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Inflammation, Immunity and Redox Signaling

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1. Introduction

It has long been known that several types of antioxidants also possess anti-inflammatory properties indicating a strong relationship between inflammation and oxidative stress. Reactive oxygen species (ROS) generated by inflammatory cells not only help to kill pathogens but also act on the inflammatory cells themselves, altering the intracellular redox balance and functioning as signaling molecules involved with the regulation of inflammatory and immunomodulatory genes. Indeed, at the transcriptional level, ROS play a key role in the control of nuclear factor kappa B (NF-κB), activator protein-1 (AP-1), and other transcription factors involved in gene expression of both inflammatory and immune mediators. More interestingly, ROS and also reactive nitrogen species (RNS) can either activate or inactivate these transcription factors by chemically modifying critical amino acid residues within these proteins or on residues of accessory proteins of the respective signaling pathways. The interest in the molecular mechanisms involved in redox regulation of inflammatory and immune responses has gone beyond the transcription factors as target proteins. Proteins involved in signaling cascades that ultimately culminate in the production of inflammatory and immune mediators have been investigated as redox sensors and therefore targets for ROS and or RNS modulation. For instance, NLRP3 inflammasome is a cysteine-rich multidimeric protein that participates in the formation of a molecular platform for caspase1-dependent IL-1β secretion. It has been suggested that IL-1β production and secretion in monocytes is a redox regulated event. However, the mechanisms of production and the nature of ROS involved in inflammasome activation are still unknown. This chapter will discuss some of the latest concepts on how ROS and RNS can modulate the inflammatory and immune responses at the molecular level, from redox regulated transcription factors to redox sensitive proteins involved in inflammatory and immune signaling pathways.

2. Chemistry, source and biological activity of reactive oxygen and nitrogen species

By definition, free radicals are reactive molecules that can exist independently and have one or more unpaired electrons (Halliwell and Gutteridge 2007). On the other hand the term "oxidant" is used in reference to any substance that can abstract an electron or hydrogen atom from other molecules, regardless of having an unpaired electron. These chemical

species readily react with macromolecules in the biological systems by oxidizing them. In addition, they can react with metals, other oxidants, and reducing substances found in the intracellular milieu and generate many other reactive species. Within cells, "free radicals" and other oxidants can be formed by several sources, include enzymatic and non-enzymatic and also as a byproduct of biochemical reactions. Because of the complexity of the chemistry of these species, especially in biological systems, the terms reactive oxygen species (ROS) and reactive nitrogen species (RNS) will be used in this chapter to refer to the species that are derived from oxygen or nitrogen respectively. ROS include oxygen radicals such as diatomic oxygen (O_2), superoxide anion ($O_2^{-\bullet}$), hydroxyl radical ($^{\bullet}OH$), and peroxide ion (O_2^{2-}). In addition to non-radicals, species such as hyperchlorous acid (HOCl), hydrogen peroxide (H_2O_2), and ozone (O_3) are commonly present in biological systems and are also considered ROS. RNS include nitrogen-derived molecules, represented by the nitric oxide (NO) and its more oxidized counterparts. In biological systems, NO is catalyzed by a family of NADPH-dependent enzymes known as nitric oxide synthases (NOS). In mammals, there are three NOS isoforms named as neuronal (nNOS or NOS1), inducible (iNOS or NOS2) and endothelial (eNOS or NOS3). Depending on the circumstances or amount that is generated, NO can act as signaling molecule ("low input NO") or as defense substance against pathogens ("high input NO"). As a signaling molecule NO is generated in small amounts and exerts its function through generally reversible mild chemical reactions with protein amino acids or prosthetic groups, including reactions with heme centers in metalloproteins and thiol groups of cysteine amino acid. However, when NO is generated in high amounts and/or accompanied by other ROS, such as $O_2^{-\bullet}$, it undergoes multiple and complex oxidative reactions forming more oxidative and detrimental molecules, such as peroxynitrite ($ONOO^-$), which is highly reactive to biomolecules (reaction 1).

$$\cdot NO + O_2^{\cdot -} \longrightarrow ONOO^- \quad (1)$$

The concentration of NO is regulated both by its consumption in chemical reactions as well as its production in the cellular microenvironment. Consequently, as important as the enzymes that generate NO directly are the enzymes that control NOS activity indirectly. One of the indirect control mechanisms for NOS activity, in particular for iNOS, is the availability of its required substrate, L-arginine. L-arginine is not only a substrate for NOS, but also for arginases, enzymes that hydrolyze L-arginine to L-ornithine and urea (Diagram 1) (Lerzynski et al. 2006).

Diagram 1. Simplified reaction demonstrating substrate competition between the enzymes NOS and arginase.

