Brain Pathology 10: 153-162(2000)

SYMPOSIUM: Role of Inflammation Following Stroke and Neurotrauma

Redox Regulation of Nuclear Factor Kappa B: Therapeutic Potential for Attenuating Inflammatory Responses

John W. Christman[1], Timothy S. Blackwell[1], and Bernhard H.J. Juurlink[2]

[1] Department of Medicine; Division of Allergy, Pulmonary, and Critical Care Medicine; Vanderbilt University School of Medicine; Nashville, TN
[2] Department of Anatomy and Cell Biology, University of Saskatchewan, Saskatoon, SK

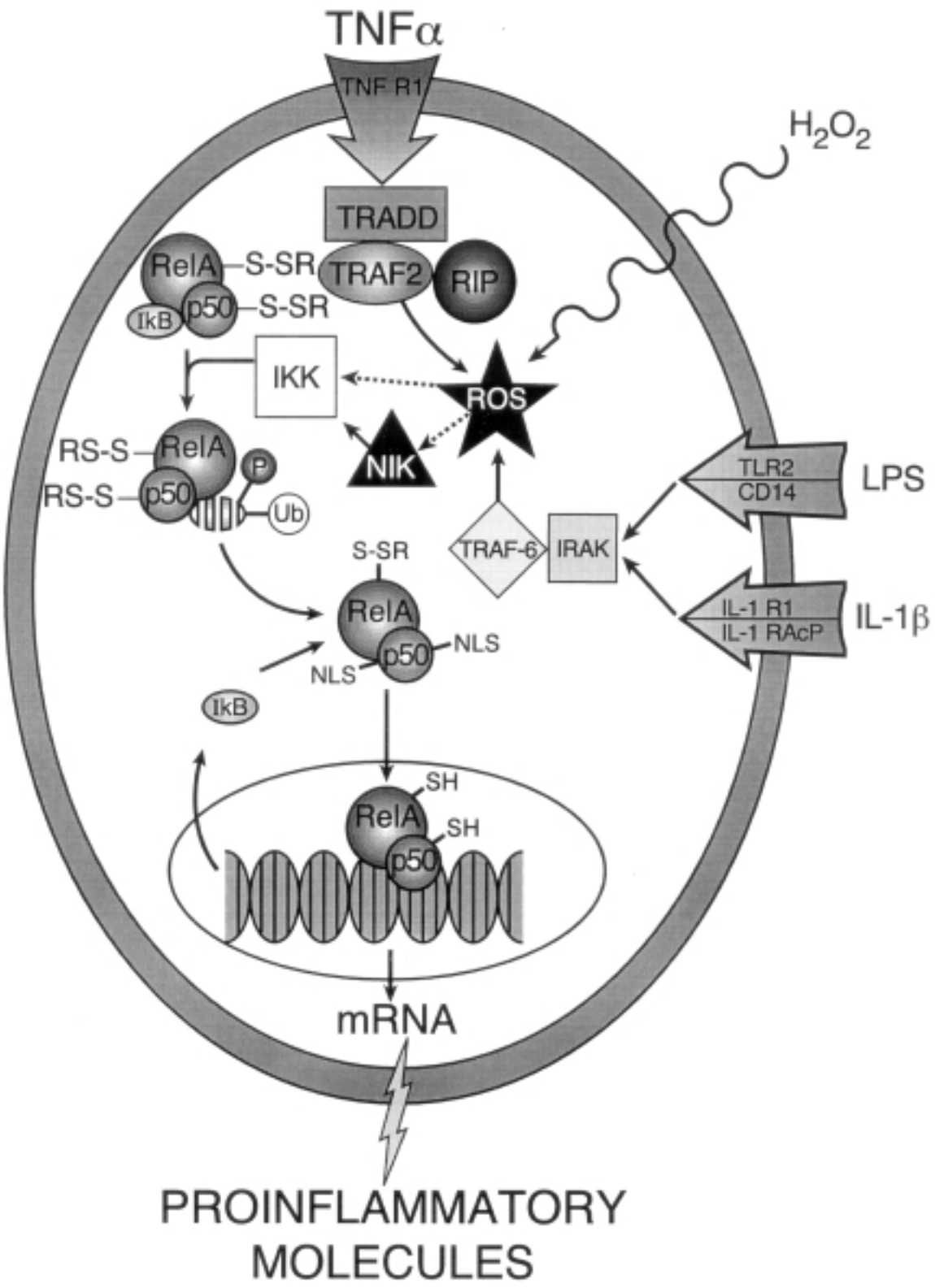
Nuclear factor kappa B (NF-κB) is a protein transcription factor that is required for maximal transcription of a wide array of pro-inflammatory mediators that are involved in the pathogenesis of stroke. The purpose of this review article is to describe what is known about the molecular biology of NF NF-κB and to review current understanding of the interaction between reactive oxygen species (ROS) in NF-κB. ROS seem to play a duel role by participating in the NF-κB activation cascade and by directly modulating DNA binding affinity. Exogenous and endogenous antioxidants are effective in blocking activation of NF-κB and preventing the consequences of pro-inflammatory gene expression. Phase II enzymes either directly or indirectly play a major *in vivo* role in minimizing oxidative stress by scavenging peroxides, peroxide breakdown products and dicarbonyls and in regeneration of lipid peroxidation chain-breaker, vitamin E. Dietary phase II enzyme inducers have been demonstrated to increase phase II enzyme activities in a variety of tissues. These data, together, suggest that phase II enzyme inducers could have therapeutic value for ameliorating inflammatory conditions.

Introduction

Nuclear factor kappa B (NF-κB) is a protein transcription factor that is required for maximal transcription of a wide array of pro-inflammatory molecules which are thought to be important in the generation of acute inflammation; such molecules include cell adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), enzymes such as inducible nitric oxide synthase (iNOS) and cycloxygenase-2 (COX-2), cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumour necrosis factor-α (TNFα), and chemokines such as regulated upon normal T-cell expressed and secreted protein (RANTES), monocytes chemoattractant protein-1(MCP-1), interleukin-8 (IL-8) (8, 14). Since these pro-inflammatory molecules are predominantly regulated at the level of transcription, by inference, NF-κB is a critical intracellular mediator of the inflammatory cascade. Most reported studies on the role of NF-κB activation in the inflammatory process have employed *in vitro* cell culture systems, e.g., (27, 60). Recently, data from animal models suggest involvement of NF-κB in cerebral ischemia-reperfusion injury (11) and response to neurotrauma (6). Enhanced NF-κB activation seems to be involved in the pathogenesis of human cerebral infarction (73), and Alzhemier dementia (9, 36) where activation of NF-κB is correlated with increased COX-2 gene expression (40). Additional data suggest that the atherosclerotic lesion is associated with activation of NF-κB (10). These accumulating data support the thesis that activation of NF-κB contributes to inflammatory disorders associated with the human brain.

