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Review

Science review: Redox and oxygen-sensitive transcription factors in the regulation of oxidant-mediated lung injury: role for nuclear factor-κB

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Abstract

The primary role of pulmonary airways is to conduct air to the alveolar epithelium, where gas exchange can efficiently occur. Injuries to airways resulting from inhalation of airborne pollutants and parenteral exposure to ingested pollutants that cause oxidative stress have the potential to interfere with this process. A progressive rise of oxidative stress due to altered reduction–oxidation (redox) homeostasis appears to be one of the hallmarks of the processes that regulate gene transcription in lung physiology and pathophysiology. Reactive metabolites serve as signaling messengers for the evolution and perpetuation of the inflammatory process that is often associated with cell death and degeneration. Redox-sensitive transcription factors are often associated with the development and progression of many human disease states and inflammatory-related injury, particularly of the lung. The present review elaborates on the role of the redox-sensitive and oxygen-sensitive transcription factor NF-κB in mediating lung injury. Changes in the pattern of gene expression through regulatory transcription factors are crucial components of the machinery that determines cellular responses to oxidative and redox perturbations. Additionally, the discussion of the possible therapeutic approaches of antioxidants, thiol-related compounds and phosphodiesterase inhibitors as anti-inflammatory agents will thereby help understand the oxidant/redox-mediated lung injury mechanisms.

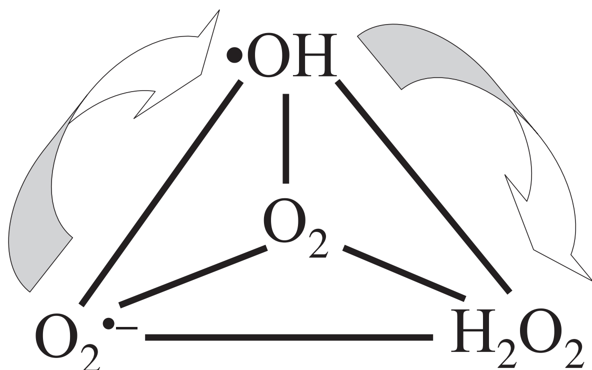
Keywords antioxidant, injury, lung, NF-κB, oxygen, redox, transcription factors

Molecular oxygen is an environmental signal that regulates cellular energetics, development and differentiation [1]. Oxygen plays univalent roles: while it is indispensable to obtain the essential chemical energy in the form of ATP, it is often transformed into highly reactive forms that are deleteriously toxic (Fig. 1). To defend themselves from the cytotoxic actions of free radicals, cells have acquired multiplicity in endogenous antioxidant systems. These defense mechanisms include reduction–oxidation (redox) enzymatic systems and combating antioxidant molecules [1]. The term 'oxidative regulation' has thus been proposed to indicate the active role of redox modifications of proteins in regulating their functions. Redox reactions of biomolecules, mostly proteins, used to be considered as 'oxidative stress' are now considered as 'signals', and they contain biological information that is necessary for maintaining cellular homeostasis [1,2]. Altering gene expression is the most fundamental way for a cell to respond to extracellular signals and/or changes in its environment.

Regulation of the signaling responses is governed at the genetic level by transcription factors that bind to control regions of target genes and alter their expression.

AP-1 = activating protein-1; ARDS = acute respiratory distress syndrome; BALF = bronchoalveolar lavage fluid; CF = cystic fibrosis; CREB = cAMP-responsive element binding protein; EMSA = electrophoretic mobility shift assay; ICAM-1 = intercellular adhesion molecule-1; IFN = interferon; IκB-α = inhibitory-κB alpha; IL = interleukin; iNOS = inducible nitric oxide synthase; LPS = lipopolysaccharide–endotoxin; MnSOD = manganese superoxide dismutase; NF-κB = nuclear factor-κB; PDTC = pyrrolidine dithiocarbamate; RANTES = regulated upon activation, normal T-cell expressed and secreted; redox = reduction–oxidation; ROS = reactive oxygen species; Sp-1 = serum protein-1; TNF = tumor necrosis factor.

Figure 1



Molecular oxygen and its revolving triangular axis of reactive species and free radicals.

Transcription factors are endogenous substances, usually proteins, that are effective in the initiation, stimulation or termination of the genetic transcriptional process [2]. While in the cytoplasm, the transcription factor is incapable of promoting transcription. A signaling event, such as a change of the state of phosphorylation, then occurs that results in protein subunit translocation into the nucleus. Signal transduction therefore involves complex interactions of multiple cellular pathways [2]. In particular, redox-sensitive transcription factors have gained an overwhelming interest momentum over the years, ever since the onset of the burgeoning field of free radical research and oxidative stress. The reason for this is that redox-sensitive transcription factors are often associated with the development and progression of many human disease states and inflammatory-related injury, particularly of the lung [3]. Their ultimate regulation therefore bears potential therapeutic intervention for possible clinical applications.

In the present review, I elaborate on the current understanding of redox/oxidative mechanisms mediating the regulation of key transcription factors, particularly NF-κB, that mediate a plethora of cellular functions that regulate redox-induced and oxidant-induced lung injury.

