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Review

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The role of oxidative stress during inflammatory processes

Abstract: The production of various reactive oxidant species in excess of endogenous antioxidant defense mechanisms promotes the development of a state of oxidative stress, with significant biological consequences. In recent years, evidence has emerged that oxidative stress plays a crucial role in the development and perpetuation of inflammation, and thus contributes to the pathophysiology of a number of debilitating illnesses, such as cardiovascular diseases, diabetes, cancer, or neurodegenerative processes. Oxidants affect all stages of the inflammatory response, including the release by damaged tissues of molecules acting as endogenous danger signals, their sensing by innate immune receptors from the Toll-like (TLRs) and the NOD-like (NLRs) families, and the activation of signaling pathways initiating the adaptive cellular response to such signals. In this article, after summarizing the basic aspects of redox biology and inflammation, we review in detail the current knowledge on the fundamental connections between oxidative stress and inflammatory processes, with a special emphasis on the danger molecule high-mobility group box-1, the TLRs, the NLRP-3 receptor, and the inflammasome, as well as the transcription factor nuclear factor-κB.

Keywords: cell signaling; danger signals; inflammasome; inflammation; innate immunity; oxidative stress; Toll-like receptors.

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Introduction

Inflammation represents a fundamental biological process that stands at the foreground of a large number of acute and chronic pathological conditions. Complex inflammatory networks, involving countless cellular and humoral components, orchestrate critical aspects of diseases as various as myocardial infarction, diabetes, rheumatoid arthritis, sepsis, cancer, or Alzheimer's disease to name only a few. As a direct corollary, clinical and basic research in the field of inflammation has been steadily growing, illustrated by the 415 000 references retrieved from a Medline search with the term 'inflammation', among which >215 000 are <10 years old (as of August 2013).

Inflammation occurs in response to any alteration of tissue integrity, in order to restore tissue homeostasis through the induction of various repair mechanisms. Proper regulation of these mechanisms is essential to prevent uncontrolled amplification of the initial inflammatory response and a shift from tissue repair toward collateral damage and disease development (Goldszmid and Trinchieri, 2012). In recent years, evidence has been obtained that chemical processes involving redox reactions triggering cellular oxidative stress play critical roles in the pathophysiology of inflammation (Liaudet et al., 2009; Nathan and Cunningham-Bussel, 2013), a concept that had been already proposed almost 30 years ago in a milestone review article on the biochemistry of oxidative stress (Sies, 1986). The aim of this article is to provide an overview of the major mechanisms underlying this link between oxidative stress and inflammation.

Oxidative stress and biology of free radicals

Many cellular processes are directed by reactions involving the transfer of electrons between molecules, whose redox state (the balance between oxidized and reduced forms of electrons donors and acceptors) becomes thereby

modified (Schafer and Buettner, 2001). Redox homeostasis is maintained by cellular and extracellular redox buffering systems, which include small molecule- and protein-based buffers such as the redox couples GSH/GSSG (glutathione-glutathione disulfide), cysteine/cystine, and oxidized/reduced thioredoxin (Banerjee, 2012). The balance of these buffering systems is maintained by key antioxidant enzymes, including superoxide dismutase, catalase, and the selenoproteins glutathione peroxidase and thioredoxin reductase, as well as non-enzymatic antioxidants such as α-tocopherol (vitamin E), ascorbate (vitamin C), β-carotene, and flavonoids (Steinbrenner and Sies, 2009; Chen et al., 2012).

Disruption of redox homeostasis occurs whenever an imbalance between reductants (electron donors) and oxidants (electron acceptors) develops, resulting either in a reductive stress (redox potential becomes more negative) or an oxidative stress (redox potential becomes more positive), the latter being by far the most common form of redox imbalance in biological systems (Shao et al., 2012). Oxidative stress therefore results from the generation of various oxidizing chemical species in excess of the cellular reducing capacities, and is best defined as 'an imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage' (Sies and Jones, 2007). Oxidant species include primarily free radicals, which are molecules or fragments of molecules containing one or more unpaired electrons in their molecular orbitals, and which stabilize by removing electrons from neighboring molecules. Certain non-radical species, such as hydrogen peroxide or peroxynitrite, also act as strong electron acceptors due to their highly positive redox potential. The two main families of relevant oxidants in biology are the reactive oxygen species (ROS) and the reactive nitrogen species (RNS) (Pacher et al., 2007), as summarized in Figure 1.