The purpose of this review is to describe what is known about the molecular biology of NF-κB and to review information regarding the role of reactive oxygen species (ROS) in the activation of NF-κB. ROS- mediated activation of NF-κB with production of NF-κB dependent inflammatory mediators may be important in the pathogenesis of tissue injury in focal brain ischemia (20) and other disease states. Critical aspects of the cellular response to oxidative stress will be reviewed with considerations given to potential therapeutic avenues to prevent activation of NF-κB.

Corresponding author:
B.H.J. Juurlink, Department of Anatomy and Cell Biology, University of Saskatchewan, 107 Wiggins Road, Saskatoon, SK, S7N 5E5, Canada; Tel.: 306-966-4083; Fax: 306-966-4298; E-mail: juurlink@duke.usask.ca


TNFα
TNF R1
H_2O_2
TRADD
RelA
S-SR
TRAF2
RIP
IkB
p50
S-SR
IKK
ROS
NIK
RS-S
RelA
P
RS-S
p50
Ub
TLR2
CD14
LPS
TRAF-6
IRAK
S-SR
RelA
p50
NLS
NLS
IL-1 R1
IL-1 RAcP
IL-1β
IkB
SH
RelA
SH
p50
mRNA
PROINFLAMMATORY
MOLECULES

Molecular biology of NF-κB

NF-κB is a DNA binding protein that interacts with the enhancing domain of target genes in the configuration of a dimer of two members of the NF-κB/Rel/Dorsal (NRD) family of proteins (48). Although there are five known NRD members, RelA (also called p65), cRel, RelB, p50 (also called NF-κB1) and p52 (also called NF-κB2), the classical dimer is composed of p50 and RelA. Both subunits of this heterodimer contact DNA but only RelA contains a transactivation domain that activates transcription by an interaction with the basal transcription apparatus (62). In unstimulated cells, NF-κB is sequestered in the cytoplasm because of an interaction with a member of the inhibitory (IκB) family. There are six known members of the IκB family, IκBα, IκBβ, IκBγ, and Bcl-3, and p100 and p105 (the precursors for p50 and p52, respectively) (3). All members of the IκB family contain an ankyrin repeat domain that is required for association and inhibition of NF-κB. Following cell stimulation, IκB is phosphorylated, polyubiquinated, and degraded by the 26S proteasome. IκB degradation unmasks nuclear localization peptide sequence signals (NLS) that allow NF-κB to be translocated to the nucleus, where NF-κB binds to a cognate DNA sequence (5′-GGGPuNNPyPyCC-3′) and activates gene transcription.

Though a wide variety of stimuli can activate NF-κB, among the most potent inducers are gram-negative endotoxin or lipopolysaccharide (LPS), TNF-α, and IL-1β which activate NF-κB via receptor-dependent signal transduction that involves specific intracellular protein-protein interactions. A wide array of receptor-independent stimuli, including uv radiation, physical stress or deformation, ischemia-reperfusion, and exposure to H_2O_2, are capable of activating NF-κB but the mechanism is unknown. A simplified version of the key events that link receptor dependent signaling to NF-κB activation is shown in Figure 1. As indicated above, NF-κB activation results from signaled phosphorylation and proteolytic degradation of IκB by the proteasome. The IκB kinase (IKK) signalsome consists of IKKα and IKKβ and other proteins which function to catalyze phosphorylation of serine residues on both IκBα and IκBβ. Activation of the IKK signalsome also requires a phosphorylation event that is mediated by NF-κB inducing kinase, or NIK.

A possible role for oxidative stress in activation of NF-κB

Reactive oxygen species (ROS), including hydrogen peroxide, superoxide anion and the hydroxyl radical have been implicated in the pathogenesis of most inflammatory diseases including cerebral vascular disease. Since pro-inflammatory molecules are involved in the pathogenesis of these inflammatory diseases, interactions between ROS and NF-κB might be a component of the intracellular signaling process that leads to activation. Four areas of evidence indicate that NF-κB activation is linked to the generation of ROS. First, treatment with hydrogen peroxide directly activates NF-κB in some cells (17, 63). In addition, overexpression of superoxide dismutase (SOD), the enzyme that converts superoxide anion to hydrogen peroxide, enhances the TNFα-induced activation of NF-κB (61). There is one report, however, that in SOD over-expressing transgenic mice there is attenuation of NF-κB activation in macrophages (49). Second, most of the known stimuli for NF-κB activation, including LPS, TNFα, and IL-1β, produce oxidative stress in cells (29, 70). Third, treat-

Figure 1. (Opposing page) TNF-α binds to the type 1 TNF receptor (TNFR1), which results in an association with TNFR1 associated death domain protein (TRADD), the receptor-interacting protein (RIP), and the TNF receptor-associated factor-2 (TRAF-2). These cytoplasmic proteins form an active signaling complex that interacts with NF-κB-inducing kinase (NIK). Activation of NIK results in phosphorylation of IκB kinases (IKK), which cause phosphorylation IκB. Phosphorylated IκB is targeted is targetted for destruction by the ubiquination/proteosome degradation pathway, allowing the translocation of NF-κB to the nucleus. IL-1 binds to the type 1 IL-1 receptor (IL-1R1) and the IL-1 receptor accessory protein (IL-1RAcP) which facilitates an interaction between IL-1 receptor-associated kinase (IRAK) and TNR receptor-associated factor-6 (TRAF-6). The interaction between IRAK and TRAF-6 can also be triggered by endotoxin (LPS). LPS binds with high affinity to CD-14, and to Toll-like Receptor 2 (TLR2). These proteins form an active signally complex that also result in activation of NIK and IKK leading to the sequence of events that results in activation of NF-κB. Activation of NF-κB results in expression of mRNA of a variety of pro-inflammatory mediators which are involved in the pathogenesis of lung inflammation. IκB is also induced by NF-κB activation and contributes to the down-regulation of this intracellular signaling cascade. Also depicted are the potential points in this intracellular cascade where reactive oxygen species (ROS) modulate the activation process. Any process that increases intracellular ROS, like TNF, IL-1 or H_2O_2, might influence the activity of NIK or the IKK signalsome. An interrupted line links ROS , NIK, and IKK because this point of interaction has not been proven. Modulation of the sulfhydryl (SH) group in the conserved Cys^{62} in RelA and p50 by cellular redox can dramatically alter DNA binding, oxidation (-S-SR) decreases binding affinity while a reductive process (-SH) is critical for strong NF-κB binding to DNA. This sulfhydryl group is shown in the cytoplasm in the oxidized state (-S-SR) and in the nucleus in the reduced state (SH) to correspond with effects on binding activity.