Arginase, classically known as an enzyme within the urea cycle in the liver, is also found in many other cells and tissues including inflammatory cells (Munder 2009). There are two distinct isoforms of mammalian arginase, arginase I and arginase II (Morris 2009). The expression and activity of arginases are induced in murine models of allergic airways disease, as well as in patients with asthma (Zimmermann and Rothenberg 2006). It has been indicated that limitation of L-arginine availability, caused by activation of arginase, could

contribute to the loss of NO bioactivity (Ricciardolo et al. 2005; Maarsingh et al. 2006). In fact, the inhibition of arginase by the pharmacological arginase inhibitor, *S*-(2-boronoethyl)-L-cysteine (BEC), decreased arginase activity and caused alterations in NO homeostasis, which were reflected by increases in NO-modified proteins in the lungs from inflamed mice. In addition, the same inhibitor enhanced perivascular and peribronchiolar lung inflammation, mucus metaplasia, genes of inflammatory chemokines, such as chemokine (C-C motif) ligand 20 (CCL20, which attracts neutrophils and dendritic cells) and keratinocyte chemoattractant (KC, responsible for attracting neutrophils). These results suggest that inhibition of arginase activity enhanced a variety of inflammatory parameters, possibly by altering NO homeostasis (Diagram 2) (Ckless et al. 2008). At the molecular level, arginase manipulation in lung epithelial cells can also impact NO homeostasis and affect inflammatory responses. In this scenario, the reduction of arginase activity enhances the general cellular content of NO and NO-modified proteins, including augmentation of NO-modified nuclear factor kappa B (NF-κB), which has a major role in regulating immune and inflammatory responses. Interestingly, the effects of arginase inhibition on NF-κB is reversed by the generic NOS inhibitor, N-ω-nitro-L-arginine methyl ester (L-NAME), suggesting a causal role for NO in the attenuation of NF-κB induced by arginase suppression. Conversely, overexpression of arginase I decreases cellular NO-derivative content which causes decrease of NO-modified NF-κB. The aforementioned NO-modification on NF-κB and other proteins includes S-nitrosylation, which will be discussed in more detail later in this chapter. Collectively, this study points out to a regulatory mechanism wherein NF-κB is controlled through arginase dependent regulation of NO levels, which may impact on chronic inflammatory diseases (Ckless et al. 2007).

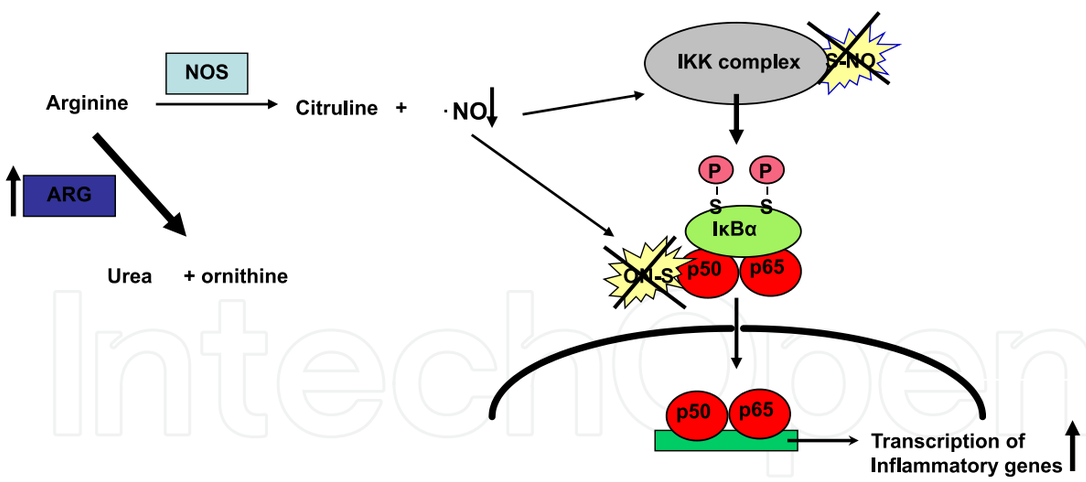

Diagram 2. Mechanism by which arginase might control NF-κB activity by decreasing NO availability.

ROS can also be generated endogenously by several enzymatic and non-enzymatic reactions. In pathological, and perhaps physiological states, the major non-mitochondrial ROS sources are the enzymes called oxidases, which includes NADPH oxidases, and cyclooxygenases among others. In general the production of ROS by these non-mitochondrial enzymes is dependent on a stimulus. Therefore in non-stimulated or in physiological conditions little is known about participation of these enzymes in ROS

generation. The generation of ROS by these non-mitocondrial enzymes in inflamed states will be discussed further in this chapter.

The mitochondria can be a significant source of superoxide and nitric oxide in eukaryotic cells. The mitochondrial contribution to the pool of free radicals varies depending on cell function, and actively respiring mitochondria contribute more to the pool than do inactive mitochondria. Given that the standard reduction potential of O_2 to $O_2^{-\bullet}$ is -0.160 V and the standard reduction potentials of the redox centers in the respiratory chain range from -0.32 V to +0.39 V, and the presence of substantial transition metals in the redox centers, it comes as no surprise that there is significant reactive species generation in this environment (Halliwell and Gutteridge 2007). Superoxide is generated on the outer mitochondrial membrane, on both surfaces of the mitochondrial intermembrane space, and within the matrix. Although superoxide generated within the matrix is dismutated by the many antioxidant defenses within the matrix, superoxide generated in the intermembrane space and on the surface of the outermembrane of the mitochondria may be carried into the cytoplasm by voltage-dependent anion channels (Halliwell and Gutteridge 2007). Under normal physiological conditions, electrons flow through the respiratory chain generating a proton gradient, pumping protons into the intermembranous space between the inner and outer mitochondrial membrane. The gradient then creates ATP as the protons flow through the ATP synthase. During times of low ADP concentration, proton flow through the ATP synthase is disrupted causing electron flow through the respiratory chain to slow, and the chain to become more reduced. It seems that the reduced state of the chain increases the rate of autoxidation of the redox centers by O_2, forming $O_2^{\bullet -}$. It has been suggested that within the mitochondrial matrix, a unique form of NOS exists (Bustamante et al. 2007). The putative formation of NO in this environment has significant consequences due to its binding affinity to heme groups in the cytochromes of the respiratory chain. Additionally, the simultaneous production of O_2^- and NO can result in the production of peroxynitrite, which might inhibit important enzymes and disrupt mitochondrial integrity. Since the generation of NO requires oxygen, the rate of its generation is O_2 dependent (Halliwell and Gutteridge 2007).