The family of ROS

The superoxide anion radical ($O_2^{\cdot-}$) is the primary ROS molecule formed in biological systems through the univalent reduction of molecular oxygen. Secondary ROS arise through the addition of a second and third electron,

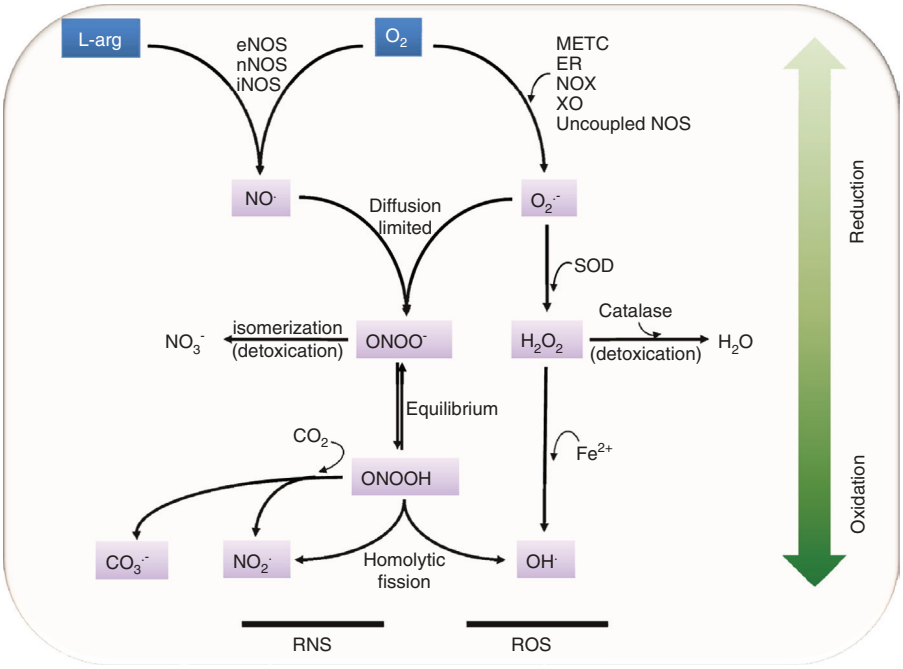

Figure 1 Molecular pathways of RNS and ROS generation.
Nitric oxide ($NO^{\cdot}$) is formed from L-arginine and molecular oxygen (O_2) by the activity of various isoforms of NO synthase (endothelial, neuronal, and inducible NOS). The superoxide radical ($O_2^{\cdot-}$) is formed during cellular metabolism in the mitochondrial electron transport chain (METC) and in the ER, or as a product of the enzymatic activities of NADPH oxidase (NOX), xanthine oxidase (XO), and uncoupled NOS. $O_2^{\cdot-}$ is dismutated by superoxide dismutase (SOD) enzymes to hydrogen peroxide (H_2O_2), which can either be detoxified to water by catalase or be converted to the hydroxyl radical ($OH^{\cdot}$) in the presence of metal (iron-mediated Fenton reaction). $NO^{\cdot}$ and $O_2^{\cdot-}$ spontaneously and rapidly react to form the strong oxidant peroxynitrite ($ONOO^-$). $ONOO^-$ can be detoxified by isomerization to nitrate (NO_3^-), or may form secondary radicals through homolytic fission (rupture of a covalent bond) or through reaction with carbon dioxide (CO_2) of its conjugated acid peroxynitrous acid (ONOOH), yielding the carbonate radical ($CO_3^{\cdot-}$), the nitrogen dioxide radical ($NO_2^{\cdot}$) or the $OH^{\cdot}$ radical.

yielding, respectively, hydrogen peroxide (H_2O_2) and the hydroxyl radical $OH^{\cdot}$), through enzymatic- and metal-catalyzed (Fenton) reactions (Jomova et al., 2010). Furthermore, $O_2^{\cdot-}$ promotes the formation of the potent oxidant and nitrating species peroxynitrite, following its spontaneous reaction with nitric oxide (see below).