Reduction–oxidation concepts: the paradigm of oxidative siege

The conceptual idea of free radical-mediated injury gains a new dimension. The human body with its various organs, and particularly the lungs, is under attack from a free radical-invoked condition generally referred to as 'oxidative stress' [1,2] (Fig. 2). Each human organ and each human cell is influenced by oxidative stress, which is separated into internal conditions (inflammation, autoimmune reactions, dysregulation of metabolism, ischemia) and external conditions (microbiological organism, electromagnetic radiation, mechanical-induced stress, thermal-induced stress, chemical-induced stress) [2].

Oxidative damage defines the consequences of a mismatch between the production of the reactive oxygen species (ROS) and the reactive nitrogen species, and the ability to defend against them. Major sources of ROS/reactive nitrogen species include, but are not exclusive to or limited to, mitochondrial oxidative metabolism, phospholipid metabolism and proteolysis [1,2].

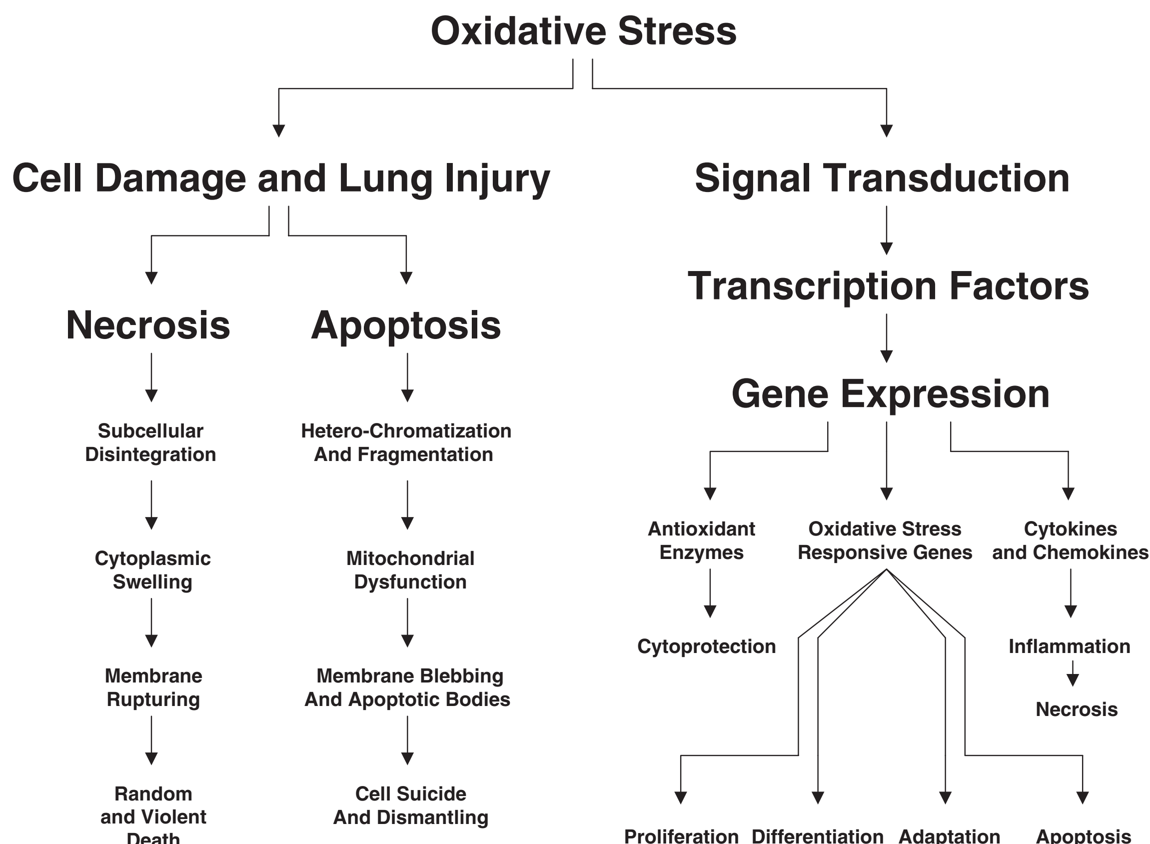
Biological systems are protected from the threat of oxidative assault by a diversity of mechanisms designed to suppress pernicious oxidative pathways. Raised against the challenges are an extensive and highly effective array of protective agents and defense antioxidant mechanisms. These comprise numerous small molecular weight antioxidants to forestall initiation of oxidative damage and/or to limit its propagation, enzymes that convert and detoxify free radicals, enzymes to repair oxidative damage when it occurs and mechanisms to route damaged molecules for destruction and replacement [1,2]. Antioxidant processes usually work by direct scavenging of the initiating pro-oxidant species. Each tissue, for instance, has an antioxidative potential, which is determined by the balance between oxidant-casing agents and those exerting an enzymatic antioxidant and non-enzymatic antioxidants to indicate a need for such protection. A healthy cell, therefore, is one in which the antioxidant systems effectively keep the level of pro-oxidants below a critical, nonpernicious threshold [1–3].