Due to the increased ROS and RNS generation present in the mitochondria, antioxidant defenses have evolved to protect the integrity of the organelle. A family of metalloenzymes known as superoxide disumutases (SODs) catalyzes the formation of H_2O_2 and O_2 from O_2^- and water. Although the dismutation of O_2^- to H_2O_2 and water will occur spontaneously, SODs increase the rate of this diffusion in a controlled-manner. Within the mitochondrial matrix, a specific manganese containing SOD eliminates O_2^- from the matrix and the inner side of the inner mitochondrial membrane. Superoxide concentration in the intermembrane space is regulated by three mechanisms; a CuZnSOD, cytochrome c, and spontaneous dismutation induced by the lower pH of this area. In physiological conditions, H_2O_2 is rapidly decomposed by glutathione peroxidase and in some cell types by catalase. However if H_2O_2 accumulates it can be detrimental because it is the main precursor of the highly reactive hydroxyl radical, formed by interaction with reduced transition metals. In general the integrity of the mitochondrial membrane is maintained by a second glutathione peroxidase, known as phopholipid-hydroperoxide glutathione. This specialized peroxidase reduces lipid peroxides associated with the membrane. In addition to the "classical" antioxidant enzymes mitochondria integrity is also preserved by mitochondrial proteins that participate in the respiratory electron chain transport. It appears that the cytochrome c

electron carriers have a detoxifying role against ROS, ubiquinol (QH_2) can act as a reducing agent in the elimination of peroxides in the presence of succinate, an intermediate of citric acid cycle. Non-enzymatic antioxidant systems also play a role in protecting mitochondrial integrity. The inner mitochondrial membrane contains high levels of vitamin E, a powerful antioxidant and inhibitor of free radical propagation reactions.

3. Redox chemistry in inflammation states

It is well known that chronic inflammatory diseases are associated with enhanced ROS and RNS production exemplified by elevated levels of NO and H_2O_2 in the site of inflammation (Antczak et al. 1997; Emelyanov et al. 2001). These oxidants can be generated by enzymes abundant not only in inflammatory cells but also in non-inflammatory cells (Janssen-Heininger et al. 2008). ROS and RNS generation in the inflammation site is typically induced as part of a defensive reaction intended to clear infectious and environmental threats, including microbial agents and particulate material. The resident and inflammatory hematopoietic-derived cells in the tissues possess oxidant-generating enzyme systems, including NADPH oxidase, which activity is mediated through the catalytic subunit $gp91^{phox}$ (NOX2) (Bedard and Krause 2007; van der Vliet 2008). This enzyme is capable of generating $O_2^{\bullet -}$, which spontaneously or enzymatically dismutates to H_2O_2 to further induce oxidation. ROS generation by non-hematopoietic NOXs is also very important. The non-hematopoietic ROS are generated by a family of enzymes, including, NOX1, NOX3, and NOX4, which function distinct from $gp91^{phox}$ (van de Veerdonk et al. 2010). In addition, some cell types such as epithelial cells have been described to produce active DUOX enzymes capable of generating H_2O_2 (van der Vliet 2008). Stimulated cells such as respiratory epithelium, neutrophils, and macrophages are also capable to produce NO in high amounts via iNOS (Janssen-Heininger et al. 2002). Induction of both iNOS and NADPH oxidases at the inflammation site leads to simultaneous increases in $O_2^{\cdot -}$ and NO that combine to form $ONOO^-$, which ultimately can react with tyrosine residues in protein, forming the more stable product, nitrotyrosine (Diagram 3) (Brennan et al. 2002). Indeed, increased levels of NO in exhaled breath and protein nitration have been observed in patients with asthma, correlating NO with inflammation (Reszka et al. 2011).

NO is highly diffusible, allowing it to potentially form $ONOO^-$ in areas spatially separated from the site of NO synthesis, limited only by its potent capacity to react with macromolecules (Lancaster and Gaston 2004). In addition, the reaction of NO with molecular oxygen (O_2) yields nitrite (NO), which can be oxidized by hemeperoxidases to form NO_2, thereby perpetuating the capacity for NO_2 reactivity. NO_2 is also formed when eosinophil peroxidase and myeloperoxidase, from eosinophils and neutrophils, respectively, consume NO and H_2O_2 (van der Vliet et al. 1999; Brennan et al. 2002). The environment can also be a source of NO_2. In fact high levels of this gas can be found in the atmosphere, and it is associated with poor air quality caused by pollution in highly industrialized areas. NO_2 primarily interacts with airway surface macromolecules, forming stable footprints of reactivity including the protein tyrosine modifications nitrotyrosine and dityrosine (Brennan et al. 2002), perhaps altering protein function. In addition, $ONOO^-$ or NO_2 can decompose to form $^{\bullet}OH$ and H_2O_2, which can facilitate further oxidation and participate in intracellular signaling events. Therefore, exogenous or endogenous-generated ROS and RNS may directly and indirectly affect cells that participate in the inflammatory and immune processes.