ment with N-acetylcysteine (NAC), α-lipoic acid, membrane permeable hydroxyl scavengers, metallothionein and the iron chelator, PDTC, blocks NF-κB activation that is induced by a wide variety of stimuli, e.g., (17, 52, 59, 71). These antioxidants are also effective in attenuating inflammation in rodent models of neutrophilic lung and cerebral inflammation (7, 11, 30, 38, 66). Fourth, overexpression of catalase (61), an enzyme that scavenges hydrogen peroxide, as well as overexpression of glutathione peroxidase (37), an enzyme that scavenges hydrogen peroxide as well as organic peroxides using glutathione (GSH) as the electron donor, inhibits the cytokine-induced activation of NF-κB. Further, over expression of γ-glutamylcysteine synthetase, the rate-limiting enzyme for GSH synthesis attenuates TNFα-induced NF-κB activation (41).

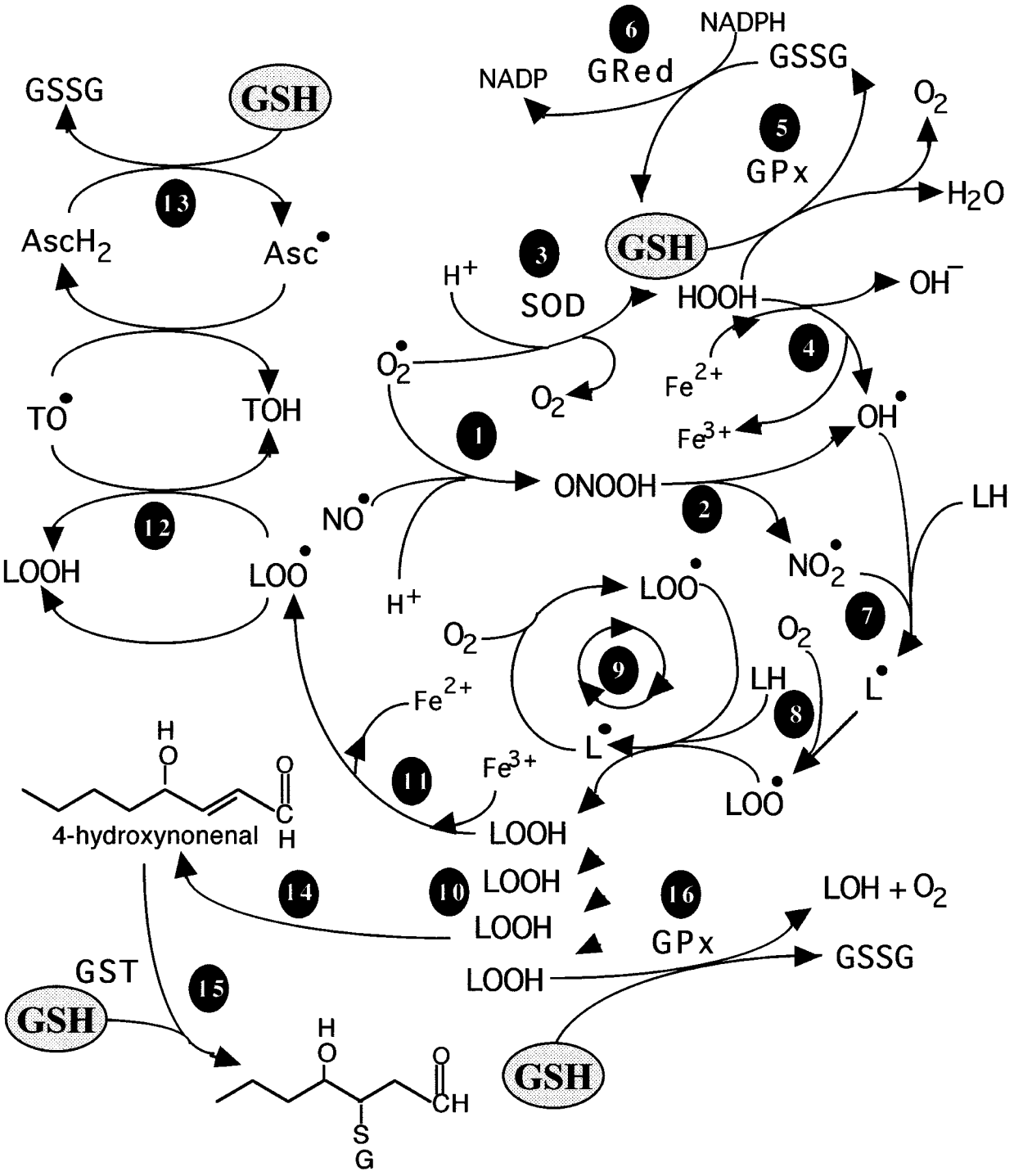
These observations, together, suggest that ROS act as a common second messenger following cellular exposure to agents that induce NF-κB activation (22). However, the common point of the interaction between ROS on the NF-κB activation pathway has not been completely defined (see Figure 1). The most like scenario is that ROS promote the activation pathway by activating a critical redox-sensitive kinase, probably NIK or the IKK signalsome since these molecules lead to phosphorylation of critical serine residues in IκB, resulting in liberation of cytoplasmic RelA/p50 heterodimers. However, the exact biochemical nature of the interaction between ROS and kinase activity of IKKα, IKKβ, or NIK has not yet been delineated. It is possible, though not proven, that the redox state of critical cysteine residues could reversibly modulate kinase activity and regulates NF-κB activation.

Besides NIK and IKK signalsome, a second potential point of impact of ROS on the NF-κB activation pathway has been suggested using cell-free *in vitro* systems. These studies have shown that redox changes can have a direct effect on DNA binding activity. The N-terminal region of NRD proteins, including p50 and RelA, contain a cysteine residue at position 62 (Cys^{62}) which is critical for DNA binding activity but is not essential for dimerization or interaction with IκB family members (43, 44, 76). While mutation of Cys^{62} to Ser does not affect DNA-binding activity, mutation to Asp markedly impairs DNA binding activity in response to redox changes (50). This implies that the conserved Cys^{62} in the N-terminal region of NRD homology domain is susceptible to redox changes that govern binding activity to DNA. Modulation of the sulfhydryl (SH) group in Cys^{62} by cellular redox can dramatically alter DNA binding. Oxidation (-S-SR) decreases binding affinity while a reductive process (-SH) is critical for strong NF-κB binding to its cognate DNA sequence(44). These observations suggest that cellular oxidative stress has a paradoxical action on NF-κB by facilitating activation yet inhibiting DNA binding activity (71). Under conditions of oxidative stress, the sulfhydryl group of Cys^{62} should be in oxidized state in the cytoplasm, but conversion to the reduced state must occur in the nucleus in order for strong DNA binding to occur. Technological limitations will need to be overcome before this *in vitro* observation can be confirmed in whole animal or human studies.