The role of NF-κB in oxidant-mediated lung injury

The expression of genes in response to oxidative stress-related transducing signals from surface receptors is predominantly determined by the conditions of the cell microenvironment. NF-κB is among the most important transcription factors shown to respond directly to oxidative stress conditions [1,2,4]. Although the transcription factor NF-κB was originally recognized in regulating gene expression in B-cell lymphocytes [5], subsequent investigations have demonstrated that it is one member of a ubiquitously expressed family of *Rel*-related transcription factors that serve as critical regulators of inflammatory-related genes such as tumor necrosis factor (TNF) and IL-1 (Fig. 3) [6].

The *Rel*/NF-κB transcription factors are a family of structurally related eukaryotic transcription factors that are involved in the control of a vast array of processes, such as immune and inflammatory responses, developmental processes, cellular growth and programmed cell death (apoptosis). In addition, these factors are active in a number of disease states, including cancer, arthritis, inflammation, asthma, neurodegenerative diseases and cardiovascular abnormalities [6]. The immunoregulatory approach aimed at targeting the NF-κB signaling pathway therefore remains of particular interest, and selective modulation of this transcription factor may bear a typical therapeutic approach for the control and regulation of inflammatory-associated diseases

Figure 2



A general schematic showing the regulation of cellular processes in response to oxidative stress. Reactive oxygen species may induce cell damage (lung injury) or may initiate a cascade of adaptive signaling mechanisms that ultimately lead to proliferation, differentiation, adaptation or apoptosis. This stands in sharp contrast with the disorderly manner of necrotic, violent death that might be incurred by excessive oxidative stress.

[6]. A hypothetical schematic depicting the role of NF-κB in oxidant-induced lung injury is displayed in Fig. 4.

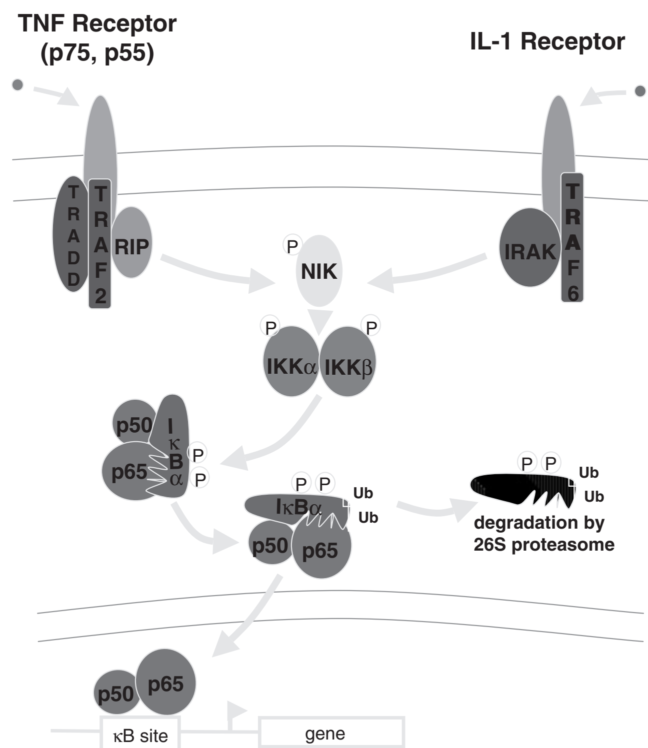
Free radicals and hyperoxia

The lung is particularly exposed to various inhaled toxic products whose toxicity can be, at least partly, mediated by the generation of free radicals [1–4]. The oxidants burden can also result from lung metabolism of xenobiotics or from activation of phagocytes. Free radicals are mainly derived from a univalent sequential reduction of molecular oxygen. Mitochondria are the main location of intracellular production, which may also result from auto-oxidation of small molecules or the function of some enzymes.

To prevent the deleterious effects of free radicals produced by normal metabolism, cells are equipped with an antioxidant system composed of enzymes (superoxide dismutase, catalase, glutathione peroxidase) and nonenzymatic substances (glutathione, iron chelators, vitamin E, vitamin C, ceruleoplasmin) [4,7,8]. Targets of free radical toxicity are phospholipids, by initiation of lipid peroxidation, and proteins that may be activated or inactivated via oxidation of sulfhydryl residues. Another target is the blueprint of life, DNA, with possible strand breaks or mutation. Transcription activities can also be altered, and it has recently been reported that some transcription factors such as NF-κB can be activated by oxidants [1,4,6].

