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REVIEW ARTICLE

Oxidative stress and gene expression in sepsis

J. Macdonald, H. F. Galley* and N. R. Webster

Academic Unit of Anaesthesia and Intensive Care, Institute of Medical Sciences, Foresterhill, Aberdeen AB25 2ZD, UK

**Corresponding author. E-mail: h.f.galley@abdn.ac.uk*

Dysregulation of the immuno-inflammatory response, as seen in sepsis, may culminate in host cell and organ damage. Lipopolysaccharide from Gram-negative bacterial cell walls induces gene activation and subsequent inflammatory mediator expression. Gene activation is regulated by a number of transcription factors at the nuclear level, of which nuclear factor κB appears to have a central role. The redox (reduction–oxidation) cellular balance is important for normal cellular function, including transcription factor regulation. In sepsis, a state of severe oxidative stress is encountered, with host endogenous antioxidant defences overcome. This has implications for cellular function and the regulation of gene expression. This review gives an overview of the mechanisms by which transcription factor activation and inflammatory mediator overexpression occur in sepsis, together with the events surrounding the state of oxidative stress encountered and the effects on the host's antioxidant defences. The effect of oxidative stress on transcription factor regulation is considered, together with the role of antioxidant repletion in transcription factor activation and in sepsis in general. Other interventions that may modulate transcription factor activation are also highlighted.

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Sepsis and its sequelae continue to be the main causes of morbidity and mortality in the intensive care unit (ICU), with an estimated 400 000–500 000 patients developing sepsis in this setting each year in Europe and the USA. Sepsis is part of a spectrum of conditions ranging from the systemic inflammatory response syndrome (SIRS) to septic shock and multiple organ dysfunction syndrome (MODS). The definitions of these conditions have been rationalized, leading to the common language currently used.[17] The mortality associated with these conditions ranges from around 26% in patients with SIRS to around 82% in septic shock,[82] and shows no sign of decreasing despite optimal current therapy. Sepsis therefore continues to have significant clinical and financial implications and remains an area that attracts intense research interest. Regulation and coordination of the immuno-inflammatory response by cytokines and other mediators is essential for host defence. The underlying molecular events are complex and culminate in altered gene expression. Dysregulation of this response may occur in sepsis, leading to excessive or inappropriate release of mediators and ultimately host cell and organ damage. There is convincing evidence of severe oxidative stress in patients with sepsis. As oxygen free radicals and other reactive oxygen species appear to be involved as messengers in cellular signal transduction and gene activation, this has implications for the expression and control of the immuno-inflammatory response in sepsis. Therapeutic intervention with antioxidant therapy to alter signal transduction and mediator production, and hence the course of sepsis, is also a possibility.

Oxidative stress and antioxidant protection mechanisms

Under normal physiological conditions, a homeostatic balance exists between the formation of reactive oxidizing/oxygen species and their removal by endogenous antioxidant scavenging compounds.[50] Oxidative stress occurs when this balance is disrupted by excessive production of reactive oxygen species, including superoxide, hydrogen peroxide and hydroxyl radicals, and/or by inadequate antioxidative defences,[49] including superoxide dismutase (SOD), catalase, vitamins C and E, and reduced glutathione (GSH). Both may occur in sepsis.

Reactive oxygen species

When molecules are oxidized during metabolism, the oxygen molecule itself is reduced to water, giving rise to intermediate reactive oxygen species:

$O_2 \rightarrow$	$O_2^{\cdot -} \rightarrow$	$H_2O_2 \rightarrow$	$^{\cdot}HO \rightarrow$	H_2O
Molecular oxygen	Superoxide anion radical	Hydrogen peroxide	Hydroxyl radical	Water

($^{\cdot}$ = unpaired electron of a free radical)

Superoxide and hydroxyl are described as free radicals because they have an atom or molecule which has one or more unpaired electron(s); this renders the free radical highly reactive and potentially toxic.

Superoxide is converted to hydrogen peroxide by the enzyme SOD. In the absence of transition metal ions, hydrogen peroxide is fairly stable. It does, however, allow neutrophils to oxidize chloride ions, via myeloperoxidase, into hypochlorous acid, providing additional cytotoxic activity. Excess hydrogen peroxide is normally converted harmlessly to water by the action of catalase, glutathione peroxidase and other peroxidases. Hydroxyl radicals can be formed by the reaction of superoxide with hydrogen peroxide in the presence of metal ions (usually iron or copper). Hydroxyl free radicals are much more reactive than superoxide anions.[100] Iron-catalysed hydroxyl generation requires that the iron is in its reduced, ferrous form (Fe^{2+}), whereas most iron existing in cells and plasma is in the oxidized form (Fe^{3+}). As well as its involvement with hydrogen peroxide in hydroxyl radical formation, superoxide can also reduce Fe^{3+} to Fe^{2+}, thereby further promoting hydroxyl production. However, most iron in the plasma exists in a bound form as a protective measure, as it is the free component which is able to participate in biochemical reactions. Biology has used molecules for iron metabolism (haem proteins), storage (ferritin) and transport (transferrin) that lock the iron in a state where free radical production cannot occur.

Under normal physiological conditions, the majority of reactive oxygen species are formed during cellular respiration and by activated phagocytic cells, including neutrophils, involved in the inflammatory response. Reactive oxygen species have physiologically essential roles in mitochondrial respiration, prostaglandin production pathways and host defence.[100] The four-electron reduction of oxygen occurs in the mitochondrial electron transport system of all aerobically respiring cells. The enzyme catalysing this reaction (cytochrome *c* oxidase) contains the transition metals iron and copper in its active site. These ions can be paramagnetic and contain stable unpaired electrons. By using the unpaired electrons in these transition metals to control the oxygen reactions, mitochondria prevent the unwanted release of oxygen-derived free radicals.

