Review

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

Severinghaus-Radiometer Research Laboratories, Molecular Neuroscience Research Division, Department of Anesthesia and Perioperative Care, University of California at San Francisco, School of Medicine, San Francisco, California, USA

Correspondence: John J Haddad, haddadj@anesthesia.ucsf.edu

Published online: 14 October 2002

Critical Care 2002, 6:481-490 (DOI 10.1186/cc1839)

Available online http://ccforum.com/content/6/6/481

© 2002 BioMed Central Ltd (Print ISSN 1364-8535; Online ISSN 1466-609X)

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.
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.
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% O₂ by the pre-exposure of animals to 85% O₂ [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...
The role of oxidative stress, cytokines, and other inflammatory signals in regulating the NF-kB 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.

Within the context of lung pathophysiology, in addition, Schwartz et al. recently reported that the expression of pro-inflammatory 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-kB (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-kB are present in the enhancer/promoter sequences of multiple proinflammatory cytokines, activation of NF-kB 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-kB 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-kB 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-kB 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 eicosanoids thromboxane A2 and leukotriene B4. 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.

Hyeroxia (hyperbaric levels of oxygen) and reactive species are potentially exacerbating in lung injury. Regarding the mechanisms reported in hypoxia-mediated lung injury, it was suggested that hypoxia-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 hypoxia, 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-[5R-hydroxyhexyl]-3,7-dimethyloxanthine), 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 promotor 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 Nω-(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 [125I]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.
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...
l 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 isohelein 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 defences 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.

**Acknowledgements**

The author’s own publications therein cited are, in part, financially supported by the Anonymous Trust (Scotland), the National Institute for Biological Standards and Control (England), the Tenerus Trust (Scotland), the UK Medical Research Council (MRC, London), the Wellcome Trust (London) (Stephen C Land, Department of Child Health, University of Dundee, Scotland, UK) and the National Institutes of Health (NIH; Bethesda, USA) (Philip E Bickler, Department of Anesthesia and Perioperative Care, University of California, San Francisco, California, USA). The work of the author was performed at the University of Dundee, Scotland, UK. This review was written at UCSF, California, USA. JIH held the Georges John Livanos prize (London, UK) under the supervision of Stephen C Land and the NIH award fellowship (California, USA) under the supervision of Philip E Bickler. The author also appreciatively thanks Jennifer Schuyler (Department of Anesthesia and Perioperative Care) for her excellent editing and reviewing of this manuscript. I also thank my colleagues at UCSF (San Francisco, California, USA) and the American University of Beirut (AUB, Beirut, Lebanon) who have criticised the work for enhancement and constructive purposes.