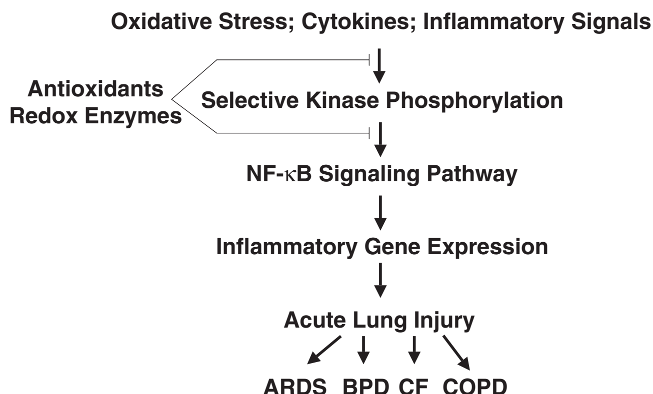
Under these circumstances, free radicals may be considered second messengers [1]; however, they may also be damaging signals. In this respect, lung oxygen toxicity has been extensively studied over the past few decades. Particularly, oxygen-induced lung lesions are, by nature, nonspecific; it is possible, for example, to induce a resistance to 100% O_2 by the pre-exposure of animals to 85% O_2 [7]. This tolerance phenomenon is associated with increased lung content in antioxidant substances. The mechanisms of gene regulation of antioxidant enzymes are still poorly understood in eukaryotes, however. Overproduction of free radicals in the lung is also involved in various clinical settings such as ischemia-reperfusion, exposure to ozone or nitrous oxide, acute respiratory distress syndrome (ARDS), drug-induced lung toxicity, pathogenesis of chronic obstructive pulmonary disease, asthma, cancer and aging [7,8]. The precise role of free

Figure 3



The *Rel*/NF-κB signal transduction pathway. Various signals, such as inflammatory cytokines, converge on activation of the inhibitory-κB kinase (IKK) complex via the upstream NF-κB inducing kinase (NIK). The IKK-α/IKK-β complex (signalsome) then phosphorylates inhibitory-κB (I-κB) at two N-terminal serines, which signals it for ubiquitination (Ub) and phosphorylation (P) by the ^{26}S proteasome system. Freed NF-κB (p50–p65; NF-κB$_1$–*Rel*A complex) enters the nucleus, binds specific κB moieties and activates gene expression. IRAK, IL-1 receptor-associated kinase; RIP, receptor-regulated intramembrane proteolysis; TNF, tumor necrosis factor; TRADD, TNF receptor-associated death domain; TRAF, TNF receptor-associated factor.

Figure 4



The role of oxidative stress, cytokines and other inflammatory signals in regulating the NF-κB signal transduction pathways in mediating oxidant-induced lung injury and disease conditions. ARDS, acute respiratory distress syndrome; BPD, bronchopulmonary dysplasia; CF, cystic fibrosis; COPD, chronic obstructive pulmonary disease.

radicals among other mechanisms of lung injury is still unclear. A better knowledge of free radical mechanisms of toxicity and of antioxidant regulation is therefore needed to develop antioxidant therapeutic strategies.

Inflammatory cytokines such as TNF-α and IL-1 can each activate NF-κB (Fig. 3) and can induce gene expression of manganese superoxide dismutase (MnSOD), a mitochondrial matrix enzyme that can provide critical protection against hyperoxic lung injury [7–9]. The regulation of MnSOD gene expression is not well understood. Since the redox status can modulate NF-κB [4] and potential κB site(s) exist in the MnSOD promoter, it was observed that the activation of NF-κB and increased MnSOD expression were potentiated by thiol reducing agents [9]. In contrast, thiol oxidizing or alkylating agents both inhibited NF-κB activation and elevated MnSOD expression in response to TNF-α and IL-1 [9]. Since diverse agents had similar effects on the activation of NF-κB and MnSOD gene expression, it was hypothesized that the activation of NF-κB and MnSOD gene expression are closely associated events and that reduced sulfhydryl groups are required for cytokine mediation of both processes [9].

Within the context of lung pathophysiology, in addition, Schwartz *et al.* recently reported that the expression of proinflammatory cytokines is rapidly increased in experimental models of ARDS, in patients at risk for ARDS and in patients with established ARDS [10]. For instance, it was demonstrated that the increased *in vivo* activation of the nuclear transcriptional regulatory factor NF-κB (but not that of NF-IL-6, cAMP-responsive element binding protein [CREB], activating protein-1 [AP-1], or serum protein-1 [Sp-1]) in alveolar macrophages from patients with ARDS is specific. Because binding sequences for NF-κB are present in the enhancer/promoter sequences of multiple proinflammatory cytokines, activation of NF-κB may contribute to the increased expression of multiple cytokines in the lung in the setting of established ARDS [10].

Antioxidant treatment in oxidant-induced lung injury has been widely observed to suppress NF-κB activation and the protracted neutrophilic lung inflammation [7,10,11]. For instance, after *in vivo* 6 mg/kg lipopolysaccharide–endotoxin (LPS) treatment, the lung NF-κB activation peaked at 2 hours and temporally correlated with the expression of cytokine-induced neutrophil chemoattractant mRNA in the lung tissue [11]. Treatment with the antioxidant *N*-acetyl-L-cysteine, an antioxidant thiol and a precursor of glutathione, 1 hour before LPS treatment, resulted in decreasing lung NF-κB activation in a dose-dependent manner and diminishing cytokine-induced neutrophil chemoattractant mRNA expression in the lung tissue. Treatment with *N*-acetyl-L-cysteine significantly suppressed LPS-induced neutrophilic alveolitis, indicating

that the NF-κB pathway may well represent an attractive therapeutic target for strategies to control neutrophilic inflammation and lung injury [11].