In sepsis, there are several potential sources of reactive oxygen species, including the mitochondrial respiratory electron transport chain, xanthine oxidase activation as a result of ischaemia and reperfusion, the respiratory burst associated with neutrophil activation, and arachidonic acid metabolism. Activated neutrophils produce superoxide as a cytotoxic agent as part of the respiratory burst via the action of membrane-bound NADPH oxidase on molecular oxygen. Neutrophils also produce the free radical nitric oxide ($NO^{\cdot}$), which can react with superoxide to produce peroxynitrite, itself a powerful oxidant, which may decompose to form the hydroxyl radical. Under ischaemic conditions followed by subsequent reperfusion, the enzyme xanthine oxidase catalyses the formation of uric acid with the coproduction of superoxide. Superoxide release results in the recruitment and activation of neutrophils and their adherence to endothelial cells, which stimulates the formation of xanthine oxidase in the endothelium, with further superoxide production.

During oxidative stress, damage mediated by reactive oxygen species can occur. Oxidation of DNA and proteins may take place, along with membrane damage, because of lipid peroxidation, leading to alterations in membrane permeability, modification of protein structure and functional changes.[105] Oxidative damage to the mitochondrial membrane can also occur, resulting in membrane depolarization and the uncoupling of oxidative phosphorylation, with altered cellular respiration.[70] This can ultimately lead to mitochondrial damage, with release of cytochrome *c*, activation of caspases and apoptosis (programmed cell death).

Endogenous antioxidant defences

Antioxidants are central to the redox balance in the human body. They do not act in isolation, but synergistically. Primary antioxidants prevent oxygen radical formation, whether by removing free radical precursors or by inhibiting catalysts, e.g. glutathione peroxidase and catalase. Secondary antioxidants react with reactive oxygen species which have already been formed, either to remove or inhibit them, e.g. vitamins C and E. Endogenous antioxidant defences exist at a number of locations, namely intracellularly, on the cell membrane and extracellularly (reviewed by Gutteridge and Mitchell).[50]

Intracellular antioxidants

The SOD enzymes are a family of metalloenzymes which rapidly promote the conversion of superoxide to hydrogen peroxide. Three forms of SOD are recognized to be important: copper–zinc SOD (cytoplasm), manganese SOD (mitochondria) and extracellular SOD (extracellular matrix). Catalase and glutathione peroxidase, a selenium-containing enzyme which requires the presence of reduced GSH for its action, catalyse the conversion of hydrogen peroxide to water. Reduced GSH (L-γ-glutamyl-L-cysteinyl-glycine) contains a thiol (sulphydryl) group. The intracellular antioxidants and the role of reduced GSH (including synthesis and recycling) are shown in Figure 1. GSH also

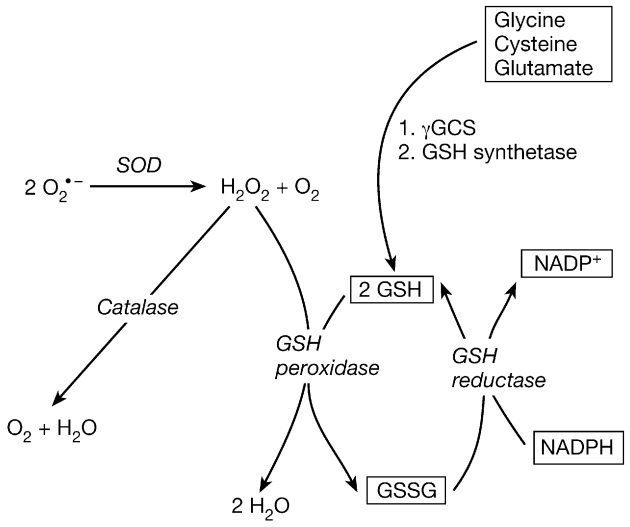

Fig 1 A schematic diagram showing the interaction between intracellular antioxidants. $O_2^{\bullet-}$=superoxide anion radical; SOD=superoxide dismutase; H_2O_2=hydrogen peroxide; GSH=reduced GSH (L-γ-glutamyl-L-cysteinyl-glycine); GSSG=oxidized GSH; γGCS=γ-glutamylcysteine synthetase.

has direct antioxidant activity, through donation of hydrogen ions, to repair damaged DNA. Oxidative stress and modulation of GSH/GSSG (GSSG=oxidized GSH) levels also up-regulate gene expression of several other antioxidant proteins, such as manganese SOD, glutathione peroxidase, thioredoxin and metallothionein.

Membrane antioxidants

The hydrophobic lipid interior of membranes requires a different spectrum of antioxidants. Fat-soluble vitamin E (α-tocopherol) is the most important antioxidant in this environment. β-Carotene, lycopene and co-enzyme Q have also been implicated as membrane antioxidants. Lipid-soluble antioxidants are important in preventing membrane polyunsaturated fatty acids from undergoing lipid peroxidation, which leads to loss of membrane integrity.

Extracellular antioxidants

Reactive oxygen species may also be present in the extracellular compartment, especially as a result of neutrophil activation. The plasma and red cell components of blood both act as antioxidants; red cells have a copper–zinc SOD-dependent pathway for the inactivation of superoxide, and catalase and glutathione peroxidase for dealing with hydrogen peroxide. A number of metal-binding plasma proteins function as valuable antioxidants in addition to their transport roles, including apotransferrin, lactoferrin and caeruloplasmin. Albumin is also effective via its oxidizable thiol group, which permits radical scavenging, and the binding of reactive transition metal ions. A number of important smaller molecules are present in the plasma, which act as secondary antioxidants. These include vitamin E, vitamin C (ascorbic acid), uric acid and bilirubin. Ascorbic acid interacts with superoxide to form dehydroascorbic acid. Vitamin C may also reduce Fe^{3+} to Fe^{2+}, which can then be involved in iron-catalysed hydroxyl generation, thereby implicating vitamin C as both a pro-oxidant and an antioxidant.