**References**

1. D’Angio CT, Finkelstein JN: Oxygen regulation of gene expression: a study in opposites. Mol Genet Metab 2000, 71:371-380.
2. Alder V, Yin Z, Tew KD, Ronai Z: Role of redox potential and reactive oxygen species in stress signaling. Oncogene 1999, 18:6104-6111.
3. Crapo JD, Harmes AG, Sherman MP, Musson RA: Pulmonary immunobiology and inflammation in pulmonary diseases. Am J Respir Crit Care Med 2000, 162:1983-1986.
4. Haddad JJ, Oliver RE, Land SC: Antioxidant/pro-oxidant equilibria regulates HIF-1α and NF-κB redox sensitivity: evidence for inhibition by glutathione oxidation in alveolar epithelial cells. J Biol Chem 2000, 275:21130-21139.
5. Sen R, Baltimore D: Multiple nuclear factors interact with the immunoglobulin enhancer sequences. Cell 1986, 48:705-716.
6. Baldwin AS: The transcription factor NF-κB and human disease. J Clin Invest 2001, 107:3-6.
7. Houssot B: Free radicals and respiratory pathology. C R Acad Sci 1994, 2:321-327.
8. Morris PE, Bernard GR: Significance of glutathione in lung disease and implications for therapy. Am J Med Sci 1994, 307: 119-127.
9. Das KC, Lewis-Molock Y, White CW: Thiol modulation of TNF-α and IL-1induced MnSOD gene expression and activation of NF-κB. Mol Cell Biochem 1995, 148:45-57.
10. Schwartz MD, Moore EE, Moore FA, Shenkar R, Moine P, Haenel JB, Abraham E: Nuclear factor-κB is activated in alveolar macrophages from patients with acute respiratory distress syndrome. Crit Care Med 1996, 24:1285-1292.
11. Blackwell TS, Blackwell TR, Holden EP, Christman BW, Christman JW: In vivo antioxidant treatment suppresses nuclear factor-κB activation and neutrophil lung inflammation. J Immunol 1996, 157:1630-1637.
12. Ghio AJ, Marshall BG, Diaz JL, Hasegawa T, Samuelson W, Povia D, Kennedy TP, Plantodoci CA: Tyloxapol inhibits NF-κB and cytokine release, scavenges HOCI and reduces viscosity of cystic fibrosis sputum. Am J Respir Crit Care Med 1996, 154: 779-795.
13. Shea LM, Beehler C, Schwartz M, Shenkar R, Tuder R, Abraham E: Hyperoxia activates NF-κB and increases TNF-α and IFN-γ gene expression in mouse pulmonary lymphocytes. J Immunol 1996, 157:3902-3908.
14. Moine P, Shenkar R, Kaneko D, Le Tubo Y, Abraham E: Systemic blood loss affects NF-κB activation in peripheral blood mononuclear cells in mice: involvement of catecholamines in nuclear factor-κB regulation and cytokine expression. J Clin Invest 1999, 95:1516-1524.
15. Le Tulzo Y, Shenkar R, Kaneko D, Kennedy TP, Piantodosi CA: Tyloxapol inhibits NF-κB nuclear translocation and increases IκB-κB in alveolar macrophages from patients with acute respiratory distress syndrome. Crit Care Med 1996, 24:1285-1292.
16. Nathens AB, Bitar R, Davreux C, Bujard M, Marshall JC, Abraham E: Pyrrolidine dithiocarbamate attenuates endotoxin-induced acute lung injury. Am J Respir Cell Mol Biol 1997, 17:608-616.
17. Wong HR, Wispe JR: The stress response and the lung. Am J Physiol 1997, 273:L1-9.
18. Wong HR, Ryan M, Wispe JR: Stress response decreases NF-κB nuclear translocation and increases IκB-κB expression in A549 cells. J Clin Invest 1997, 99:2423-2428.
19. Lentsch AB, Shanker TK, Sarma V, Ward PA: In vivo suppression of NF-κB and preservation of IκB-κB by interleukin-10 and dexamethasone in mice. J Immunol 1997, 159:2443-2448.
20. Lentsch AB, Czermak BJ, Bless NM, Ward PA: NF-κB activation during IgG immune complex-induced lung injury: requirements for TNF-α and IL-β but not complement. Am J Pathol 1998, 152:1237-1238.
21. Lentsch AB, Czermak BJ, Jordan JA, Ward PA: Regulation of acute lung inflammatory injury by endogenous IL-13. J Immunol 1999, 162:1071-1076.
22. Ayad O, Stark JM, Fiedler MM, Menendez IY, Ryan MA, Wong HR: The heat shock response inhibits RANTES gene expression in cultured human lung epithelium. J Immunol 1998, 161:2594-2599.
23. Lentsch AB, Crouch LD, Jordan JA, Czermak BJ, Yun EC, Guo R, Sarma V, Diehl KM, Ward PA: Regulatory effects of interleukin-11 during acute lung inflammatory injury. J Leukoc Biol 1999, 66:151-157.
24. Pugin J, Dunn I, Jollivet P, Tassaux D, Magenat JL, Nicod LP, Chevrolet JC: Activation of human macrophages by mechanical ventilation in vitro. Am J Physiol 1998, 275:L1040-L1050.
25. Haddad JJ, Vuk RM, A novel MKP-1 inhibitor with anti-inflammatory actions (Vertex Pharmaceuticals). Curr Opin Investig Drugs 2001, 2:1070-1076.