Role of GSH in the management of oxidative stress

That overexpression of γ-glutamylcysteine synthetase attenuates cytokine-induced activation of NF-κB (41) points to a potential therapeutic approach to minimize inflammatory responses. To consider this requires

Figure 2. (Opposing page) The many roles of glutathione (GSH) in the management of oxidative stress. Outlined are some of the sources of oxidative stress and mechanisms used by the cell to minimize oxidative stress. To avoid clutter, no attempt has been made to balance the equations. Superoxide anion can interact with the nitric oxide radical (reaction 1) to give rise to peroxynitrous acid. Peroxynitrous acid gives rise to strong oxidants such as the hydroxyl radical and the nitrogen dioxide radical (reaction 2). Superoxide anion is dismutated by the enzyme superoxide dismutase (SOD) to hydrogen peroxide (reaction 3). Transition metal ions can convert hydrogen peroxide to the hydroxyl anion and the hydroxyl radical (reaction 4). The most efficient way to scavenge hydrogen peroxide (reaction 5) is catalyzed by the enzyme glutathione peroxidase (GPx) that requires GSH as the electron donor. The oxidized-glutathione (GSSG) is normally reduced back to GSH (reaction 6) by the enzyme glutathione reductase (GRed) that uses NADPH as the electron donor. Strong oxidants such as the hydroxyl or nitrogen dioxide radical can extract an electron from a polyunsaturated fatty acid (LH) to give rise to a carbon-centred lipid radical (reaction 7). Such a lipid radical can interact with molecular oxygen to give rise to a lipid peroxyl radical (reaction 8) that in turn can interact with another polyunsaturated fatty acid giving rise to another carbon-centred lipid radical, hence initiating a chain of lipid peroxidations (reaction 9) that results in many of the polyunsaturated fatty acids of cell membranes to form lipid hydroperoxides (10). Such lipid hydroperoxides can interact with transition metal ions to form new lipid peroxyl radicals (reaction 11). Lipid peroxyl radicals are normally reduced by vitamin E (TOH) resulting in the formation of a lipid hydroperoxide and a vitamin E radical (reaction 12). The vitamin E radical is reduced by ascorbic acid ($AscH_2$) and the oxidized ascorbate is reduced by GSH (reaction 13). Lipid hydroperoxides can also break down into aldehydes such the strong oxidant 4-hydroxynonenal (reaction 14) that is rendered into an innocuous glutathiyl adduct (reaction 15) by the phase II enzyme glutathione S-transferase (GST). Lipid hydroperoxides are reduced to alcohols and molecular oxygen (reaction 16) by GPx using GSH as the electron donor. Note the many critical roles that GSH plays in the scavenging of strong oxidants.


GSSG
GSH
13
AscH2
Asc•
TO•
TOH
12
LOOH
LOO•
6
NADPH
NADP
GRed
GSSG
O2
5
GPx
H2O
GSH
3
H+
SOD
HOOH
OH−
O2•
O2
4
Fe2+
Fe3+
OH•
1
ONOOH
NO•
2
LH
NO2•
H+
LOO•
7
O2
O2
9
LH
L•
Fe2+
8
L•
11
Fe3+
LOO•
4-hydroxynonenal
LOOH
14
10
LOOH
LOOH
16
LOH + O2
GPx
GSSG
GST
15
LOOH
GSH
GSH

a brief review of some of the critical roles that GSH plays in the management of oxidative stress (note Figure 2). This has been reviewed in depth elsewhere (33, 34).

One of the sources of oxidative stress is the incomplete reduction of oxygen to the superoxide anion. During normal metabolism approximately 3% of oxygen is incompletely reduced to the superoxide anion (18), this is increased when there is prolonged rises in intracellular Ca^{2+} due to futile mitochondrial Ca^{2+} cycling (58). The CNS, which is only about 2% of the body mass, consumes 20% of basal oxygen usage (67); hence, even in the absence of perturbation there is a large amount of superoxide anion being produced. Other sources of superoxide anion such as the respiratory burst of leukocytes (13) are reviewed in (33, 34). Although relatively innocuous, the superoxide anion can interact with other compounds such as the nitric oxide radical (reaction 1) to give rise to peroxynitrous acid (69) which gives rise to strong oxidants (reaction 2) that can readily oxidize macromolecules such as polyunsaturated fatty acids (reaction 7). For this and other reasons it is important for cells to scavenge superoxide anions; this is done via superoxide dismutase (reaction 3) where superoxide anions are converted to hydrogen peroxide (19). Hydrogen peroxide, although a relatively weak oxidant, in the presence of transition metal ions can be converted to the strong oxidant, the hydroxyl radical (24) (reaction 4). The most efficient means of scavenging hydrogen peroxide is mediated by the selenoprotein glutathione peroxidase (GPx) (79). GPx can scavenge both hydrogen peroxide (reaction 5) as well as organic peroxides (reaction 16). Furthermore, peroxide scavenging requires GSH as the electron donor, with increasing efficiency of peroxide scavenging with increasing cellular GSH concentration (12). Normally, the oxidized-glutathione (GSSG) is reduced by glutathione reductase (GRed) using NADPH as the electron donor (reaction 6). Under conditions of severe oxidative stress, much of the GSSG formed reacts with protein sulfhydryls forming glutathiyl-protein adducts (65); thus, the cell becomes very dependent upon *de novo* synthesis for the formation of GSH.

Since free transition metal ions tend to be localized at polyanionic sites such phospholipids of cell membranes, one of the major problems associated with the production of strong oxidants such as the hydroxyl radical is oxidation of polyunsaturated fatty acids (24). As seen in reaction 7 of Figure 2, the extraction of an electron from an unsaturated lipid by a strong oxidant results in the formation of a carbon-centred lipid radical that can interact with molecular oxygen to form a lipid peroxyl radical (reaction 8) that in turn can interact with an unsaturated lipid forming a new carbon-centred lipid radical as well as a lipid peroxide. The initiation of this peroxidation chain reaction (reaction 9) will rapidly convert the majority of unsaturated lipids to lipid hydroperoxides.