A continuous generation of $O_2^{\cdot-}$ occurs in the mitochondria, due to the 'leakage' of electrons from the electron transport chain, mainly at the ubiquinone binding sites of complex I and complex III, resulting in the one-electron reduction of oxygen (Drose and Brandt, 2012). Under physiological conditions, approximately 2% of oxygen consumed is thus diverted to $O_2^{\cdot-}$ as a by-product of mitochondrial respiration. Oxidative damage is prevented by the rapid scavenging of $O_2^{\cdot-}$ by the mitochondrial enzyme manganese superoxide dismutase (Dhar and St Clair, 2012). The production of ROS may, however, significantly increase under various pathological conditions, for example, hypoxia or inflammation, which promote damage to electron transfer within the mitochondrial respiratory chain (Alfadda and Sallam, 2012). Besides mitochondria, the endoplasmic reticulum (ER) is another organelle contributing to cellular ROS generation. ER promotes protein folding through the formation of disulfide bonds within proteins, mediated by the enzymes protein disulfide isomerase and oxidoreductin 1, and coupled to the transfer of electrons to molecular oxygen, yielding H_2O_2. This process becomes significant under conditions of ER stress (triggered, for instance, by inflammatory cytokines or high glucose concentration), during which accumulation of misfolded proteins within the ER triggers a protective program termed the unfolded protein response, associated with an increased ER-dependent ROS generation (Laurindo et al., 2012; Popov, 2012).

The production of $O_2^{\cdot-}$ also depends on the activity of several enzyme systems, which include primarily the various isoforms of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX), present in virtually every cell type. They exist as seven distinct isoforms (NOX1–NOX5 and the dual oxidases DUOX1 and DUOX2), structurally composed of a core catalytic subunit and various regulatory subunits ($p40^{phox}$, $p47^{phox}$, $p67^{phox}$, $p22^{phox}$, NOXA1, NOXO1, DUOXA1, and DUOXA2) determining spatial organization, membrane location, subcellular expression, and activation of the enzyme (Drummond et al., 2011; Segal et al., 2012). Activation of NOX catalyzes the transfer of electrons from cytosolic NADPH to molecular oxygen to form $O_2^{\cdot-}$. For NOX4 and DUOX1-2, the primary ROS produced is H_2O_2 instead of $O_2^{\cdot-}$.

ROS produced by NOXs regulate multiple cellular (e.g., differentiation, proliferation, and migration) and physiological (e.g., vascular tone, oxygen sensing) processes (Drummond et al., 2011; Segal et al., 2012). Excess ROS production and oxidative stress occurs when overactivation of NOX occurs in response to stimuli as diverse as hyperglycemia, angiotensin II, growth factors, hormones (Schramm et al., 2012), and most significantly inflammatory cytokines such as interleukin (IL)-1β (Ginnan et al., 2013) and TNFα (Frey et al., 2002). In addition, activated neutrophils and macrophages produce large amounts of ROS through the activation of NOX2 during the so-called oxidative burst, which is essential for eliminating invading pathogens but which may also become a significant pathway of tissue injury under sterile inflammatory conditions associated with the activation of phagoyctes (Segal et al., 2012).

A second important enzymatic source of ROS is the xanthine dehydrogenase (XDH)/xanthine oxidase (XO) system. XDH is a metalloflavoenzyme converting xanthine and hypoxanthine into uric acid during the catabolism of purine nucleotides, using NAD^+ as an electron acceptor. Under inflammatory conditions and during tissue ischemia, XDH is converted to XO by oxidation of cysteine residues or by limited proteolysis (Engerson et al., 1987; Pritsos, 2000). XO does not reduce NAD^+ but uses O_2 as an electron acceptor, thereby producing large amounts of $O_2^{\cdot-}$ and H_2O_2 during purine catabolism (Nishino et al., 2008). The crucial role of XO-dependent oxidant generation is underscored by the great therapeutic potential of various pharmacological XO inhibitors, as recently reviewed (Pacher et al., 2006). Finally, additional enzymes may contribute to the formation of ROS, including cytochrome P450, cyclooxygenase, and uncoupled NO synthase, as detailed below.