Furthermore, cystic fibrosis (CF) patients are known to develop progressive cytokine-mediated inflammatory lung disease, with abundant production of thick, tenacious, protease-rich and oxidant-rich purulent airway secretions that are difficult to clear, even with physiotherapy. In the search for a potential treatment, Ghio *et al*. tested tyloxapol, an alkylaryl polyether alcohol polymer detergent, previously used as a mucolytic agent in adult chronic bronchitis [12]. Tyloxapol inhibited the activation of NF-κB, reduced the resting secretion of the chemokine IL-8 in cultured human monocytes and inhibited LPS-stimulated release of TNF-α, IL-1β, IL-6, IL-8, granulocyte-macrophage colony-stimulating factor and the eiconsanoids thromboxane A_2 and leukotriene B_4. It has also been shown that tyloxapol is a potent antioxidant scavenger for the hydroxyl radicals (•OH) [12]. Tyloxapol effectively scavenged the oxidant hypochlorous acid *in vitro* and protected against hypochlorous acid-mediated lung injury in rats. In addition, tyloxapol also reduced the viscosity of CF sputum (from 463 ± 133 to 128 ± 52 centipoise) [12]. Tyloxapol, therefore, may be potentially useful as a new anti-inflammatory therapy for CF lung disease and could possibly promote clearance of secretions in the CF airway in a NF-κB-dependent manner.

Hyperoxia (hyperbaric levels of oxygen) and reactive species are potentially exacerbating in lung injury. Regarding the mechanisms reported in hyperoxia-mediated lung injury, it was suggested that hyperoxia-associated production of ROS might lead to neutrophil infiltration into the lungs and to increased pulmonary proinflammatory cytokine expression [7]. However, the initial events induced by hyperoxia, thereby leading to acute inflammatory lung injury, remain incompletely characterized. To explore this issue, Shea *et al*. examined nuclear transcriptional regulatory factor (NF-κB and NF-IL-6) activation and cytokine expression in the lungs following 12–48 hours of hyperoxia exposure [13]. Evidently, no substantial increases in cytokine (IL-1β, IL-6, IL-10, transforming growth factor beta, TNF-α, IFN-γ) expression nor in NF-κB activation were found after 12 hours of hyperoxia (relatively early events). Following 24 hours of hyperoxia, however, NF-κB activation and increased levels of TNF-α mRNA were present in pulmonary lymphocytes. By 48 hours of hyperoxia, the amounts of IFN-γ and TNF-α protein as well as mRNA were increased in the lungs and NF-κB continued to show activation, even though no histological abnormalities were detected [13]. These results showed that hyperoxia activates NF-κB in the lungs before any increase in proinflammatory cytokine protein occurs, and they further suggest that NF-κB activation may represent an initial event in the proinflammatory sequence induced by hyperoxia. Increased expression of proinflammatory cytokines therefore appears to be an important factor contributing to the development of acute lung injury.

Another approach adopted to protect against oxidant-induced lung injury was reported on the effect of phosphodiesterase inhibitors, believed to play a critical role in modulating the intracellular dynamic ratios of cAMP and cGMP, which are involved in regulating the inflammatory process associated with oxidative stress [14–25]. For example, lisofylline (1-[5*R*-hydroxyhexyl]-3,7-dimethylxanthine), a nonselective phosphodiesterase inhibitor, was shown to decrease lipid peroxidation *in vitro* and to suppress proinflammatory cytokine expression *in vivo* in models of lung injury due to sepsis, blood loss and oxidative damage [26–37]. In a murine hyperoxia model, the effects of lisofylline on the activation of NF-κB and CREB, on the expression of proinflammatory cytokines in the lungs and on the circulating levels of oxidized free fatty acids were examined, as well as its effects on hyperoxia-induced lung injury and mortality. Treatment with lisofylline inhibited hyperoxia-associated increases in TNF-α, IL-1β and IL-6 in the lungs as well as decreasing the levels of hyperoxia-induced serum-oxidized free fatty acids [38]. Although hyperoxic exposure produced activation of both NF-κB and CREB in lung cell populations, only CREB activation was reduced in the mice treated with lisofylline. Furthermore, lisofylline diminished hyperoxia-associated increases in lung wet-to-dry weight ratios and improved survival in animals exposed to hyperoxia [38]. These results suggest that lisofylline ameliorates hyperoxia-induced lung injury and mortality through inhibiting CREB activation, membrane oxidation and proinflammatory cytokine expression in the lungs.

Hemorrhage and resuscitation

In murine models, for example, mRNA levels of proinflammatory and immunoregulatory cytokines, including IL-1α, IL-1β, transforming growth factor beta 1 and TNF-α, are increased in intraparenchymal lung mononuclear cells 1 hour after hemorrhage [39]. Binding elements for the nuclear transcriptional regulatory factors, NF-κB, CCAAT/enhancer binding protein beta, Sp-1, AP-1 and CREB are present in the promoter regions of numerous cytokine genes, including those whose expression is increased after blood loss.