Oxidative stress in sepsis

There now exists a considerable body of evidence for redox imbalance and oxidative stress in human sepsis, demonstrating increased markers of oxidative damage, direct evidence of free radical production using electron paramagnetic resonance analysis, xanthine oxidase activation, increased redox reactive iron, abnormal handling of exogenous antioxidants, and low concentrations of individual endogenous antioxidants. Early work by Takeda and colleagues[94] found reduced plasma α-tocopherol levels accompanied by increased plasma thiobarbituric acid-reactive substance (TBARS) levels in critically ill patients compared with controls, suggesting increased lipid peroxidation. Goode and colleagues[45] investigated antioxidant status in patients with septic shock. They reported reduced plasma concentrations of retinol (vitamin A), tocopherol (vitamin E), β-carotene and lycopene in these patients compared with healthy controls. They also found increased plasma TBARS in patients who developed three or more dysfunctional secondary organs, suggesting increased lipid peroxidation. Borrelli and colleagues[18] documented that plasma vitamin C was significantly decreased in ICU patients who developed multiple organ failure compared with those who did not; plasma concentrations of vitamin E, copper and zinc, however, did not differ between the two groups. Galley and colleagues reported increased redox reactive iron concentrations in patients with sepsis or septic shock, coupled with lowered plasma levels of vitamin C[40 41] and elevated lipid peroxides. [42] Later work has, however, disputed the presence of redox reactive iron in the plasma of patients with septic shock.[69 101] Cowley and colleagues[27] described decreased total antioxidant potential in patients with sepsis and secondary organ dysfunction, associated with non-survival. However, a subsequent study reported that although total antioxidant capacity was decreased in patients with sepsis, it was increased in patients with septic shock, which was attributed to high bilirubin levels.[75] Increased xanthine oxidase activity has been reported in patients with sepsis or SIRS in both the adult[39] and paediatric[10 71] populations. Galley and colleagues[39] found xanthine oxidase activation and high free radical concentrations in septic patients compared with both healthy volunteers and non-infected patients. Batra and colleagues[10] found an increase in xanthine oxidase, SOD and glutathione peroxidase activity in neonates with sepsis, suggesting increased production of reactive oxygen species in this population. However, malondialdehyde levels (a marker of lipid peroxidation) were also increased, suggesting that the elevations of these antioxidant enzymes were not so effective as to prevent cellular damage.

GSH metabolism is altered in sepsis. Rapid depletion of intracellular GSH in human and animal endothelial and epithelial cells occurs in response to tumour necrosis factor α (TNF-α) *in vitro* because of oxidation of GSH to GSSG, followed by rebound increases in GSH synthesis as a result of up-regulation of the enzyme γ-glutamylcysteine synthetase (γGCS). GSH turnover is increased early in sepsis in rats, with increased GSH synthesis in a number of tissues (especially the liver), but with lower blood GSH concentrations.[64] In a rat lipopolysaccharide (LPS) endotoxic shock model, oxidative stress was apparent, with decreased plasma antioxidant capacity, potentiated by depletion of liver GSH.[21] In children with sepsis, whole blood GSH concentrations and synthesis rates were found to be decreased,[63] while blood GSH redox ratios (GSSG:GSH) were found to be increased,[71] suggesting increased oxidative stress. Glutathione peroxidase is a selenium-containing enzyme (selenoenzyme) and selenium depletion is therefore likely to be crucial in antioxidant defences secondary to reduced glutathione peroxidase activity. Selenium itself inhibits transcription and proinflammatory gene expression.[51] Selenium excess (toxicity), however, has also been linked to oxidative stress.[91] Forceville and colleagues[35] reported early and prolonged decreases in plasma selenium concentrations in patients with SIRS, associated with a three-fold increase in morbidity and mortality.

Gene expression and sepsis

Activation of the immune and inflammatory systems occurs in response to both infectious and non-infectious stimuli. In sepsis, Gram-negative and, increasingly, Gram-positive organisms are important causative microbes. Infection initially results in stimulation of the innate (non-specific) immune response, mediated mainly via circulating and tissue inflammatory cells, such as monocytes/macrophages and neutrophils. These cells normally exist in a non-activated state but are rapidly activated in response to bacteria, their products or inflammatory mediators, to become highly active phagocytes. LPS (endotoxin) is the principle component of the cell wall of Gram-negative bacteria, and exotoxins are from Gram-positive bacteria.

