentially efficacious approach for treating mustard lung toxicity.

Biologics against TNFα are widely used clinically to treat immunoinflammatory diseases, including rheumatoid arthritis, psoriasis, and Crohn's disease, with minimal toxicity.[98,99] TNFα blocking agents have also proven beneficial in patients with the lung diseases such as severe asthma, COPD, and sarcoidosis.[19,100] Based on these findings, anti-TNF antibody has been evaluated as a countermeasure against mustard lung toxicity. Treatment of rats with anti-TNFα antibody (15 mg/kg, IV, every 8 days) blunts mustard-induced structural alterations in the lung at all post-exposure times (3–28 days) examined.[78,86] Thus, parenchymal lesions are reduced in size and intensity, and deposits of plasma proteins decreased; occlusion of the bronchiolar lumen by fibrillar membrane, ulceration of bronchial epithelium, acute inflammation, edema, bronchoalveolar, and goblet cell hyperplasia and hypertrophy, bronchiectasis, interstitial thickening, macrophage accumulation, squamous cell metaplasia, mesothelial cell proliferation, and emphysema are attenuated.[78,86] Anti-TNFα antibody also reduces NM-induced collagen deposition, peribronchial and parenchymal fibrosis, and numbers of fibrotic lesions in the lung.[78]

Further studies demonstrated that anti-TNFα reduces mustard-induced alveolar-epithelial barrier dysfunction, oxidative stress, and increases in inflammatory proteins and profibrotic cytokines in the lung, along with the numbers of proinflammatory macrophages, while antiinflammatory macrophages important in wound healing are increased or unaffected.[78,86] Small live animal imaging techniques, including magnetic resonance imaging (MRI) and computed tomography (CT) imaging, confirmed the efficacy of anti-TNFα antibody in blunting NM-induced lung injury and fibrosis.[101] Thus, anti-TNFα antibody treatment of rats was found to reduce the percentage of injured lung within 1 day of NM exposure and subsequent development of fibrosis. Together, these data demonstrate that inhibiting TNFα represents an efficacious approach to mitigating acute lung injury, inflammatory macrophage activation, oxidative stress, and lung remodeling induced by mustard vesicants.

Role of TNFα in Radiation-Induced Lung Injury

Radiation exposure causes acute lung injury, which progresses to pneumonitis within weeks to months and fibrosis within months to years.[102] Early injury is characterized by damage to the alveolar wall, interstitial edema, and an accumulation of proteins and inflammatory cells in the lung lining fluid; this is followed by thickening of alveolar walls, solid lesions with collagen deposits, and bronchiectasis as the pathology develops. Mechanistically, radiation-induced lung injury involves DNA damage and the generation of cytotoxic reactive oxygen and nitrogen species.[103,104] This is associated with loss of epithelial and endothelial barrier function, an accumulation of inflammatory cells in the lung that produce mediators such as IL-1, IL-6, IL-13, IL-17, TNFα, and TGFβ that can further damage the tissue and/or contribute to tissue remodeling and fibrogenesis.[102,105–110] Radiation also affects pulmonary endothelial cell function as measured by decreases in the activity of angiotensin converting enzyme (ACE) and plasminogen activator (PLA).[111] This is accompanied by increases in lung wet weight, protein and hydroxyproline content, and eicosanoids.