To investigate early transcriptional mechanisms that may be involved in regulating pulmonary cytokine expression after hemorrhage, Shenkar and Abraham examined *in vivo* the activation of these nuclear transcriptional factors among intraparenchymal lung mononuclear cells obtained in the immediate posthemorrhage period [39,40]. Activation of NF-κB and CREB, but not of CCAAT/enhancer binding protein beta, Sp-1 or AP-1, was present in lung mononuclear cells isolated from mice 15 min after hemorrhage. Inhibition of xanthine oxidase, an enzyme that generates ROS, by prior feeding with either an allopurinol-supplemented or a tungsten-enriched diet, prevented hemorrhage-induced activation of CREB but not of NF-κB. These results clearly demonstrate that hemorrhage leads to rapid *in vivo* activation in the lung of CREB through a xanthine oxidase-dependent mechanism and of NF-κB through other pathways, and they suggest that the activa-

tion of these transcriptional factors may have an important role in regulating pulmonary cytokine expression and the development of acute lung injury after blood loss [14].

In concert with these observations, it has been reported that systemic blood loss affects NF-κB regulatory mechanisms in the lungs. For instance, NF-κB is activated in the lungs of patients with ARDS [10,15]. In experimental models of acute lung injury, activation of NF-κB contributes to the increased expression of immunoregulatory cytokines and other pro-inflammatory mediators in the lungs. Moine *et al*. examined cytoplasmic and nuclear NF-κB counter-regulatory mechanisms in lung mononuclear cells, using a murine model in which inflammatory lung injury develops after blood loss [15]. Sustained activation of NF-κB was present in lung mononuclear cells over the 4-hour period after blood loss. The activation of NF-κB after hemorrhage was accompanied by alterations in levels of the NF-κB regulatory proteins inhibitory-κB alpha (IκB-α) and Bcl-3. Cytoplasmic and nuclear IκB-α were increased and nuclear Bcl-3 was decreased during the first hour after blood loss, but by 4 hours posthemorrhage the cytoplasmic and nuclear IκB-α levels were decreased and the nuclear levels of Bcl-3 were increased. Inhibition of xanthine oxidase activity in otherwise unmanipulated and unhemorrhaged mice resulted in increased levels of IκB-α and in decreased amounts of Bcl-3 in nuclear extracts from lung mononuclear cells. Moreover, no changes in the levels of nuclear IκB-α or Bcl-3 occurred after hemorrhage when xanthine oxidase activity was inhibited [15], indicating that blood loss, at least partly through xanthine oxidase-dependent mechanisms, produces alterations in the levels of both IκB-α and Bcl-3 in lung mononuclear cell populations. The effects of hemorrhage on proteins that regulate activation of NF-κB may therefore contribute to the frequent development of inflammatory lung injury in this setting.

In parallel, resuscitation from hemorrhagic shock induces profound changes in the physiologic processes of many tissues and activates inflammatory cascades that include the activation of stress transcriptional factors and the upregulation of cytokine synthesis. This process is accompanied by acute organ damage (e.g. to the lungs and the liver). It was demonstrated that the inducible nitric oxide synthase (iNOS) is expressed during hemorrhagic shock. Hierholzer and colleagues, in this respect, postulated that nitric oxide production from iNOS would participate in proinflammatory signaling [16]. It was found using the iNOS inhibitor N_6-(iminoethyl)-L-lysine or using iNOS knockout mice that the activation of NF-κB and the signal transducer and activator of transcription, and that increases in IL-6 and granulocyte colony-stimulating factor mRNA levels in the lungs and livers measured 4 hours after resuscitation from hemorrhagic shock, were iNOS dependent. Furthermore, iNOS inhibition resulted in a marked reduction of lung and liver injury produced by hemorrhagic shock [16]. iNOS is thus essential for the upregulation of the inflammatory response in resuscitated hemorrhagic shock and participates in end organ damage under these conditions.

Polymorphonuclear leukocyte-mediated oxidant injury

Lung injury is, in part, due to polymorphonuclear leukocyte-mediated oxidative tissue damage. By means of NF-κB activation, oxidants may also induce several genes implicated in the inflammatory response [1–4,31–39] (Fig. 5). The dithiocarbamates are antioxidants with potent inhibitory effects on NF-κB.

It was postulated that the pyrrolidine derivative pyrrolidine dithiocarbamate (PDTC), a nonthiol antioxidant, would attenuate lung injury following intratracheal challenge with LPS through its effect as an antioxidant and an inhibitor of gene activation. Rats were given 1 mmol/kg PDTC by intraperitoneal injection, followed by intratracheal administration of LPS. The transpulmonary flux of [^{125}I]albumin (the permeability index) was used as a measure of lung injury. Northern blot analysis of total lung RNA was performed to assess induction of TNF-α and intercellular adhesion molecule-1 (ICAM-1) mRNA as markers of NF-κB activation. The effect of *in vivo* treatment with PDTC on LPS-induced NF-κB DNA-binding activity in macrophage nuclear extracts was evaluated with the electrophoretic mobility shift assay (EMSA).