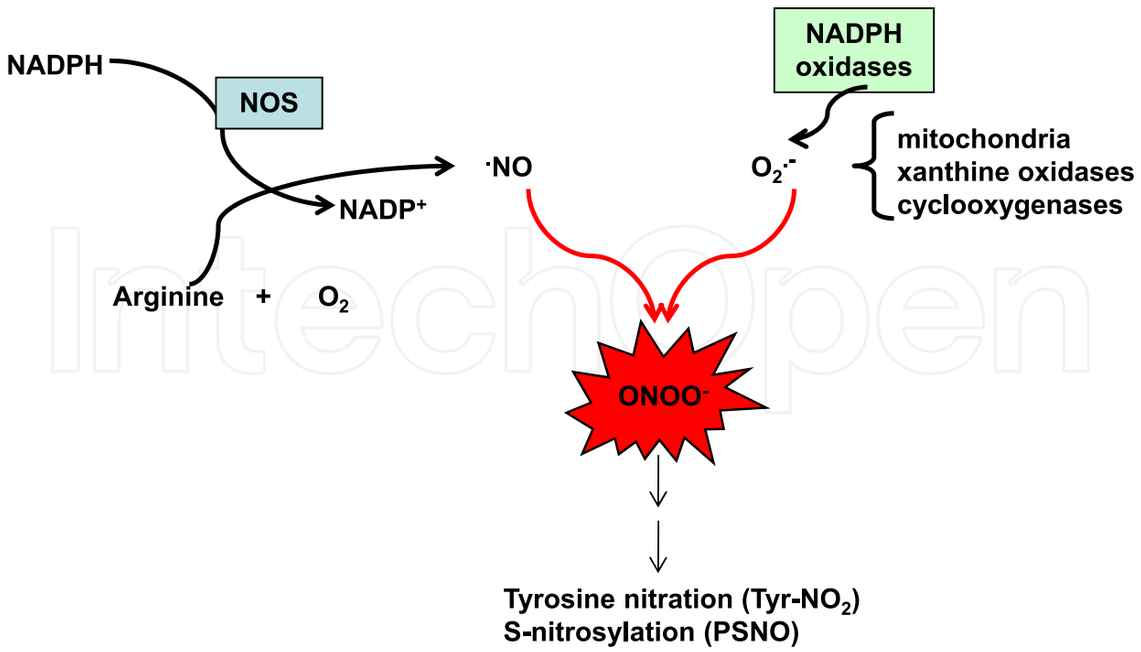

Diagram 3. Generation of peroxynitrite ($ONOO^-$) in the inflammation site and potential reactions with proteins.

The direct detection of ROS and RNS *in situ* of inflammation is an extremely challenging task. To overcome this difficulty, the detection of stable oxidation endproducts in proteins, such as nitrotyrosine, cysteine sulfenic (Cys-SOH), sulfinic ($Cys\text{-}SO_2H$) and sulfonic ($Cys\text{-}SO_3H$) acids is a feasible approach to indicate the presence of ROS and RNS. The chemistry of these oxidations is especially complex. NO generated endogenously by NOS can react indirectly with the tripeptide glutathione (GSH) and convert it to the S-nitrosothiol, called S-nitrosoglutathione (GSNO). Consequently, NO can exert its effects directly or through its derivatives (GSNO), by mediating protein S-nitrosylation (Diagram 4). By definition,

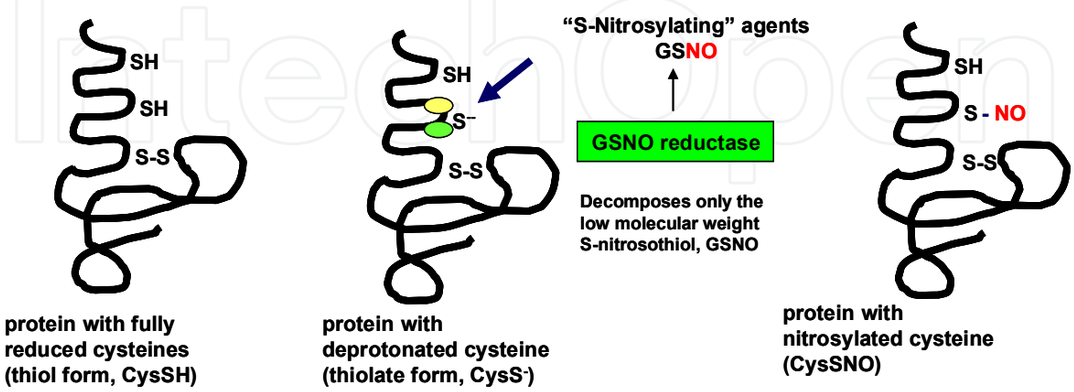

Diagram 4. Mechanism by which GSNO mediates S-nitrosylation of proteins and the role of enzyme GSNO reductase on the equilibrium of S-nitrosylated proteins.

protein S-nitrosylation is a covalent binding of NO to a cysteine residues in the proteins and it has been considered a mechanism of protein regulation (Numajiri et al. 2011). Up to date there is no identified enzyme that specifically and directly decomposes PSNO, however the enzyme GSNO reductase decomposes the S-nitrosothiol, GSNO and indirectly controls the protein S-nitrosylation. In more oxidized states, NO is further oxidized to ONOO- which is very well known as a nitrating agent of tyrosine residues in proteins (See above). The presence of these oxidized proteins has been considered "a biomarker" of inflammation.