Lipid peroxidation creates many problems for the cells. Not least of which is that membrane function is greatly altered (45, 57, 77). The lipid peroxides can interact with transition metal ions giving rise to new lipid peroxyl (reaction11) or alkoxyl radicals that can initiate new rounds of lipid peroxidation. It is essential for the cell to scavenge lipid radicals. This is most efficiently done by scavenging (reaction 12) of lipid peroxyl radicals by vitamin E (TOH) with the endproducts being a lipid hydroperoxide and a vitamin E radical. Although the vitamin E radical is innocuous, it is necessary to reduce the radical to vitamin E to enable the vitamin E molecule to scavenge another lipid peroxyl radical. The reduction of the vitamin E radical is mediated by ascorbic acid (51), which is oxidized in the process. GSH plays a major role in reducing oxidized-ascorbate (reaction 13) (46). This is a second major means by which GSH plays an important role in minimizing oxidative stress.

Lipid peroxides can break down to give rise to a variety of harmful molecules. Such molecules include pro-inflammatory isoprostanoids (39, 42) and aldehydes (16) including strong oxidants (reaction 14) such as 4-hydroxynonenal (68). α,β-Unsaturated aldehydes such as 4-hydroxynonenal are scavenged by the formation of a glutathiyl adducts catalyzed by various glutathione S-transferases (GSTs) (23, 32). GSTs have also been shown to form glutathiyl adducts with lipid epoxides and hydroperoxides (86). Scavenging of lipid peroxidation products is a third major means whereby GSH plays an important role in minimizing oxidative stress.

Oxidative stress also promotes the formation of highly reactive dicarbonyls from sugars (82). GSH also plays a critical role here since scavenging of dicarbonyls is via the GSH-dependent glyoxalase system (74).

Even with the brief outline above, it is clear that GSH plays many important roles in the management of oxidative stress. As noted earlier, during tissue oxidative stress GSSG forms glutathiyl-protein adducts (65) and thus *de novo* synthesis of GSH becomes of importance in the minimization of oxidative stress following a perturbation.

Phase II enzyme induction as a potential therapeutic approach

The rate-limiting enzyme in GSH synthesis, γ-glutamyl-cysteine synthase (47), is a phase II enzyme (21, 83). Further, the GSTs are also phase II enzymes (25, 53, 55). The overall function of phase II enzymes is the elimination of strongly electrophilic compounds. The transcription of phase II enzymes is under the control of the antioxidant (or electrophile) response element (31, 54, 55). Antioxidant response element-mediated expression is activated by a protein complex that is comprised of Nrf1 and Nrf2 proteins in conjunction with either small Maf or Jun proteins (28, 80). Activation of this transcriptional complex is mediated by certain electrophilic compounds known as phase II enzyme inducers. Such inducers include Michael reaction acceptors such as α,β-unsaturated aldehydes (75), diphenols that can be oxidized to Michael acceptor quinone groups (56) and hydroperoxides (55). Further, depletion of GSH augments inducer activity suggesting that redox alterations mediate part of the activation of the transcriptional complex (85).

Phase II enzyme inducers can be encountered in our diet. Such inducers include: the phytoestrogens genistein present in high amounts in soy flour and enterolactone, a metabolite of the major lignan secoisolaricidiglucoside present in flax seeds (81); the flavonoid kaempferol (78) found in high amounts in certain crucifers (26); the isothiocyanate sulforaphane found in high amounts in certain crucifers (85); the polyphenolic ellagic acid (4) found in high amounts in strawberries and raspberries (15); and green tea polyphenolics (35). Since most of the research on such phase II enzyme inducers have been carried out on cultured cells, the question arises whether dietary intake of such compounds can increase phase II enzyme activities in tissues. Dietary intake of 2(3)-*tert*-butyl-4-hydroxyanisole (5, 72), soy flour (2), green tea polyphenolics (35), ellagic acid (1), and the isothiocyanate sulforaphane (84) increase phase II enzyme activities in a variety of tissues, although the CNS was not examined in these studies. In only one of the above studies was the effect of a phase II enzyme inducer on GSH levels examined and here it was demonstrated that intake of 2(3)-*tert*-butyl-4-hydroxyanisole caused an increase in liver GSH which was more pronounced in female than male mice (64). 2(3)-*Tert*-butyl-4-hydroxyanisole is metabolized to tert-butylhydroquinone, a classical phase II enzyme inducer (56).

That phase II enzyme inducers can increase phase II enzyme activity suggests that they ought to be investigated for their therapeutic potential to minimize oxidative stress and thereby minimize the activation of NF-κB and hence, ameliorate the inflammatory response following insult to the CNS.

Concluding remarks

The various speakers at the 5th Altschul Symposium have clearly outlined that inflammation is a major factor causing tissue damage in a variety of disease processes including stroke, neurotrauma, and acute respiratory distress syndrome. There is an abundance of evidence that acute inflammatory responses are mediated by activation of NF-κB. In this review we have outlined the mechanisms of activation of NF-κB and demonstrated that involvement of a strong oxidant plays a role in the activation of NF-κB. A consideration of the pathways that lead to strong oxidant production demonstrates the critical roles GSH and GSTs play in minimizing oxidative stress. This suggests that elevating GSH and GSTs in tissues experiencing perturbations should tend to minimize oxidative stress and, hence, ameliorate the inflammatory response. Since the rate-limiting enzyme for GSH synthesis and GSTs are phase II enzymes, we suggest that phase II enzyme inducers be investigated for their therapeutic potential. One would anticipate that since these compounds can be encountered in our diet, there might well be minimal side-effects when these compounds are used therapeutically.

Acknowledgements

J.W. Christman and T.S. Blackwell thank the Cystic Fibrosis Foundation, the U.S. Department of Veterans Affairs, and Grant No. HL 61419, National Heart Lung and Blood Institute, National Institutes of Health for research support. B.H.J. Juurlink thanks the Medical Research Council of Canada (Grant No. 13467), the Neurotrauma Initiative, Saskatchewan and the Saskatchewan Agriculture and Food's Agriculture Development Fund for research support.