Radiation-induced lung injury is characterized by increases in TNFα and TNFR1.[112–114] This is observed early (1–3 hours) after exposure to a single dose of radiation[6,113] and aligned with increases in numbers of TUNEL-positive epithelial cells and upregulation of cleaved capase-3, markers of apoptosis. Radiation-induced increases in TNFα persist in the lung up to 24 hours post-exposure; subsequently, TNF levels return to baseline. This is followed by secondary, more exaggerated increases 2–24 weeks post-exposure coinciding with radiation-induced histopathological changes in the lung, including diffuse alveolitis, inflammatory cell accumulation, thickening of alveolar walls, depletion of type II epithelial cells, fibroblast proliferation, and interstitial and alveolar deposition of extracellular matrix.[6,106,108,114–118] Fractionated radiation exposure (single high dose divided into low dose radiation over several days) is also associated with increases in TNF levels at early times but at reduced levels when compared to a single high dose. Cumulative TNFα levels after fractionated radiation, however, are greater and more persistent.[113] Of note, early increases in TNFα precede radiation-induced increases in IL-1α and IL-6, suggesting that TNFα plays a role in the initiation of the inflammatory cytokine cascade.[6]

Blocking TNFα Mitigates Radiation-Induced Lung Injury, Pneumonitis, and Fibrosis

Treatment of mice with a TNFR1-specific antisense oligonucleotide (ASO) has been reported to reduce radiation-induced increases in TUNEL-positive cells and expression of cleaved caspase-3.[113] This correlates with a reduction in collagen deposition and restoration of pulmonary function 8 weeks post-exposure. Mice lacking TNFR1 are also resistant to radiation-induced alterations in lung function.[113] Similarly, gene therapy using a plasmid vector encoding mouse soluble TNFR1 (psTNFR1) reduces radiation-induced lung fibrosis and mortality.[112] In response to radiation, mice bearing mutations in the TNFα signaling pathway also exhibit an attenuated breathing rate.[116]

Pharmacologic inhibition of TNFα has also been found to mitigate radiation-induced lung injury and inflammation. For example, Rube et al. (2002)[119] reported that PTX downregulates radiation-induced increases in lung TNFα. PTX treatment has also been reported to delay radiation-induced apoptosis in the lung.[118] Relatively greater numbers of SP-D expressing cells, which are important in suppressing pulmonary inflammatory responses, have been noted 1–5 weeks after radiation exposure in mice returning to control levels after 8–12 weeks.[118] PTX treatment significantly enhances numbers of SP-D expressing cells at all time points examined after radiation exposure. This is associated with a delayed accumulation of neutrophils in the lung and a reduction in radiation-induced alveolar septal thickness up to 12 weeks after exposure.[118,120] PTX treatment also reduces radiation-induced increases in lung wet weight and protein content and improves lung perfusion, which enhances tissue oxygenation and wound healing.[111,121,122] Taken together, these findings show that TNF plays a role in radiation-induced lung injury; moreover, inhibition of TNFα or TNFR1 can mitigate the deleterious effects of radiation. Further investigations on the efficacy of biologics against TNFα may lead to new treatments for radiation-induced lung pathology.

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Conclusions

TNFα is a key mediator of local damage and inflammation in the lung. Both mustard vesicant- and radiation-induced lung injury are associated with increases in TNFα. A wide range of responses, including apoptosis, mitosis, chemotaxis, angiogenesis, extracellular matrix production, and release of cytokines and chemokines, are triggered when TNFα binds to its receptor on target cells. Given that tissues and cells are exposed to complex mixtures of inflammatory mediators, it is likely that blocking TNFα immediately after the injury may be beneficial. As increases in TNFα have been linked to both acute and chronic manifestations of toxic injury, multiple sequential doses of biologics against TNFα may be required to keep inflammation in check. Anti-TNFα biological therapies have been used to treat immuno-inflammatory diseases of the skin, joints, and gut. These treatments have also been effective to varying degrees in patients with chronic lung diseases.[19,100,123,124] Further research and clinical investigation with anti-TNFα therapy in acute and chronic pulmonary toxicity induced by mustard vesicants and radiation may prove useful for the development of successful treatment strategies using TNF targeting agents.

Author contributions. Rama Malaviya: literature search, original manuscript draft writing, review & editing; Jeffrey D. Laskin: manuscript review & editing; Rita Businaro: manuscript review & editing; Debra L. Laskin: conceptualization, funding acquisition, manuscript review & editing.

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