The redox changes on cysteine residues are very dynamic and complex. In fact, S-nitrosothiols, such as GSNO can also mediate another Cys oxidation, called S-glutathionylation. Similar to S-nitrosylation, protein S-glutathionylation (PSSG) is the covalent modification of cysteines with the tri-peptide, glutathione (GSH). The formation of PSSG follows a more transient form of Cys-protein oxidation, which can by initiated by H_2O_2 which oxidizes protein cysteine to its thiolate state (Cys^-) and further to the unstable sulfenic acid (Cys-SOH). In the presence of high amounts of ROS, cysteine residues can also be further overoxidized to Cys-SO_2H and Cys-SO_3H. The susceptibility of cysteine to oxidation is proportionally dependent on the low *pKa* of this amino acid, indicating substantial specificity to these oxidation events. Different from protein S-nitrosylation, protein S-glutathionylation can be decomposed by specific enzymes. In physiologic settings, glutaredoxins act to specifically reverse S-glutathionylated proteins (Diagram 5). Similarly, the thioredoxin (Trx) system of enzymes catalyzes the reversible reduction of disulfides, thereby resulting in a reduced thiol on target protein, and a disulfide on Trx, which is subsequently reduced by thioredoxin reductase. The presence of these and other enzymes to

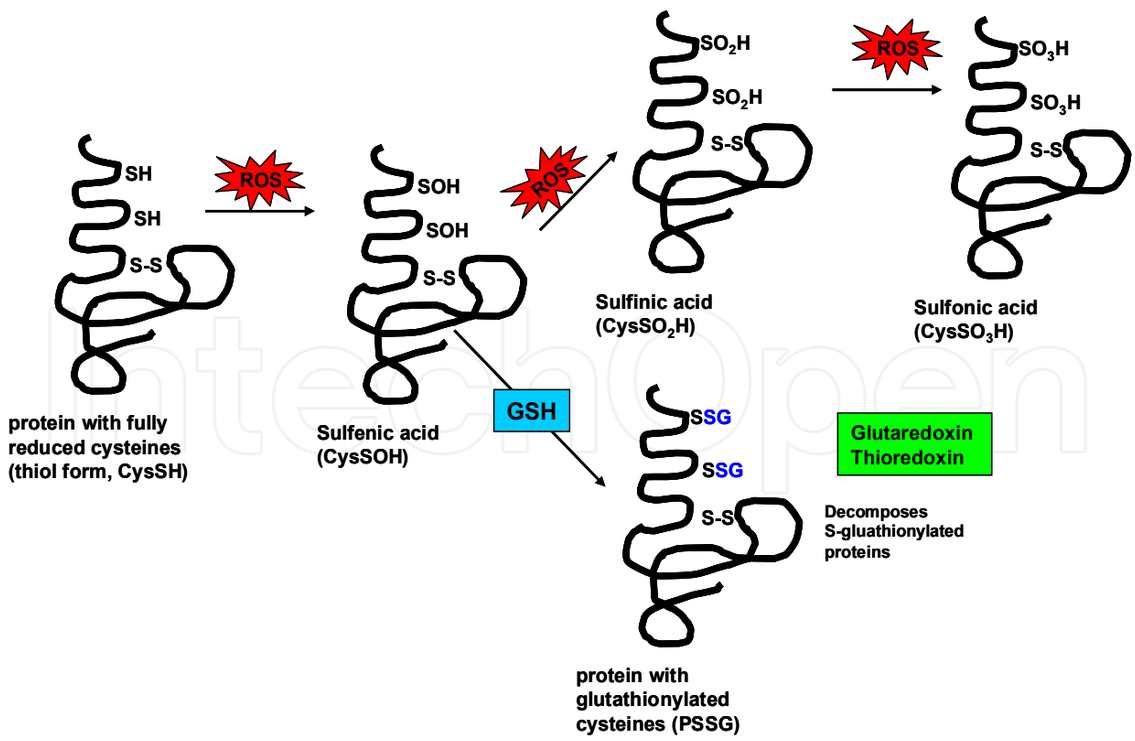

Diagram 5. Simplified mechanism by which protein S-glutathionylation is mediated in biological systems.

directly or indirectly regulate the oxidation state of protein cysteines gives additional acceptance to the relevance of protein oxidation events in cell biology and disease (Janssen-Heininger et al. 2008).

Since protein S-glutathionylation is reversible and perhaps regulated by specific enzymes, this post-translational modification has emerged as a regulatory mechanism of proteins. While certain redox changes that occur in the inflamed and adjacent cells may well contribute to the inflammatory disease process, the exact mechanisms by which ROS and RNS participate in the inflammatory and immune responses has remained unknown. One emerging scenario is centered on the role of oxidants as signaling molecules critical to tissue homeostasis and innate host defense. This concept of ''redox biology'' is based on the recently gained appreciation that NO and H_2O_2 cause specific oxidations in target cysteines within proteins, which exert regulatory functions, depending on the protein that is being targeted. Proteins with several functions, ranging from membrane proteins and proteases to transcription factors, are regulated via S-nitrosylation, S-glutathionylation, or other forms of cysteine oxidation. In this context, the NF-κB is the most represented transcription factor that is redox regulated.