26. Haddad JJ, Land SC, Tarnow-Mordi WO, Zembala M, Kowalczyk D, Lauterbach R: Immunopharmacological potential of selective phosphodiesterase inhibition. I. Differential regulation of lipopolysaccharide-mediated pro-inflammatory cytokine (interleukin-6 and tumor necrosis factor-α) biosynthesis in alveolar epithelial cells. J Pharmacol Exp Ther 2002, 300:559-566.
27. Haddad JJ, Land SC, Tarnow-Mordi WO, Zembala M, Kowalczyk D, Lauterbach R: Immunopharmacological potential of selective phosphodiesterase inhibition. II. Evidence for the involvement of an inhibitory xB/nuclear factor-xB-sensitive pathway in alveolar epithelial cells. J Pharmacol Exp Ther 2002, 300: 567-576.
28. Departmen E, Belfrage P, Manganiello VC: Structure, localization and regulation of cGMP-inhibited phosphodiesterase (PDE5). J Biol Chem 1997, 272:6823-6826.
29. Demolins-Mason CD: Cyclic nucleotide phosphodiesterase inhibitors. Exp Opin Ther Patents 1998, 5:417-430.
30. Essayan DM: Cyclic nucleotide phosphodiesterase (PDE) inhibitors and immunomodulation. Biochem Pharmacol 1999, 57:965-973.
31. Houslay MD, Milligan G: Tailoring cAMP-signalling responses through isofomr diversity. Trends Biochem Sci 1997, 22: 217-224.
32. Lauterbach R, Szymura-Oleksiak J: Nebulized pentoxifylline in successful treatment of five premature neonates with bronchopulmonary dysplasia. Eur J Pediatr 1999, 158:607-610.
33. Lauterbach R, Pawlik D, Kowalczyk W., Hellwich E, Zembala M: Effect of the immunomodulating agent, pentoxifylline, in the treatment of sepsis in prematurely delivered infants: a placebo-controlled, double-blind trial. Crit Care Med 1999, 27: 807-814.
34. Perry MJ, Higgs GA: Chemotherapeutic potential of phosphodiesterase inhibitors. Curr Opin Chem Biol 1998, 2:472-481.
35. Rogers DF, Laurent GJ: New ideas on the pathophysiology and treatment of lung disease. Thorax 1998, 53:200-203.
36. Pittet JF, Wiener-Kronish JP, Serirok V, Matthey MA: Resistance of the alveolar epithelium to injury from septic shock in sheep. Am J Respir Crit Care Med 1995, 151:1093-1100.
37. George CL, Fantuzzi G, Bursten S, Lear L, Abraham E: Effects of lisofylline on hyperoxia-induced lung injury. J Am Physiol 1999, 276:L776-L785.
38. Chenekar R, Abraham E: Hemorrhage induces rapid in vivo activation of CREB and NF-κB in murine intraparenchymal lung mononuclear cells. Am J Respir Cell Mol Biol 1997, 16:145-152.
39. An JR, Marshall JC, Jimenez M, Shek PN, Zagorski J, Rotstein OD: Hemorrhagic shock primes for increased expression of cytokine-induced neutrophil chemotactant in the lung: role in pulmonary inflammation following lipopolysaccharide. J Immunol 1998, 161:440-447.
40. Loperwe-Woodford SK, R, K: Acute hyoxia increases alveolar macrophage tumor necrosis factor activity and alters NF-κB expression. Am J Physiol 1999, 276:L909-L916.
41. Blackwell TS, Debek JP, Venkatarkrishnan A, Schot DJ, Harley DH, Pinson CW, Williams P, Washington K, Christiansen JW, Chapman WC: Acute lung injury after hepatic cryo-ablation: correlation with NF-κB activation and cytokine production. Surgery 1999, 126:518-526.
42. Lentsch AB, Yoshidome H, Warner RL, Ward PA, Edwards MJ: Secretory leukocyte protease inhibitor in mice regulates local and remote organ inflammatory injury induced by hepatic ischemia/reperfusion. Gastroenterology 1999, 117:953-961.
43. Baabum C, Lemjabbar H, Longpré M, Li D, Gensch E, McNamara N: Control of mucin transcription by diverse injury-induced signaling pathways. Am J Respir Crit Care Med 1999, 159:S44-S48.
44. Yoshidome H, Kato A, Edwards MJ, Lentsch AB: Interleukin-10 inhibits pulmonary NF-κB activation and lung injury induced by hepatic ischemia-reperfusion. Am J Physiol 1999, 277:L919-L923.
45. Armstrong VE, Opentanova IL, Minchenko AG, Lefer AM: Tissue factor expression in vital organs during murine traumatic shock: role of transcription factors AP-1 and NF-κB. Anesthesiology 1999, 91:1844-1852.
46. Chapman WC, Debek JP, Wright Pinson C, Washington MK, Atkinson JB, Venkatarkrishnan A, Blackwell TS, Christians JW: Hepatic cryo-ablation, but not radio-frequency ablation, results in lung inflammation. Ann Surg 2000, 231:752-761.
47. Chapman WC, Debek JP, Blackwell TS, Gainer KA, Christians JW, Pinson CW, Brigham KL, Parker RE: Hepatic cryo-ablation
induced acute lung injury: pulmonary hemodynamic and permeability effects in a sheep model. Arch Surg 2000, 135:667-672.