The molecular mechanisms by which LPS induces gene activation, and hence inflammatory mediator expression, have been reviewed recently.[48] Briefly, LPS initially binds to the acute-phase LPS-binding protein (LBP) in the plasma, the level of which appears to rise in response to the insult. Bound LPS is subsequently delivered to the monocyte (and neutrophil) CD14 surface receptor. LPS then interacts with the transmembrane signal transduction receptor Toll-like receptor 4 (TLR4), which exists in complex with the accessory protein MD-2. TLR2 has been implicated as the receptor for Gram-positive exotoxin.[34] This complexing and binding of LPS subsequently activates a number of intracellular signalling pathways, including the IκB kinase (IKK)–nuclear factor κB (NFκB) pathway and three mitogen-activated protein kinase (MAPK) pathways. These pathways phosphorylate and activate various transcription factors (see below), including NFκB/Rel proteins, activator protein 1 (AP-1) and nuclear factor–interleukin 6 (NF-IL-6), thereby allowing rapid gene induction and the expression of inflammatory mediators, including cytokines, chemokines, lipid mediators, inducible nitric oxide synthase (type II NOS), enzymes and adhesion molecules. Cytokines are low molecular weight soluble proteins which are synthesized and secreted directly in response to inflammatory stimuli. As well as initiating the immuno-inflammatory response, they also coordinate and modulate the nature, amplitude and duration of this response. Cytokines have a variety of target cells, with their specific actions dependent upon the stimulus, the cell type and the presence of other inflammatory mediators and receptors.[43] The key characteristics which cytokines exhibit are redundancy, pleiotropy, synergy and antagonism. Recently, a number of other mechanisms by which LPS induces gene transcription and inflammatory mediator expression have been described, including mechanisms operating TREM-1 (triggering receptor expressed on myeloid cells 1),[19 20] macrophage transmembrane potassium channels,[15] and the intracellular cytoplasmic proteins Nod1 and Nod2.[53] An outline of the proposed molecular mechanisms by which LPS stimulates the host inflammatory response is seen in Figure 2.

Gene expression and oxidative stress

The synthesis of messenger RNA (mRNA) from template DNA is called gene transcription, and occurs in the cell nucleus. After modification, mRNA is transported to the cytoplasm, where it is translated into a protein, such as a cytokine or an enzyme. Gene transcription is controlled by various transcription factors, which are DNA-binding proteins, and several transcription factors can control the production of one protein. Translocation of a transcription factor to the cell nucleus and subsequent binding to a target gene(s) results in protein synthesis. Although a number of transcription factors may be linked to the altered gene activation seen in sepsis, including AP-1 and NF-IL-6, the one that has been described in most detail is NFκB (a family of proteins belonging to the Rel family). Substantial *in vitro* and *in vivo* evidence suggests a pivotal role for NFκB in sepsis and SIRS.

Nuclear factor κB

NFκB is a ubiquitous transcription factor which is crucial for normal immune system function, regulating the activation of genes necessary to provide rapid and appropriate responses. However, inappropriate, increased and/or prolonged activation of NFκB, resulting in the overexpression of mediator proteins, may account for the deleterious effects seen in sepsis.

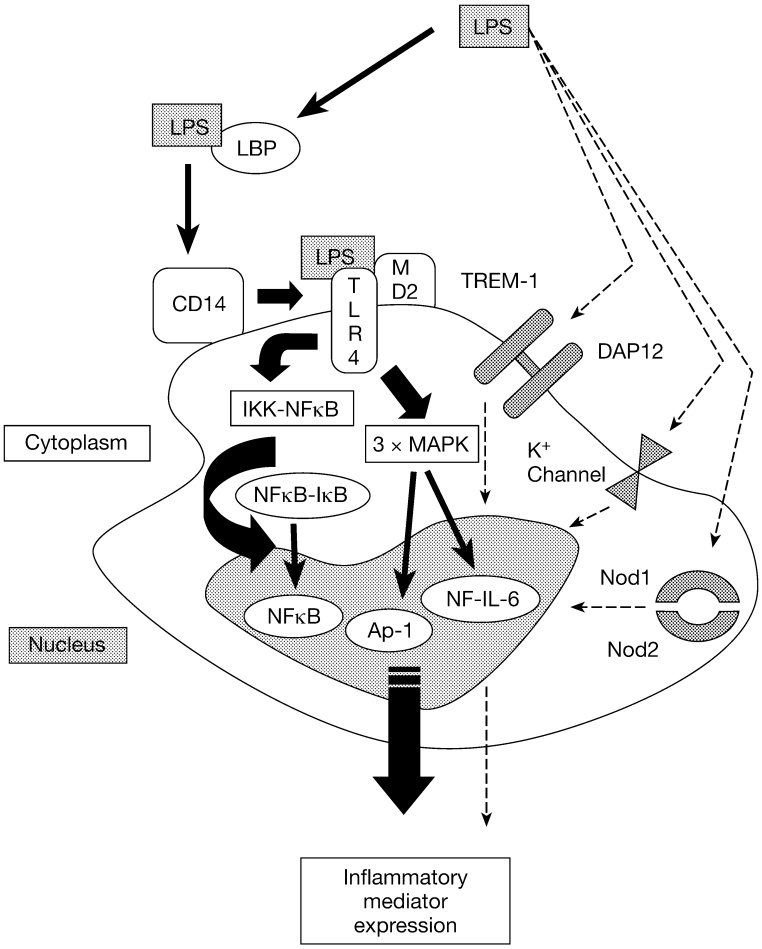

Fig 2 A schematic diagram showing LPS stimulation of the host inflammatory response in monocytes, macrophages and neutrophils. NFκB=nuclear factor κB; AP-1=activator protein 1; NF-IL-6=nuclear factor-interleukin-6; LPS=lipopolysaccharide; LBP=LPS-binding protein; CD14=CD14 surface receptor; TLR4=Toll-like receptor 4; MD-2=accessory protein MD-2; IKK-NFκB=IκB kinase–NFκB pathway; NFκB-IκB=NFκB–inhibitory protein IκB complex; MAPK=mitogen-activated protein kinase pathway; TREM-1=triggering receptor expressed on myeloid cells 1; DAP12=transmembrane adapter molecule DAP12; Nod1 and Nod2=intracellular cytoplasmic proteins Nod1 and Nod2.