References

1. Ahn, D, Putt, D, Kresty, L, Stoner, GD, Fromm, D, Hollenberg, PF (1996) The effects of dietary ellagic acid on rat hepatic and esophageal mucosal cytochromes P450 and phase II enzymes. *Carcinogenesis* 17: 821-8
2. Appelt, LC, Reicks, MM (1997) Soy feeding induces phase II enzymes in rat tissues. *Nutr Cancer* 28: 270-5
3. Baeuerle, PA (1998) IkappaB-NF-kappaB structures: at the interface of inflammation control (comment). *Cell* 95: 729-31

4. Barch, DH, Rundhaugen, LM, Pillay, NS (1995) Ellagic acid induces transcription of the rat glutathione S-transferase- Ya gene. *Carcinogenesis* 16: 665-8
5. Benson, AM, Hunkeler, MJ, Talalay, P (1980) Increase of NAD(P):quinone reductase by dietary antioxidants: possible role in protection against carcinogenesis. *Proc Natl Acad Sci USA* 77: 5216-5220
6. Bethea, JR, Castro, M, Keane, RW, Lee, TT, Dietrich, WD, Yezierski, RP (1998) Traumatic spinal cord injury induces nuclear factor-κB activation. *J Neurosci* 18: 3251-3260
7. Blackwell, TS, Blackwell, TR, Holden, EP, Christman, BW, Christman, JW (1996) In vivo antioxidant treatment suppresses nuclear factor-kappa B activation and neutrophilic lung inflammation. *J Immunol* 157: 1630-7
8. Blackwell, TS, Christman, JW (1997) The role of nuclear factor-kappa B in cytokine gene regulation. *Am J Respir Cell Mol Biol* 17: 3-9
9. Boissiere, F, Hunot, S, Faucheux, B, Duyckaerts, C, Hauw, JJ, Agid, Y, Hirsch, EC (1997) Nuclear translocation of NF-kappaB in cholinergic neurons of patients with Alzheimer's disease. *Neuroreport* 8: 2849-52
10. Brand, K, Page, S, Rogler, G, Bartsch, A, Brandl, R, Knuechel, R, Page, M, Kaltschmidt, C, Baeuerle, PA, Neumeier, D (1996) Activated transcription factor nuclear factor-kappa B is present in the atherosclerotic lesion. *J Clin Invest* 97: 1715-22
11. Carroll, JE, Howard, EF, Hess, DC, Wakade, CG, Chen, Q, Cheng, C (1998) Nuclear factor-kappa B activation during cerebral reperfusion: effect of attenuation with N-acetylcysteine treatment. *Brain Res Mol Brain Res* 56: 186-91
12. Carsol, MA, Pouliquen-Sonaglia, I, Lesgards, G, Marchis-Mouren, G, Puigserver, A, Santimone, M (1997) A new kinetic model for the mode of action of soluble and membrane- immobilized glutathione peroxidase from bovine erythrocytes--effects of selenium. *Eur J Biochem* 247: 248-55
13. Chanock, SJ, El Benna, J, Smith, RM, Babior, BM (1994) The respiratory oxidase burst. *J Biol Chem* 269: 24519-24522
14. Christman, JW, Lancaster, LH, Blackwell, TS (1998) Nuclear factor kappa B: a pivotal role in the systemic inflammatory response syndrome and new target for therapy (see comments). *Intensive Care Med* 24: 1131-8
15. Daniel, EM, Krupnick, AS, Heur, Y-H, Blinzler, JA, Nims, RW, Stoner, GD (1989) Extraction, stability, and quantitation of ellagic acid in various fruits and nuts. *J Food Comp Analys* 2: 338-349
16. Esterbauer, H, Zollner, H, Schauer, RJ (1990) Aldehydes formed by lipid peroxidation: mechanisms of formation, occurrence, and determination., In: *Membrane Lipid Peroxidation* (Vigo-Pelfrey, C ed, pp. 239-268. CRC Press: Boca Raton, FL
17. Flohé, L, Brigelius-Flohe, R, Saliou, C, Traber, MG, Packer, L (1997) Redox regulation of NF-kappa B activation. *Free Radic Biol Med* 22: 1115-26
18. Fridovich, I (1986) Biological effects of the superoxide radical. *Arch Biochem Biophys* 247: 1-11
19. Fridovich, I (1995) Superoxide radical and superoxide dismutases. *Ann Rev Biochem* 64: 97-112
20. Gabriel, C, Justicia, C, Camins, A, Planas, AM (1999) Activation of nuclear factor-kappaB in the rat brain after transient focal ischemia. *Brain Res Mol Brain Res* 65: 61-9
21. Galloway, DC, Blake, DG, Shepherd, AG, McLellan, LI (1997) Regulation of human gamma-glutamylcysteine synthetase: co-ordinate induction of the catalytic and regulatory subunits in HepG2 cells. *Biochem J* 328: 99-104
22. Gius, D, Botero, A, Shah, S, Curry, HA (1999) Intracellular oxidation/reduction status in the regulation of transcription factors NF-kappaB and AP-1. *Toxicol Lett* 106: 93-106
23. Goon, D, Saxena, M, Awasthi, YC, Ross, D (1993) Activity of mouse liver glutathione S-transferases toward trans,trans- muconaldehyde and trans-4-hydroxy-2-nonenal. *Toxicol Appl Pharmacol* 119: 175-80
24. Gutteridge, JMC (1992) Iron and oxygen radicals in brain. *Ann Neurol* 32: S16-S21
25. Hayes, JD, Pulford, DJ (1995) The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit Rev Biochem Mol Biol* 30: 445-600
26. Hertog, lMGL, Hollman, PCH, Katan, MB (1992) Contents of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *J Agr Food Chem* 40: 2379-2383
27. Howard, EF, Chen, Q, Cheng, C, Carroll, JE, Hess, D (1998) NF-kappa B is activated and ICAM-1 gene expression is upregulated during reoxygenation of human brain endothelial cells. *Neurosci Lett* 248: 199-203
28. Itoh, K, Chiba, T, Takahashi, S, Ishii, T, Igarashi, K, Katoh, Y, Oyake, T, Hayashi, N, Satoh, K, Hatayama, I, Yamamoto, M, Nabeshima, Y (1997) An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. *Biochem Biophys Res Comm* 236: 313-322
29. Iuvone, T, D'Acquisto, F, Van Osselaer, N, Di Rosa, M, Carnuccio, R, Herman, AG (1998) Evidence that inducible nitric oxide synthase is involved in LPS- induced plasma leakage in rat skin through the activation of nuclear factor-kappaB. *Br J Pharmacol* 123: 1325-30
30. Ivanov, V, Merkenschlager, M, Ceredig, R (1993) Antioxidant treatment of thymic organ cultures decreases NF-kappa B and TCF1(alpha) transcription factor activities and inhibits alpha beta T cell development. *J Immunol* 151: 4694-704
31. Jaiswal, AK (1994) Antioxidant response element. *Biochem Pharmacol* 48: 439-444
32. Jensson, H, Guthenberg, C, Alin, P, Mannervik, B (1986) Rat glutathione transferase 8-8, an enzyme efficiently detoxifying 4- hydroxyalk-2-enals. *FEBS Lett* 203: 207-9
33. Juurlink, BHJ (1999) Management of oxidative stress in the CNS: The many roles of glutathione. *Neurotox Res* 1: in press