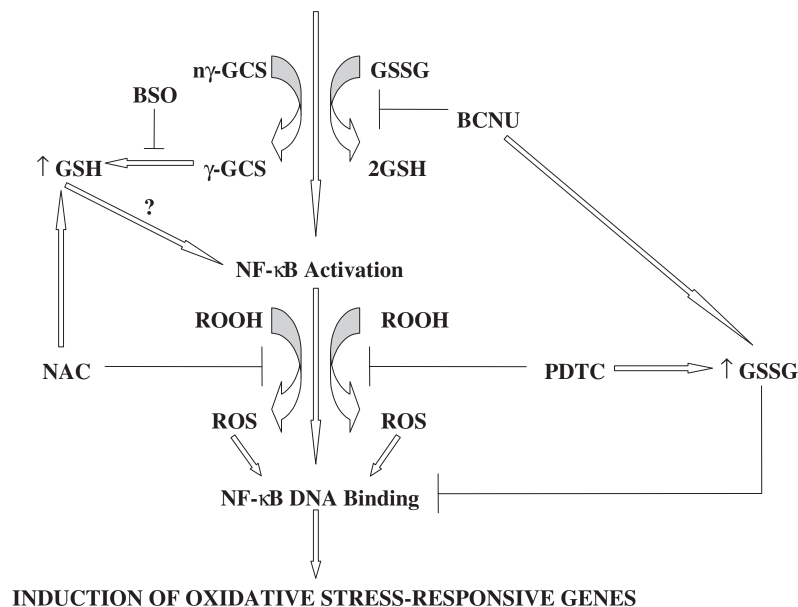
PDTC administration attenuated LPS-induced increases in lung permeability (permeability index = 0.16 ± 0.02 for LPS versus 0.06 ± 0.01 for LPS + PDTC) [17]. TNF-α levels and polymorphonuclear leukocyte counts in the bronchoalveolar lavage fluid (BALF) were unaffected, as were whole-lung TNF-α and ICAM-1 mRNA expression. In addition, PDTC had no effect on NF-κB activation as evaluated with the EMSA. PDTC reduced lung lipid peroxidation as assessed by levels of malondialdehyde, without reducing the neutrophil oxidant production [17].

It is concluded that PDTC attenuates LPS-induced acute lung injury; this effect occurs independently of any effect on NF-κB. PDTC reduced oxidant-mediated cellular injury, however, as demonstrated by a reduction in the accumulation of malondialdehyde. The administration of PDTC may therefore represent a novel approach to limiting neutrophil-mediated oxidant injury.

Stress response

The stress response is a highly conserved cellular defense mechanism defined by the rapid and specific expression of stress proteins, with concomitant transient inhibition of non-stress protein gene expression [36,37]. The stress proteins mediate cellular and tissue protection against diverse cytotoxic stimuli. The stress response and stress proteins confer protection against diverse forms of cellular and tissue injury, including acute lung injury [18]. The stress response can inhibit nonstress protein gene expression, and therefore transcriptional inhibition of proinflammatory responses could be a mechanism of protection against acute lung injury.

Figure 5



Schematic diagram of NF-κB activation circuits and oxygen-signaling mechanisms. Reduction of oxidized glutathione (GSSG) to glutathione (GSH), which is blocked by 1,3-*bis*-(2-chloroethyl)-1-nitrosourea (BCNU), leads to increasing intracellular stores of GSSG, a potent inhibitor of NF-κB transcription factor DNA binding. The pathway leading to the formation of GSH by the action of γ-glutamylcysteine synthetase (GCS) is blocked by L-buthionine-(*S,R*)-sulfoximine (BSO), inducing an irreversible inhibition of NF-κB activation. Reactive oxygen species (ROS) are key components of the pathways leading to the activation of NF-κB, whose binding activity is obliterated by *N*-acetyl-L-cysteine (NAC) and pyrrolidine dithiocarbamate (PDTC), potent scavengers of ROS. Although NAC is elevating reduced GSH, it is unknown whether this mechanism induces NF-κB activation independently from the antioxidant effects of this inhibitor. PDTC elevates GSSG concentration by GSH oxidation, a pro-oxidant effect characteristic of dithiocarbamates, thereby mediating NF-κB inhibition. Upon NF-κB DNA binding, cascades of hyperoxia-responsive genes are activated, which have the potential to modulate cellular response to oxidative injury. ROOH, highly reactive peroxide.

To explore this possibility, Wong *et al.* determined the effects of the stress response on nuclear translocation of NF-κB. In cancerous epithelial A549 cells, the induction of the stress response decreased TNF-α-mediated NF-κB nuclear translocation [19]. TNF-α also initiated NF-κB nuclear translocation by causing dissociation of IκB-α from NF-κB and by rapid degradation of IκB-α. Prior induction of the stress response, however, inhibited TNF-α-mediated dissociation of IκB-α from NF-κB and subsequent degradation of IκB-α. Induction of the stress response also increased expression of IκB-α [19]. It seems that the stress response affects NF-κB-mediated gene regulation by at least two independent mechanisms: the stress response stabilizes IκB-α and it induces the expression of IκB-α. The composite result of these two effects is to decrease NF-κB nuclear translocation, and this suggests that the protective effect of the stress response against acute lung injury involves a similar effect on the IκB-α/NF-κB pathway.