NFκB is a dimer consisting of two Rel subunits, and there are five known mammalian NFκB-Rel proteins. The classical NFκB dimer contains the proteins p50 (NFκB1) and p65 (Rel A). However, the subunit composition of NFκB can vary, with differing effects on gene regulation depending upon the specific combinations.[2] NFκB exists in the cytoplasm in an inactive form, complexed with an inhibitory protein from the IκB family, which includes IκBα, IκBβ and IκBε. The IκB proteins mask a nuclear localization signal on NFκB proteins, thereby preventing the translocation of NFκB to the nucleus. NFκB can be activated in cells by a number of inflammatory stimuli in addition to LPS, including cytokines [such as TNF-α, interleukin (IL) 1β, 11 and 17], reactive oxidant species (especially hydrogen peroxide), protein kinase C activators, viruses, UV light and ionizing radiation.[8]

NFκB activation is achieved via phosphorylation and degradation of the inhibitory IκB protein through the action of specific kinases, the NFκB-inducible kinases (NIK), IKK-1 and IKK-2.[2] Degradation of IκB is ultimately accomplished by attachment of ubiquitin residues and proteolysis, unmasking the nuclear localization signal, thus allowing translocation of NFκB to the cell nucleus; translocation may involve the protein BCL-2.[98] NFκB is then able to bind to its target genes to initiate transcription, translation and hence protein synthesis. The key regulatory step in the activation of NFκB by inflammatory stimuli is the activation of the IKK complexes.[59] There are many genes which contain sequence-specific NFκB binding sites in their promoter regions, a selection of which are shown in Table 1.[2 25 48] It should be noted, however, that the presence of an NFκB binding site in a gene does not necessarily imply regulation by NFκB. Feedback loops have been identified which may amplify the response to the initial stimulus (positive feedback) or regulate NFκB activation (negative feedback).[12] In addition, LPS can induce synthesis of anti-inflammatory cytokines such as IL-10 (and also IL-4 and IL-13), thereby blocking NFκB activation and

Table 1 Genes containing sequence-specific NFκB binding sites in their promoter regions

Cytokines and growth factors	Tumour necrosis factor α
	IL-1β, IL-2, IL-6, IL-12
	Colony stimulating factors
	Granulocyte colony stimulating factor
	Interferon-β
	Vascular endothelial growth factor
Chemokines	IL-8, growth-related oncogene (-α, -β,- γ)
	Regulated upon activation, normal T-cell expressed and secreted (RANTES)
	Monocyte chemoattractant protein 1
Cell adhesion molecules	E-selectin
	Intercellular cell adhesion molecule 1
	Vascular cell adhesion molecule 1
Enzymes	Type II nitric oxide synthase, cyclooxygenase 2, phospholipase A2
	Matrix metalloproteinases
Immunoreceptors	Tissue factor, IL-2 receptor α
	Major histocompatibility complex class I
Acute phase proteins	C-reactive protein, lipopolysaccharide binding protein

IL=interleukin.

inhibiting cytokine production,[99] providing an extracellular mechanism for negative feedback. NFκB activation and regulation are summarized in Figure 3.

Redox regulation of NFκB and oxidative stress

The intracellular redox (reduction–oxidation) state is important physiologically in terms of maintaining cellular homeostasis. The major intracellular regulator of redox homeostasis is GSH, which acts via reversible oxidation of an active thiol group.[6] NFκB has been implicated in the upregulation of the expression of the rate-limiting enzyme for GSH synthesis, γGCS, in response to various stimuli, including inflammatory cytokines (IL-1β and possibly TNF-α).[47] Another important thiol-containing compound involved in redox homeostasis is thioredoxin. Thioredoxin has a large number of functions other than its originally identified role as a hydrogen donor for ribonucleotide reductase, which is essential for DNA synthesis. Every thioredoxin contains the amino acid sequence Trp–Cys–Gly–Pro–Cys, and the two cysteine residues can be reversibly oxidized to form a disulphide bridge. Because of its general protein disulphide reductase activity, thioredoxin can regulate enzymes and transcription factors by thiol redox control. After the dissociation of IκB, reduction of NFκB by thioredoxin is necessary for the binding of NFκB to DNA in the nucleus. However, thioredoxin seems to play dual and opposing roles in the regulation of NFκB, because, in the cytoplasm, thioredoxin interferes with the signal to IκB kinases and blocks the degradation of IκB. Thioredoxin also contributes indirectly to AP-1 activation via the nuclear redox factor Ref-1 (redoxfactor 1).

At least two transcription factors, NFκB and AP-1, have been identified as being regulated by the intracellular redox state.[87] It has been hypothesized that intracellular redox changes may have important effects on the activation of these transcription factors and the subsequent inflammatory response. Sun and Oberley[92] reviewed the redox regulation of transcription factors, including AP-1 and NFκB. Transcription factor redox regulation appears to occur via highly conserved cysteine residues in their DNA binding domains. In general, in intact cells, antioxidants and oxidant species appear to have opposite effects, antioxidants decreasing NFκB activation while dramatically increasing AP-1 activation (AP-1 acting as a secondary antioxidant response factor), and oxidant species, conversely, strongly activating NFκB while still moderately activating AP-1.[67]

However, there are several points which should be noted in terms of redox regulation of NFκB. Data are conflicting, with cell type-specific differences. In addition, most of the evidence of redox signalling is indirect, being based on the inhibition of NFκB activation by antioxidants, and direct evidence has not been reported.[59] The relative importance of sites within the NFκB activation pathway which are amenable to redox influence remains to be elucidated. It is postulated that NFκB regulation may occur at various sites, including the specific kinases (NIK/IKK/MAPKs) and phosphorylation steps, protease activity, nuclear translocation, and gene transcription.

NFκB and sepsis

Several cell culture and animal studies have demonstrated a link between sepsis and NFκB activation. In a recent animal study, LPS administration resulted in NFκB activation in several organs, associated with an increase in both lung tissue mRNA and protein expression of a variety of NFκB-regulated cytokines, including TNF-α and IL-6.[13] As oxidative stress regulates NFκB activation and evidence of such stress has been demonstrated in patients with sepsis, it is likely that increased NFκB, and hence upregulation of cytokines, will occur in these patients.