4. Redox regulation of transcription factors controlling immune and inflammatory responses

The transcription factor NF-κB has been considered the master regulator of both innate and adaptive immune responses and has been demonstrated to play a critical role in allergic airways disease. In addition, this transcription factor has been known for a long time as a "redox sensitive transcription factor". Therefore, understanding the various facets of redox regulation of NF-κB and its targets offers the potential to advance our understanding of immune and inflammatory processes. The family of NF-κB comprises five related proteins: p50, p52, RelA (also known as p65), c-Rel, and RelB. These factors can homo- and heterodimerize through the rel homology domain. Only RelA, c-Rel, and RelB contain a transcriptional activation domain, while p50 and p52 lack this, and can only activate transcription through heterodimerization with RelA, c-Rel, or RelB (Hayden and Ghosh 2004; Pantano et al. 2006). NF-κB is sequestered in the cytoplasm of unstimulated cells bound to IκB proteins. In response to a wide array of stimuli, IκB proteins are phosphorylated by the serine kinase; inhibitor of kappa B kinase (IKK). Phosphorylated IκBs are ubiquitinated and degraded by the 26S proteosome, unmasking the NF-κB nuclear localization signal, allowing NF-κB to accumulate in the nucleus. At least two parallel pathways of IKK-induced NF-κB have been described. In the canonical pathway, activation of IKKβ by many stimuli, including cytokine TNF-α, TLR agonists, and IL-1β leads to the phosphorylation of IkBα at Serines 32 and 36 (DiDonato et al. 1997). In the noncanonical pathway, NF-κB–inducing kinase (NIK) phosphorylates IKKα. IKKα subsequently phosphorylates p100, which causes its proteolytic processing to p52 (Karin 1999), allowing p52/RelB dimeric complexes to translocate to the nucleus (Hayden and Ghosh 2008). Activators of the noncanonical pathway include subsets of stimuli that include B cell–activating factor (BAFF), lymphotoxin b (LTb), and CD40 ligand (CD40L), as well as lipopolysaccharide (LPS). It is generally held that the noncanonical pathway is necessary for the adaptive immune response, while the canonical pathway is required for the onset of the

innate immune response (Bonizzi et al. 1999), although crosstalk between these pathways exists to control the strength and duration of the transcriptional response (Ghosh and Hayden 2008).

NF-κB activation, by diverse proinflammatory stimuli including IL-1β, has been demonstrated to require ROS in part after activation of NADPH oxidases (Bonizzi et al. 1999; Li and Engelhardt 2006), and mitochondrial ROS in certain contexts also can lead to activation of NF-κB (Chandel et al. 2001; Hughes et al. 2005). However, it is not clear whether the oxidative events triggered by those stimuli are temporal and therefore specific to components of the NF-κB pathway. In fact, the physiologic role for oxidants in the activation of NF-κB has been questioned by studies demonstrating that redox activation is cell type specific (Anderson et al. 1994; Brennan and O'Neill 1995), and that various antioxidants were nonspecific in their actions (Hayakawa et al. 2003). Evidence also exists that NADPH oxidase–induced ROS do not mediate NF-κB signaling, but lower the magnitude of its activation (Hayakawa et al. 2003). A number of reports demonstrate that oxidants can specifically inhibit the NF-κB pathway in lung epithelial cells via S-nitrosylation or S-glutathionylation of cysteine 179 of IKKβ (Reynaert et al. 2004; Reynaert et al. 2006), the same cysteine also targeted by anti-inflammatory cyclopentenone prostaglandins (Rossi et al. 2000). It is likely that additional cysteine oxidative events that include modification of p50 (Matthews et al. 1992) and RelA (Kelleher et al. 2007) also contribute to oxidative inhibition of NF-κB. These observations suggest that certain regulatory oxidative events could play important anti-inflammatory roles in tissues by limiting the activation of NF-κB. The redox regulation of NF-κB and perhaps other transcription factors and proteins involved in inflammatory and immune responses is extremely complex, and there are strong evidences that this process is cell type, ROS/RNS space-temporal dependent. A good illustration of this complexity is represented by several publications on post-translational modifications of the NF-κB pathway. In two separate studies, the same cell line but different sources of RNS were utilized to demonstrate that the NF-κB pathway is a target for redox regulation. In one study it has been demonstrated that exogenous S-nitrosothiols (an NO-derivative) cause S-nitrosylation of IKKβ inhibiting it and consequently inhibiting NF-κB (Reynaert et al 2004). In contrast, the second study demonstrated that increases in endogenous S-nitrosothiols caused by arginase inhibition do not affect the extent of IKKβ activity, but still attenuate the NF-κB pathway downstream of IKKβ (Ckless et al 2007). This apparent inconsistency may stem from potentially different chemical forms of NO, concentration, or subcellular localization that arose after arginase suppression (endogenous generation of S-nitrosothiols) compared with extracellularly delivered S-nitrosothiols and this different scenario may impact different targets of NF-κB pathways. In fact, in the study where the S-nitrosolthiols were generated endogenously, the target for S-nitrosylation was the p50 subunit of NF-κB complex. The NF-κB pathway is also a target for other type of posttranslational modification induced by ROS. Indeed in airway epithelial cells, cysteine-179 of the IKKβ regulatory kinase is a central target for oxidative inactivation by S-glutathionylation, caused by exogenous H_2O_2. The various conflicting mechanisms that are described for ROS and their role in NF-κB regulation and consequent antioxidants response to ROS generation may reflect on the complexity of the inflammatory processes. Recently it was suggested that acute inflammation possesses well balanced opposing arms, apoptosis and wound healing (Khatami 2008). Since NF-κB controls at least

in part apoptotic/antiapoptotic events of this process, misregulation of this transcription factor caused by oxidative stress could lead to an unbalance between apoptosis and wound healing and in addition to the co-existence of death and growth factors in tissues, could create an dysfunctional immune response potentially leading to chronic inflammation, autoimmune diseases and cancer. (Khatami 2008; Khatami 2009; Khatami 2011).