49. Washington K, Debek J, Gobbell C, Szitapanovits DR, Shyr Y, Olson S, Chapman WC: Hepatic cryo-ablation-induced acute lung injury: histopathologic findings. J Surg Res 2001, 95:1-7.

50. Ross SD, Kron IL, Gangemi JJ, Shockey KS, Stoler M, Kern JA, Tribble CG, Laubach VE: Attenuation of lung reperfusion injury after transplantation using an inhibitor of nuclear factor-κB. Am J Physiol Lung Cell Mol Physiol 2000, 279:L528-L536.

51. Sajjan U, Thanassoulis G, Cherapanov V, Lu A, Sjolin C, Steer B, Wu YJ, Rotstein OD, Kent G, McKerlie C, Forstner J, Downey GP: Enhanced susceptibility to pulmonary infection with Burkholderia cepacia in Cfr−/− mice. Infect Immun 2001, 69:5138-5150.

52. Lentsch AB, Ward PA: Regulation of experimental lung inflammation. Respir Physiol 2001, 128:17-22.

53. Fan J, Ye RD, Malik AB: Transcriptional mechanisms of acute lung injury. Am J Physiol Lung Cell Mol Physiol 2001, 281: L1037-L1050.

54. Kupfer JG, Arcaroli JJ, Yum HK, Nadler SG, Yang KY, Abraham E: Role of NF-κB in endotoxemia-induced alterations of lung neutrophil apoptosis. J Immunol 2001, 167:7044-7051.

55. Park SY, Le S, Park KH, Le CT, Kim YW, Han SK, Shim YS, Yoo CG: Anti-inflammatory effect of adenovirus-mediated IκB-α overexpression in respiratory epithelial cells. Eur Respir J 2001, 18:801-809.

56. Semenza GL: Oxygen-regulated transcription factors and their role in pulmonary disease. Respir Res 2000, 1:159-162.

57. Cuzzocrea S, Chatterjee PK, Mazzon E, Dugo L, Serraino I, Britti D, Mazzullo G, Caputi AP, Thiemermann C: Pyrrolidine dithiocarbarnate attenuates the development of acute and chronic inflammation. Br J Pharmacol 2002, 135:496-510.

58. Sunil VR, Connor AJ, Guo Y, Laskin JD, Laskin DL: Activation of type II alveolar epithelial cells during acute endotoxemia. Am J Physiol Lung Cell Mol Physiol 2002, 282:L872-L880.

59. Haddad JJ, Saieh-Garabedian B, Saade NE, Kanaan SA, Land SC: Chemioxyexcitation (∆pO2/ROS)-dependent release of IL-1β, IL-6 and TNF-α: evidence of cytokines as oxygen-sensitive mediators in the alveolar epithelium. Cytokine 2001, 13: 138-147.

60. Haddad JJ, Choudhary KK, Land SC: The ex vivo differential expression of apoptosis signaling cofactors in the developing perinatal lung: essential role of oxygenation during the transition from placental to pulmonary-based respiration. Biochem Biophys Res Commun 2001, 281:311-316.

61. Haddad JJ, Saieh-Garabedian B, Saade NE, Land SC: Thiol regulation of pro-inflammatory cytokines reveals a novel immunopharmacological potential of glutathione in the alveolar epithelium. J Pharmacol Exp Ther 2001, 296:996-1005.

62. Haddad JJ, Land SC: O2-evoked regulation of HIF-1α and NF-κB in perinatal lung epithelium requires glutathione biosynthesis. Am J Physiol Lung Cell Mol Physiol 2000, 278: L492-L503.

63. Haddad JJ, Land SC: The differential expression of apoptosis factors in the alveolar epithelium is redox sensitive and requires NF-κB (RelA)-selective targeting. Biochem Biophys Res Commun 2000, 271:257-267.

64. Chabot F, Mitchell JA, Gutteridge JMC, Evans TW: Reactive oxygen species in acute lung injury. Eur Respir J 1998, 11: 745-757.

65. van der Vliet A, Cross CE: Oxidants, nitrosants, and the lung. Am J Med 2000, 109:398-421.

66. Schreck R, Albermann K, Baueerle PA: Nuclear factor κB: an oxidative stress-responsive transcription factor of eukaryotic cells. Free Radic Res Commun 1992, 17:221-237.

67. Li N, Karin M: Is NF-κB the sensor of oxidative stress? FASEB J 1999, 13:1137-1143.

68. Haddad JJ, Lauterbach R, Saade NE, Saieh-Garabedian B, Land SC: α-Melanocyte-related tripeptide, Lys-D-Pro-Val, ameliorates endotoxin-induced NF-κB translocation and activation: evidence for involvement of an interleukin-1β receptor antagonist in the alveolar epithelium. Biochem J 2001, 355: 29-38.

69. Bruick RK, McKnight SL: Transcription enhanced: oxygen sensing gets a second wind. Science 2002, 295:807-808.

70. Haddad JJ: Oxygen homeostasis, thiol equilibrium and redox regulation of signalling transcription factors in the alveolar epithelium. Cell Signal 2002, 14:799-810.