Indeed, several studies have now demonstrated increased NFκB activity in isolated leucocytes from patients with sepsis or SIRS, associated with non-survival.[5 16 76] Bohrer and colleagues[16] reported that increased NFκB activity was comparable to the Acute Physiology and Chronic Health Evaluation (APACHE II)

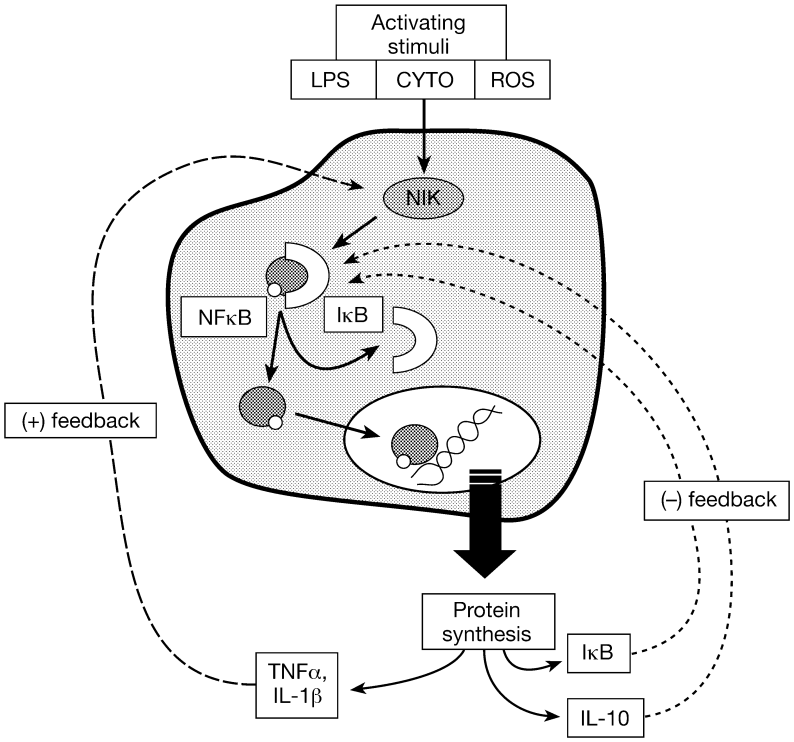

Fig 3 A schematic diagram showing NFκB activation and regulation. LPS=lipopolysaccharide; CYTO=proinflammatory cytokines; ROS=reactive oxygen species; NFκB=nuclear factor κB; NIK=NFκB inducible kinase; IκB=inhibitory protein IκB; TNF-α=tumour necrosis factor α; IL-1β=interleukin-1β; IL-10=interleukin-10.

score as a predictor of outcome and mortality from sepsis. These findings were confirmed by Arnalich and colleagues,[5] who found that NFκB activity was significantly higher in non-survivors and correlated strongly with APACHE II score. These authors also found that, of the cytokines they measured, only IL-1 receptor antagonist (IL-1ra) was of value in predicting mortality, although this is an anti-inflammatory mediator with no known effects exerted via NFκB. However, Paterson and colleagues[76] reported increased mononuclear cell NFκB activation in critically ill patients who died, which was inhibitable by treatment with the antioxidant *N*-acetylcysteine. Circulating levels of IL-8 but not IL-6 or intracellular adhesion molecule 1 (ICAM-1) were also decreased in patients receiving *N*-acetylcysteine.[77] IL-8 has been shown to be regulated at the transcriptional level by NFκB.[68]

A number of agents have been employed in both the experimental and clinical settings with the aim of reducing oxidative stress, thus inhibiting activation of transcription factors (mainly NFκB) and hence attenuating the inflammatory response.

Interventions to regulate gene expression

The clear evidence for oxidative stress in sepsis and the link with inflammatory gene expression has provided a foundation for intervention to either reduce oxidative stress or inhibit transcriptional activation.

Strategies to enhance endogenous antioxidant defences

Glutathione

N-acetylcysteine is a sulphydryl donor which can replete intracellular GSH by donating cysteine; however, the GSH synthesis enzymes are necessary. GSH monoester does not require γGCS or glutathione synthetase. *N*-acetylcysteine also has direct antioxidant activity[7] and has been safely used to treat acetaminophen overdose for two decades.[95] In animal studies, increased survival on exposure to endotoxin, decreased cytokine and adhesion molecule expression, decreased oxidative stress and inhibition of NFκB have been demonstrated in response to administration of *N*-acetylcysteine. Pretreatment with *N*-acetylcysteine before endotoxin administration resulted in decreased NFκB activation,[14] lower TNF-α release and increased survival.[104] However, in a murine caecal ligation and puncture model of sepsis, improved survival after *N*-acetylcysteine treatment was not associated with lower TNF-α or increased liver GSH content.[97] Administration of *N*-acetylcysteine along with α-tocopherol (vitamin E) suppressed NFκB activation[36] and with vitamin E and β-carotene it reduced lipid peroxidation and restored GSH levels in endotoxic rats.[54] In a rat model of lung injury, *N*-acetylcysteine

decreased lipid peroxidation and reduced the expression of several inflammatory mediators.[28]

In the clinical setting, *N*-acetylcysteine alone and in combination with other antioxidants has been shown to have variable results. In a randomized, placebo-controlled trial, Paterson and colleagues[77] found a reduction in mononuclear leucocyte NFκB activation after infusion of *N*-acetylcysteine in patients with sepsis, associated with decreases in cytokines. An earlier acute study, in which *N*-acetylcysteine was administered in conjunction with ascorbic acid and α-tocopherol in patients with septic shock, did not investigate NFκB, and neither total antioxidant capacity nor lipid peroxidation was changed.[42] More recently, Ortolani and colleagues[74] randomized patients with early septic shock to receive either placebo, i.v. GSH or GSH plus *N*-acetylcysteine. Administration of high doses of *N*-acetylcysteine plus GSH significantly decreased markers of oxidative stress (expiration of ethane and lipid peroxidation). Other randomized clinical trials have been undertaken employing *N*-acetylcysteine in septic shock.[78 88] Although Spapen and colleagues[88] did show a significant improvement in oxygenation and static lung compliance at 24 h and reduced IL-8 levels in *N*-acetylcysteine-treated patients, in none of these trials was survival improved. However, these small studies were not powered to detect changes in mortality.