5. RNS and ROS beyond transcription factors

The Interleukin 1 (IL-1) family of cytokines is critical to the host response to infection, playing a variety of roles not only in the acute phase response from the liver, but also in alterations of metabolism, induction of fever, and lymphocyte activation (Dinarello 2009). Overproduction of IL-1β, in particular, is thought to be responsible for a variety of autoinflammatory syndromes such as familial Mediterranean fever and Muckle-Wells syndrome, and is also a contributing factor in rheumatoid arthritis, gout, multiple sclerosis (in the animal model experimental autoimmune encephalomyelitis), Alzheimer's Disease, and diabetes (Gris et al.2010 ; Masters et al. 2010 ; Zhou et al. 2010 ; Griffin et al. 2006; Daheshia and Yao 2008; Clutterbuck et al. 2009). IL-1β is also a pathogenic mediator in several pulmonary disorders, including infection, asthma, ALI/ARDS, transplant rejection, COPD, PAH, sarcoidosis, asbestosis, and silicosis (Dorfmuller et al. 2003; Wanderer 2008; Soon et al. 2010). Setting IL-1β apart from other acute phase cytokines such as IL-6 and TNF-α is the requirement for processing from an inactive pro-form to an active secreted form by caspase-1 cleavage, which itself is activated by the assembly of cytoplasmic inflammasome complexes, which are multiprotein complexes that can activate caspase-1 and ultimately lead to the processing and secretion of interleukin (IL)-1β and IL-18 . A number of the NOD-like receptor (NLR) family members can form inflammasomes. One of the best-studied members of the NLR family is NLRP3 (NOD-like receptors pyrin domain-containing 3). The activation of NLRP3 inflammasome facilitates the formation of a molecular platform for caspase1-dependent secretion of IL-1β. It has been demonstrated that the mechanism of processing and secretion of mature IL-1β in myeloid cells is a multistep event. The initial event necessary is the synthesis and accumulation of the precursor proteins including pro–IL-1β and NLRP3 ("signal 1"), accomplished by a variety of stimuli, including danger- and pathogen- associated molecular pattern molecules (DAMPs and PAMPs, respectively). After priming, NLRP3 activation leads to recruitment of the adaptor protein ASC (apoptotic speck-like protein containing a CARD) and the enzyme caspase-1 to form the NLRP3 inflammasome complex ("signal 2"), which ultimately is responsible for the cleavage and secretion of IL-1β (Diagram 6) (Martinon et al. 2009). The cleavage and secretion of IL-1β can be enhanced by release of endogenous ATP that stimulates the purinergic receptor P2X7 (Piccini et al. 2008). Interestingly, several identified NLRP3 activators also trigger reactive oxygen species (ROS) production. It is well documented that activation of P2X7 is accompanied by production of ROS, produced at least in part by NADPH oxidases (Cruz et al. 2007; Dostert et al. 2008).

In the context of redox regulation of target proteins, what appears to be highly significant is the cellular location and quantity of ROS generated. Overall, several studies using antioxidants support a model in which ROS production by NLRP3 agonists drive inflammasome assembly (Tschopp and Schroder, 2010). The initial idea that NADPH

oxidases are the primary source of ROS production during inflammasome activation is becoming less accepted. Two independent studies utilizing mononlucear phagocytes from patients with granulomatous disease, who because of mutation in $p47^{phox}$ have defective NADPH activity, demonstrated that there is IL-1β secretion from these inflammatory cells upon stimulation, despite the fact that these cells cannot generate NADPH oxidase-dependent ROS. (Meissner et al. 2010; van de Veerdonk et al.,2010). Since NADPH oxidase may not be the only source of ROS in the cells, the importance of mitochondrial-derived ROS has been recently explored. The mitochondria is the main source of ROS under physiological conditions, however under conditions of cellular stress, including increases in metabolic rates, hypoxia or cellular disruption, the mitochondria can generate increased amount of ROS (Brookes et al. 2004). In fact, blockage of key enzymes of the respiratory chain leads to ROS generation and consequent NLRP3 inflammasome activation (Zhou et al. 2010). Since NLRP3 de novo synthesis is an essential step in the activity of NLRP3, the temporal generation of ROS is also another important aspect to be considered to understand the role of these species in controlling NLRP3 inflammasome activation. In fact it has been recently published that ROS are important for de novo synthesis of NLRP3, but not activation. However, this evidence does not exclude a general role for ROS in the process of NLRP3-triggered inflammation (Bauernfeind et al. 2011). Despite several high profile publications in the field, the mechanisms of production and the nature of ROS involvement in inflammasome activation remain the subject of intense scrutiny.