In another stress model of IgG immune complex-mediated lung injury, the cytokines IL-10 and IL-13 (which possess powerful anti-inflammatory activities *in vitro* and *in vivo*) have recently been shown to suppress neutrophil recruitment and ensuing lung injury by greatly depressing the pulmonary production of TNF-α when exogenously administered [20]. EMSA assessment of nuclear extracts from alveolar macrophages and whole lung tissues demonstrated that both IL-10 and IL-13 suppressed nuclear localization of NF-κB after *in vivo* deposition of IgG immune complexes. Western blot analysis indicated that these effects were due to preserved protein expression of IκB-α in both alveolar macrophages and whole lungs. Northern blot analysis of lung mRNA showed that, in the presence of IgG immune complexes, IL-10 and IL-13 augmented IκB-α mRNA expression [20–22]. These findings unequivocally suggest that IL-10 and IL-13 may operate by suppressing NF-κB activation through preservation of IκB-α *in vivo*.

Further to the effect of stress in acute lung injury, it has been observed that the β-chemokine, regulated upon activation, normal T-cell expressed and secreted (RANTES), is involved in the pathophysiology of inflammation-associated

lung injury. Although much is known regarding signals that induce RANTES gene expression, relatively few data exist regarding signals that inhibit RANTES gene expression [23]. The heat shock response, a highly conserved cellular defense mechanism, has been demonstrated to inhibit a variety of lung proinflammatory responses. The hypothesis that induction of the heat shock response inhibits RANTES gene expression was investigated. Treatment of A549 cells with TNF-α induced RANTES gene expression in a concentration-dependent manner. Induction of the heat shock response inhibited subsequent TNF-α-mediated RANTES mRNA expression and secretion of immunoreactive RANTES. In addition, transient transfection assays involving a RANTES promoter-luciferase reporter plasmid demonstrated that the heat shock response inhibited TNF-α-mediated activation of the RANTES promoter.

Inhibition of NF-κB nuclear translocation with isohelenin inhibited TNF-α-mediated RANTES mRNA expression, indicating that RANTES gene expression is NF-κB dependent, for the moment specific to A549 cells [23]. Furthermore, the induction of the heat shock response inhibited degradation of the NF-κB inhibitory protein, IκB-α, but did not significantly inhibit phosphorylation of IκB-α. These observations suggest that the heat shock response inhibits RANTES gene expression by a mechanism involving inhibition of NF-κB nuclear translocation and subsequent inhibition of RANTES promoter activation. The mechanism by which the heat shock response inhibits NF-κB nuclear translocation involves stabilization of IκB-α, without significantly affecting its phosphorylation.

Anti-inflammatory cytokine-mediated oxidant injury

Another anti-inflammatory cytokine that is involved as a regulatory element in lung injury is IL-11. For instance, the role of IL-11 was evaluated in the IgG immune complex model of acute lung injury in rats [24]. IL-11 mRNA and protein were both upregulated during the course of this inflammatory response. Exogenously administered IL-11 substantially reduced, in a dose-dependent manner, the intrapulmonary accumulation of neutrophils and the lung vascular leak of albumin. These *in vivo* anti-inflammatory effects of IL-11 were associated with reduced NF-κB activation in the lung, with reduced levels of TNF-α in the BALF and diminished upregulation of lung vascular ICAM-1. It is interesting to observe that IL-11 did not affect the BALF content of the CXC chemokines, of the macrophage inflammatory protein-2 and of the cytokine-inducible neutrophil chemoattractant. The presence of IL-11 did not affect these chemokines. However, the BALF content of the complement C5a was reduced by IL-11 [24]. These data indicate that IL-11 is a regulatory cytokine in the lung and that, like other members of this family, its anti-inflammatory properties appear to be linked to its suppression of NF-κB activation, its diminished production of TNF-α and its reduced upregulation of ICAM-1.

Conclusion and future prospects

The molecular response to oxidative stress is regulated, in part, by redox-sensitive transcription factors. The study of gene expression/regulation is critical in the development of novel gene therapies [25,41–46]. Reactive species (oxidative stress) are produced in health and disease. The antioxidant defense system (a complex system that includes intracellular enzymes, nonenzymatic scavengers, and dietary components) normally controls the production of ROS [45–54]. Oxidative stress occurs when there is a marked imbalance between the production and removal of ROS and reactive nitrogen species. This imbalance arises when antioxidant defenses are depleted or when free radicals are overproduced. A growing body of evidence also exists showing that enhancement of the oxidative stress antioxidant defense system can reduce markers of oxidative stress [55–61]. Recognition of reactive species and redox-mediated protein modifications as potential signals may open up a new field of cell regulation via specific and targeted genetic control of transcription factors, and thus could provide us with a novel way of controlling disease processes [62–70]. Dynamic variations in partial pressure of oxygen and redox equilibrium thus regulate gene expression, apoptosis signaling and the inflammatory process, thereby bearing potential consequences for screening emerging targets for therapeutic intervention.

Competing interests

None declared.

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