34. Juurlink, BHJ, Paterson, PG (1998) Review of oxidative stress in brain and spinal cord injury: suggestions for pharmacological and management strategies. *J Spinal Cord Med* 21: 309-334

35. Khan, SG, Katiyar, SK, Agarwal, R, Mukhtar, H (1992) Enhancement of antioxidant and phase II enzymes by oral feeding of green tea polyphenols in drinking water to SKH-1 hairless mice: possible role in cancer chemoprevention. *Cancer Res* 52: 4050-4052

36. Kitamura, Y, Shimohama, S, Ota, T, Matsuoka, Y, Nomura, Y, Taniguchi, T (1997) Alteration of transcription factors NF-kappaB and STAT1 in Alzheimer's disease brains. *Neurosci Lett* 237: 17-20

37. Kretz-Rémy, C, Mehlen, P, Mirault, ME, Arrigo, AP (1996) Inhibition of IkB-a phosphorylation and degradation and subsequent NFk-B activation by glutathione peroxidase overexpression. *J Biol Chem* 133: 1-11

38. Liu, SF, Ye, X, Malik, AB (1997) In vivo inhibition of nuclear factor-kappa B activation prevents inducible nitric oxide synthase expression and systemic hypotension in a rat model of septic shock. *J Immunol* 159: 3976-83

39. Liu, T-Z, Stern, A, Morrow, JD (1998) The isopstanes: Unique bioactive products of lipid peroxidation. *J Biomed Sci* 5: 415-420

40. Lukiw, WJ, Bazan, NG (1998) Strong nuclear factor-kappaB-DNA binding parallels cyclooxygenase-2 gene transcription in aging and in sporadic Alzheimer's disease superior temporal lobe neocortex. *J Neurosci Res* 53: 583-92

41. Manna, SK, Kuo, MT, Aggarwal, BB (1999) Overexpression of gamma-glutamylcysteine synthetase suppresses tumor necrosis factor-induced apoptosis and activation of nuclear transcription factor-kappa B and activator protein-1. *Oncogene* 18: 4371-82

42. Mark, RJ, Lovell, MA, Markesbery, WR, Uchida, K, Mattson, MP (1997) A role for 4-hydroxynonenal, an aldehydic product of lipid peroxidation, in disruption of ion homeostasis and neuronal death induced by amyloid b-peptide. *J Neurochem* 68: 255-264

43. Matthews, JR, Kaszubska, W, Turcatti, G, Wells, TN, Hay, RT (1993) Role of cysteine62 in DNA recognition by the P50 subunit of NF-kappa B. *Nucleic Acids Res* 21: 1727-34

44. Matthews, JR, Wakasugi, N, Virelizier, JL, Yodoi, J, Hay, RT (1992) Thioredoxin regulates the DNA binding activity of NF-kappa B by reduction of a disulphide bond involving cysteine 62. *Nucleic Acids Res* 20: 3821-30

45. Mattson, MP (1998) Modification of ion homeostasis by lipid peroxidation: roles in neuronal degeneration and adaptive plasticity. *Trends Neurosci* 21: 53-57

46. May, JM, Qu, Z-C, Whitesell, RR, Cobb, CE (1996) Ascorbate recycling in human erythrocytes: role of GSH in reducing dehydroascorbate. *Free Radic Med Biol* 20: 543-551

47. Meister, A (1989) Metabolism and function of glutathione, In: *Glutathione. Chemical, Biochemical, and Medical Aspects Vol. A*, (Dolphin, D, Avramovic´, O, Poulson, R eds), pp. 367-474. John Wiley & Sons: New York

48. Mercurio, F, Manning, AM (1999) Multiple signals converging on NF-kappaB. *Curr Opin Cell Biol* 11: 226-32

49. Mirochnitchenko, O, Inouye, M (1996) Effect of overexpression of human Cu,Zn superoxide dismutase in transgenic mice on macrophage functions. *J Immunol* 156: 1578-86

50. Mitomo, K, Nakayama, K, Fujimoto, K, Sun, X, Seki, S, Yamamoto, K (1994) Two different cellular redox systems regulate the DNA-binding activity of the p50 subunit of NF-kappa B in vitro. *Gene* 145: 197-203

51. Niki, E, Noguchi, N, Tsuchihashi, H, Gotoh, N (1995) Interaction among vitamin C, vitamin E and β-carotene. *Am J Clin Nutr* 62(Suppl): 1322S-1336S

52. Pinkus, R, Weiner, LM, Daniel, V (1996) Role of oxidants and antioxidants in the induction of AP-1, NF-kappaB, and glutathione S-transferase gene expression. *J Biol Chem* 271: 13422-9

53. Prestera, T, Holtzclaw, WD, Zhang, Y, Talalay, P (1993) Chemical and molecular regulation of enzymes that detoxify carcinogens. *Proc Natl Acad Sci USA* 90: 2965-9

54. Prestera, T, Talalay, P (1995) Electrophile and antioxidant regulation of enzymes that detoxify carcinogens. *Proc Natl Acad Sci USA* 92: 8965-9

55. Prestera, T, Zhang, Y, Spencer, SR, Wilczak, CA, Talalay, P (1993) The electrophile counterattack response: protection against neoplasia and toxicity. *Advan Enzym Reg* 33: 281-296

56. Prochaska, HJ, De Long, MJ, Talalay, P (1985) On the mechanisms of induction of cancer-protective enzymes: a unifying proposal. *Proc Natl Acad Sci USA* 82: 8232-6

57. Rauchova, H, Ledvinkova, J, Kalous, M, Drahota, Z (1995) The effect of lipid peroxidation on the activity of various membrane-bound ATPases in rat kidney. *Int J Biochem Cell Biol* 27: 251-255

58. Richter, C, Kass, GEN (1991) Oxidative stress in mitochondria: its relationship to cellular Ca2+ homeostasis, cell death, proliferation and differentiation. *Chem-Biol Interactions* 77: 1-23

59. Sakurai, A, Hara, S, Okano, N, Kondo, Y, Inoue, J, Imura, N (1999) Regulatory role of metallothionein in NF-kappaB activation. *FEBS Lett* 455: 55-8