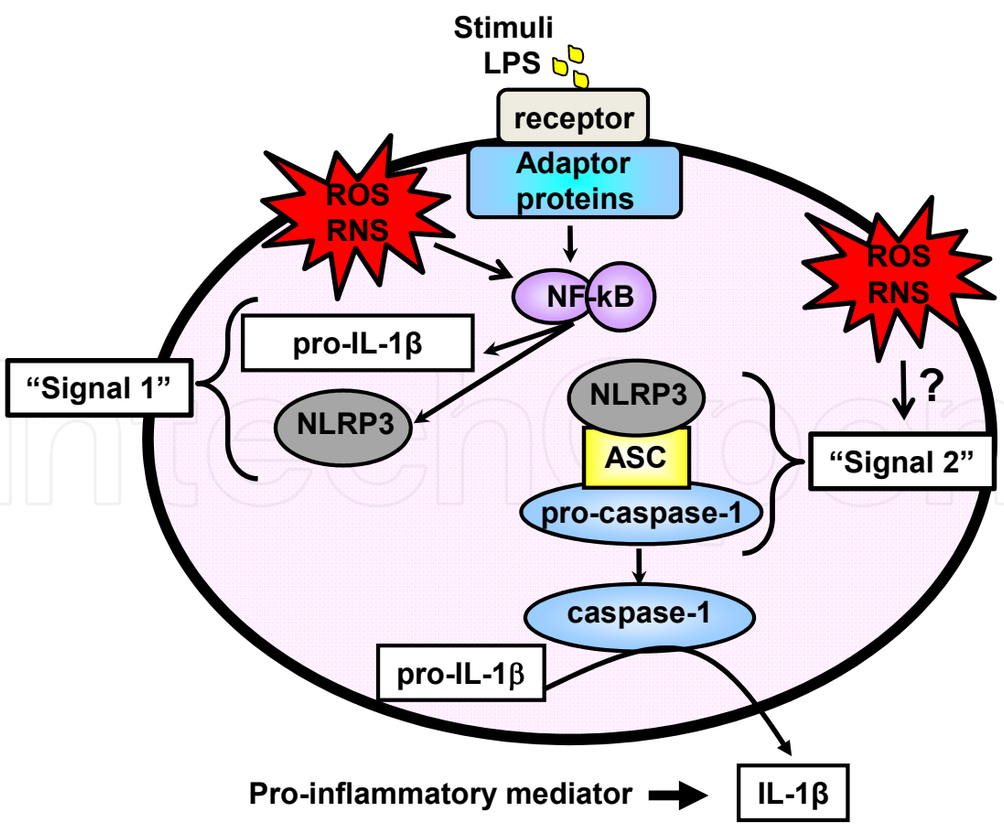

Diagram 6. Suggested mechanism of NLRP3 inflammasome activation and IL-1β secretion.

6. Conclusions

ROS and RNS are active participants in complex biological processes, including innate and immune responses. Whether the sources of ROS and RNS are environmental (exogenous sources) or are generated endogenously, they can affect several steps involved in these processes. It is critical to take in consideration that individual cells and multicellular organisms have developed intricate mechanisms to utilize ROS and RNS to modulate homeostasis and respond to threats. Therefore, generalized therapeutic and prophylactic approaches to modulate ROS and RNS generation and reactivity may not represent realistic tools to prevent or treat inflammatory diseases. Therefore, a better understanding of the sequence of events leading to specific immune and inflammatory responses, the temporal and spatial generation of ROS and RNS, and the potential molecular targets of oxidative modification, may provide crucial knowledge for the future development of more effective alternative therapeutic interventions in combination with the current ones to improve quality of life of patients with chronic inflammatory and auto-immune diseases

7. Acknowledgements

We thank Jennifer Ather and Edward Manna for editing the manuscript.

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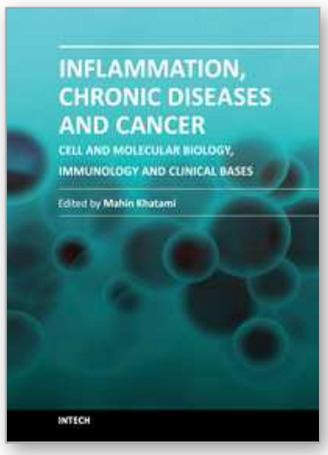

Inflammation, Chronic Diseases and Cancer - Cell and Molecular Biology, Immunology and Clinical Bases

Edited by Dr Mahin Khatami

ISBN 978-953-51-0102-4
Hard cover, 430 pages
Publisher InTech
Published online 09, March, 2012
Published in print edition March, 2012

This book is a collection of excellent reviews and perspectives contributed by experts in the multidisciplinary field of basic science, clinical studies and treatment options for a wide range of acute and chronic inflammatory diseases or cancer. The goal has been to demonstrate that persistent or chronic (unresolved or subclinical) inflammation is a common denominator in the genesis, progression and manifestation of many illnesses and/or cancers, particularly during the aging process. Understanding the fundamental basis of shared and interrelated immunological features of unresolved inflammation in initiation and progression of chronic diseases or cancer are expected to hold real promises when the designs of cost-effective strategies are considered for diagnosis, prevention or treatment of a number of age-associated illnesses such as autoimmune and neurodegenerative diseases as well as many cancers.

How to reference

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Joshua Jabaut and Karina Ckless (2012). Inflammation, Immunity and Redox Signaling, Inflammation, Chronic Diseases and Cancer - Cell and Molecular Biology, Immunology and Clinical Bases, Dr Mahin Khatami (Ed.), ISBN: 978-953-51-0102-4, InTech, Available from: http://www.intechopen.com/books/inflammation-chronic-diseases-and-cancer-cell-and-molecular-biology-immunology-and-clinical-bases/inflammation-immunity-and-redox-signaling

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