Decreased plasma selenium and glutathione peroxidase activity have been shown in patients with sepsis, and two small clinical studies of selenium repletion have been reported.[3 44] In both reports, selenium administration was associated with a decrease in organ failure scores, notably a reduction in the incidence of acute renal failure requiring haemodialysis. However, inspection of these reports suggests that they may be describing the same study, the later report published in English[3] and the earlier in German.[44] A commentary which accompanied the later paper also highlighted the fact that the study was not blinded, and that there were differences in the distribution of infecting organisms and other imbalances between the patients who received selenium and those who did not.[73]

Vitamins

Vitamin C (ascorbic acid) is a powerful electron donor, reacting with both superoxide and hydroxyl radicals. *Ex vivo* studies demonstrated regulation of cellular activity by exogenous ascorbic acid, in that the increased adherence of, and superoxide anion production by, macrophages from mice with endotoxic shock were lower in the presence of ascorbic acid.[96] In a rat caecal ligation and puncture model, exogenous administration of ascorbic acid protected against compromised microvascular perfusion. *In vitro* studies showed that ascorbic acid inhibited the replication of bacteria and prevented hydrogen peroxide injury to cultured microvascular endothelial cells.[4] In guinea-pigs, which, like humans, cannot synthesize their own vitamin C, administration of endotoxin rapidly depleted vitamin C stores; repletion prevented oxidative damage.[81] However, in another study using infusion of live bacteria, mortality was only improved in guinea-pigs receiving high doses of vitamin E; high doses of vitamin C did not improve survival.[79] Circulating concentrations of vitamin C are markedly depleted in patients with sepsis.[18 41 42 85] Markedly different handling of infused ascorbate compared with healthy subjects was reported,[41] and administration of vitamin C in conjunction with other antioxidants failed to ameliorate free radical-mediated damage.[42]

There have been several studies which have reported low circulating vitamin E (α-tocopherol) levels in sepsis. A steady decrease in circulating α-tocopherol levels in plasma was reported in a pig septic shock model in both survivor and non-survivor animals. This was accompanied by a simultaneous increase in plasma 8-iso-prostaglandin $F_{2\alpha}$, a marker of oxidative injury, and these levels increased steadily in the animals which died.[9] Low vitamin E concentration associated with evidence of oxidative stress has also been reported in patients.[45 94] Vitamin E derivatives have been shown to inhibit NFκB activation *in vitro*.[33 93] Suzuki and Packer[93] found that both α-tocopherol acetate and α-tocopherol succinate inhibited TNF-α-induced NFκB activation in a T-cell line, whereas Erl and colleagues[33] found that only α-tocopherol succinate caused a reduction in NFκB activation in human endothelial cells. α-Tocopherol did not inhibit NFκB in either study. Vitamin E has been shown to be protective in reducing the effects of oxidative stress in sepsis in a number of animal studies (reviewed by Goode and Webster).[46] High doses of vitamin E reduced mortality in guinea-pigs infused with live bacteria, as described above.[79] Monocytes from α-tocopherol-supplemented human volunteers were found to have significantly suppressed responses to LPS,[31] but clinical studies are limited. In conjunction with other antioxidants, vitamin E had little effect.[42] The practicality of giving vitamin E i.v. in critically ill patients is a problem; these authors used an i.m. preparation (Ephynal) supplied by Roche,[42] but allergic reactions to the solvent (Cremophore) can occur. The alternative route would be enteral; this approach has been used in animals.

Large molecular weight proteins

Administration of albumin in patients with sepsis was shown to lead to a sustained increase in plasma thiols, which are known to have antioxidant effects and may therefore be beneficial.[80] However, other parameters and outcomes were not reported; this is especially important considering the controversies surrounding the Cochrane systematic review of human albumin administration in the critically ill,[26] which suggested that albumin administration may increase mortality in this group of patients.

Synthetic antioxidant agents

Dimethylsulphoxide

The potent antioxidant agent dimethyl sulphoxide (DMSO) has been found to regulate transcription factor activation in

septic rats. Pretreatment with DMSO before induction of sepsis inhibited hepatic NFκB activation and ICAM-1 gene expression.[22] A subsequent study in which DMSO was administered after the septic challenge also reported reduced transcriptional activation and ICAM-1 gene expression.[23] DMSO has been studied in man in the treatment of intracranial hypertension.[65] As well as cautioning its use, the authors highlighted the mechanical difficulties in the administration of DMSO because of its solvent properties, with the resultant tendency to dissolve standard i.v. infusion systems.

Lazaroids

Lazaroids are 21-aminosteroid drugs which have potent anti-inflammatory and antioxidant properties. In rats they have been shown to suppress sepsis-induced increases in renal vascular resistance and improve renal blood flow via free radical scavenging and altered arachidonic acid metabolism.[56 57] Improved hepatic blood flow and preserved sinusoidal endothelial cell function and structure were also shown in a dog endotoxic shock model.[89] In mice, lazaroids suppressed proinflammatory gene activation in endotoxin-induced shock via the inhibition of NFκB activation, and this was shown to be mediated through inhibition of IκB degradation.[38 72] The suggestion from these animal studies is that lazaroids may have a role in the prevention or treatment of sepsis-related organ failure. However, a role, if any, for lazaroids in the management of clinical sepsis remains far from being elucidated.