60. Schmedtje, JF, Jr., Ji, YS, Liu, WL, DuBois, RN, Runge, MS (1997) Hypoxia induces cyclooxygenase-2 via the NF-kappaB p65 transcription factor in human vascular endothelial cells. *J Biol Chem* 272: 601-8

61. Schmidt, KN, Amstad, P, Cerutti, P, Baeuerle, PA (1996) Identification of hydrogen peroxide as the relevant messenger in the activation pathway of the transcription factor NFkB. *Adv Exp Med Biol* 387: 63-68

62. Schmitz, ML, Baeuerle, PA (1991) The p65 subunit is responsible for the strong transcription activating potential of NF-kappa B. *Embo J* 10: 3805-17

63. Schreck, R, Albermann, K, Baeuerle, PA (1992) Nuclear factor kappa B: an oxidative stress-responsive transcription factor of eukaryotic cells (a review). *Free Radic Res Commun* 17: 221-37

64. Sharma, R, Ahmad, H, Singhal, SS, Saxena, M, Srivastava, SK, Awasthi, YC (1993) Comparative studies on the effect of butylated hydroxyanisole on glutathione and glutathione S-transferases in the tissues of male and female CD-1 mice. *Comp Biochem Physiol C* 105: 31-7

65. Shivakumar, BR, Kolluri, SV, Ravindranath, V (1995) Glutathione and protein thiol homeostasis in brain during reperfusion after cerebral ischemia. *J Pharmacol Exp Ther* 274: 1167-73

66. Simeonova, PP, Leonard, S, Flood, L, Shi, X, Luster, MI (1999) Redox-dependent regulation of interleukin-8 by tumor necrosis factor- alpha in lung epithelial cells. *Lab Invest* 79: 1027-37

67. Sokoloff, L (1989) Circulation and energy metabolism of the brain, In: *Basic Neurochemisitry 4th ed*., (Sigel, J, Agranoff, BW, Albers, RW, Molinoff, PB eds), pp. 565-590. Raven Press: New York

68. Springer, JE, Azbill, RD, Mark, RJ, Begley, JG, Waeg, G, Mattson, MP (1997) 4-hydroxynonenal, a lipid peroxidation product, rapidly accumulates following traumatic spinal cord injury and inhibits glutamate uptake. *J Neurochem* 68: 2469-2476

69. Squadrito, GL, Pryor, WA (1995) The formation of peroxynitrite in vivo from nitric oxide and superoxide. *Chem-Biol Interactions* 96: 203-206

70. Staal, FJ, Roederer, M, Herzenberg, LA (1990) Intracellular thiols regulate activation of nuclear factor kappa B and transcription of human immunodeficiency virus. *Proc Natl Acad Sci USA* 87: 9943-7

71. Suzuki, YJ, Mizuno, M, Tritschler, HJ, Packer, L (1995) Redox regulation of NF-kappa B DNA binding activity by dihydrolipoate. *Biochem Mol Biol Int* 36: 241-6

72. Talalay, P, Zhang, Y (1996) Chemoprotection against cancer by isothiocyanates and glucosinolates. *Biochem Soc Trans* 24: 806-10

73. Terai, K, Matsuo, A, McGeer, EG, McGeer, PL (1996) Enhancement of immunoreactivity for NF-kappa B in human cerebral infarctions. *Brain Res* 739: 343-9

74. Thornalley, PJ (1998) Glutathione-dependent detoxification of alpha-oxoaldehydes by the glyoxalase system: involvement in disease mechanisms and antiproliferative activity of glyoxalase I inhibitors. *Chem Biol Interact* 111-112: 137-51

75. Tjalkens, RB, Luckey, SW, Kroll, DJ, Petersen, DR (1998) α,β-Unsaturated aldehydes increase glutathione S-transferase mRNA and protein: correlation with activation of the antioxidant response element. *Arch Biochem Biophys* 359: 42-50

76. Toledano, MB, Leonard, WJ (1991) Modulation of transcription factor NF-kappa B binding activity by oxidation-reduction in vitro. *Proc Natl Acad Sci USA* 88: 4328-32

77. Tretter, L, Adamvizi, V (1996) Early events in free radical-mediated damage of isolated nerve terminals: effects of peroxides on membrane potential and intracellular Na+ and Ca++ concentrations. *J Neurochem* 66: 2057-2066

78. Uda, Y, Price, KR, Williamson, G, Rhodes, MJ (1997) Induction of the anticarcinogenic marker enzyme, quinone reductase, in murine hepatoma cells in vitro by flavonoids. *Cancer Lett* 120: 213-6

79. Ursini, F, Maiorino, M, Brigeliuys-Flohé, R, Aumann, KD, Roveri, A, Schomburg, D, Flohé, L (1995) Diversity of glutathione peroxidases. *Methods Enzymol.* 252: 38-53

80. Venugopal, R, Jaiswal, AK (1998) Nrf2 and Nrf1 in association with Jun proteins regulate antioxidant response element-mediated expression and coordinated induction of genes encoding detoxifying enzymes. *Oncogene* 17: 3145-3156

81. Wang, W, Liu, LQ, Higuchi, CM, Chen, H (1998) Induction of NADPH:quinone reductase by dietary phytoestrogens in colonic Colo205 cells. *Biochem Pharmacol* 56: 189-95

82. Wells-Knecht, KJ, Zyzak, DV, Litchfield, JE, Thorpe, SR, Baynes, JW (1995) Mechanism of autoxidative glycosylation: identification of glyoxal and arabinose as intermediates in the autoxidative modification of proteins by glucose. *Biochemistry* 34: 3702-9

83. Wild, AC, Gipp, JJ, Mulcahy, T (1998) Overlapping antioxidant response element and PMA response element sequences mediate basal and beta-naphthoflavone-induced expression of the human gamma-glutamylcysteine synthetase catalytic subunit gene. *Biochem J* 332: 373-81

84. Zhang, Y, Talalay, P (1994) Anticarcinogenic activities of organic isothiocyanates: chemistry and mechanisms. *Cancer Res* 54: p1976s-1981s

85. Zhang, Y, Talalay, P, Cho, CG, Posner, GH (1992) A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure. *Proc Natl Acad Sci USA* 89: 2399-403

86. Zimniak, P, Singhal, SS, Srivastava, SK, Awasthi, S, Sharma, R, Hayden, JB, Awasthi, YC (1994) Estimation of genomic complexity, heterologous expression, and enzymatic characterization of mouse glutathione S-transferase mGSTA4-4 (GST 5.7). *J Biol Chem* 269: 992-1000