Pyrrolidine dithiocarbamate

Pyrrolidine dithiocarbamate (PDTC) is a water-soluble, low molecular weight substance which has antioxidant properties. It has been shown to inhibit NFκB activation in rat and mouse models of septic shock by preventing LPS-induced IκBα degradation, with subsequent inhibition of NFκB subunit translocation to the nucleus, resulting in reduced expression of type II nitric oxide synthase, TNF-α and other mediators.[58 60 61 62] Reduced mortality in PDTC-treated septic mice was attributed to both a direct antioxidant effect in addition to NFκB inhibition.[66] Again, studies involving this treatment in the clinical setting are awaited, so a role, if any, for PDTC remains unclear.

Other agents

Tempol, the natural antioxidants NAO (a water-soluble antioxidant purified from spinach) and apocynin, dimethylthiourea, phenyl-*N*-tert-butylnitrone (PBN), MDL 101,002 (a novel cyclized variant of PBN with increased antioxidant activity) and dihydroxychlorodihydrochalcone (DCDC) are all compounds with antioxidant and free radical scavenging ability. Tempol was found to attenuate the degree of multiple organ failure induced experimentally by zymosan (which enhances the formation of reactive oxygen species) in rats secondarily to its free radical scavenging activity.[29] NAO and apocynin pretreatment before an LPS challenge improved survival in a study of oxidative stress in the rat heart.[11] Dimethylthiourea decreased TNF and NFκB activity and improved survival in two rat models of Gram-negative sepsis, despite having weak hydrogen peroxide-scavenging ability.[90] PBN inhibited the LPS-mediated increase in NFκB DNA binding activity in cultured mice macrophages,[55] inhibiting type II NOS induction. Furthermore, pretreatment with PBN in an LPS-induced rat septic shock model resulted in attenuation of NFκB and AP-1 activation, with suppression of proinflammatory cytokine expression and upregulation of anti-inflammatory IL-10 expression.[83] MDL 101,002 decreased organ dysfunction and mortality caused by LPS in a rat model of sepsis, in association with marked inhibition of TNF secretion.[32] Finally, in a study of LPS-induced murine macrophages the free radical scavenger DCDC inhibited IκBα degradation and NFκB activation, resulting in decreased nitric oxide production.[52] There have been no studies in man.

Non-antioxidant transcriptional inhibitors

A number of other NFκB inhibitors have been studied in the laboratory and animal settings. Some of these are known to inhibit NFκB at a particular site in its activation pathway, whereas the inhibition of NFκB by other agents is less specific. Agents which are known to inhibit NFκB in the setting of induced inflammation and sepsis include activated protein C,[102] antisense oligonucleotides to the p65 subunit of NFκB,[84] steroids,[24 38] protease inhibitors,[1 86] D-amino acid peptide,[37] glucans (which also appear to inhibit nuclear factor-IL-6),[103] vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide.[30] Theoretically, the inhibition of NFκB by these agents may interrupt the overexpression of inflammatory mediators seen in sepsis. However, the clinical situation in the critically ill is complex, and such interventions in the treatment of sepsis have proved disappointing thus far. Clinical applications are still awaited for these novel therapies.

Conclusion

The spectrum of cellular insult and host damage seen in sepsis occurs as a result of a dysregulated immuno-inflammatory response to an infective stimulus. A complex molecular network exists which transduces this stimulus and ultimately promotes gene expression with the resultant (over)expression of inflammatory mediators. This occurs via the modulation of a number of intracellular pathways and the nuclear translocation and gene binding of transcription factors, including NFκB. NFκB appears to have a central role in the pathophysiology of sepsis, and firm evidence exists for the upregulation of NFκB activity in human sepsis. However, NFκB appears to have dual and opposing roles in sepsis, being involved in both inflammatory mediator production and antioxidant synthesis and regulation. Therefore, further investigation is needed to clarify the precise role of this transcription factor in sepsis.

Cellular injury can be promoted by reactive oxygen (and nitrogen) species, formed when endogenous antioxidant defences are overcome and redox imbalance occurs, with the resultant state of oxidative stress. Indeed, considerable evidence now exists for redox imbalance and oxidative stress in human sepsis. Furthermore, the regulation of NFκB appears to be at least in part attributable to the redox state of the cell. Thus, oxidative stress may be involved in cellular injury in sepsis through both direct and indirect mechanisms. However, most of the evidence for this redox regulation of NFκB is based indirectly upon the inhibition of NFκB by antioxidants. Conclusive direct evidence is awaited.

A number of agents have been used experimentally in both animal models of sepsis and human sepsis, with the aim of combating the products of oxidative stress and modulating transcription factor activation. Alterations in NFκB activation and/or markers of oxidative damage have been demonstrated in the use of a number of antioxidant therapies. *N*-acetylcysteine in particular has been the focus of a number of studies. However, no antioxidant therapy to date has been shown to improve survival in human sepsis, although it is our view that a properly powered study may indeed indicate benefit. The prospect of finding the ideal therapeutic antioxidant agent increases as the quest to elucidate the precise cellular and molecular interactions in sepsis continues to yield intriguing information. In particular, it is becoming apparent that a number of key mediators in sepsis have variable actions and interactions depending on the precise circumstances, a fact largely unrealized until recently. Carefully targeted modulation of redox balance, notably NFκB, may yet have a role in the future treatment of sepsis.

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