The family of RNS

The parent molecule of all RNS is the free radical $NO^{\cdot}$, generated during the conversion of L-arginine to L-citrulline by the enzyme NO synthase (NOS) in a five-electron oxidative reaction. Three distinct isoforms of NOS exist, including neuronal (type I, nNOS), endothelial (type III, eNOS), and inducible (iNOS, type II) NOS. Whereas eNOS and nNOS are constitutively expressed, iNOS is expressed *de novo* upon cell activation by inflammatory cytokines and microbial products (Forstermann and Sessa, 2012). Under physiological conditions, $NO^{\cdot}$ functions as a key cellular messenger and cytoprotective species by interacting with metals and other free radicals, and by modulating the biological activity of a myriad of proteins through S-nitrosylation of cysteine residues (Liaudet et al., 2000).

Alternatively, $NO^{\cdot}$ may become a potent cytotoxic effector following its extremely fast reaction with $O_2^{\cdot-}$ (rate constant k=0.38–1.6×10^{10} M^{-1} s^{-1}) to generate peroxynitrite, the prototype of toxic RNS (Pacher et al., 2007). The oxidative chemistry of peroxynitrite depends in large part on the secondary formation of free radicals (Figure 1), including the hydroxyl radical ($^{\cdot}OH$), the carbonate radical ($CO_3^{\cdot-}$), and the nitrogen dioxide radical ($^{\cdot}NO_2$). The latter is notably responsible for the nitration of tyrosine residues within proteins, forming nitrotyrosine, which is used as a biochemical marker ('footprint') of peroxynitrite formation in a given situation (Pacher et al., 2007; Szabo et al., 2007; Ferrer-Sueta and Radi, 2009). By analogy with the term 'oxidative stress' associated with excessive ROS formation, the terms 'nitroxidative stress' (Calcerrada et al., 2011) and 'nitrative stress' (Sugiura and Ichinose, 2011) are frequently used to describe the consequences of excessive peroxynitrite formation in a biological system, while the term 'nitrosative stress' refers to the consequences of increased NO production (Pacher et al., 2005).

The generation of peroxynitrite may be significant under inflammatory conditions, due to the simultaneous generation of increased amounts of $NO^{\cdot}$ and $O_2^{\cdot-}$ (Pacher et al., 2007). It is also noteworthy that reduced availability of L-arginine (the NOS substrate) or tetrahydrobiopterin (an essential NOS cofactor) can result in dysfunction of NO synthase, by 'uncoupling' its reductase and oxygenase domains (Kietadisorn et al., 2012). Uncoupled NOS generates $O_2^{\cdot-}$ instead of $NO^{\cdot}$, and thereby favors the formation of peroxynitrite. This process has been particularly well described in the context of inflammatory cardiovascular pathologies, most significantly atherosclerosis, as reviewed recently (Forstermann and Li, 2011; Alkaitis and Crabtree, 2012).

Biological chemistry of ROS/RNS

ROS and RNS introduce various oxidative insults to lipids, proteins, and nucleic acids, with consequences ranging from subtle modulation of cell signal transduction processes to overt biomolecular damage and cell death, as illustrated in Figure 2. The one-electron oxidation of poly-unsaturated fatty acid (PUFA) residues may trigger a chain reaction of lipid peroxidation in biomembranes, causing significant alterations of membrane permeability (Valko et al., 2007). Oxidized PUFAs also form various cytotoxic aldehydes such as malondialdehyde and 4-hydroxynonenal (Devasagayam et al., 2003). The reaction of peroxynitrite with PUFAs can generate nitrated lipids, which may

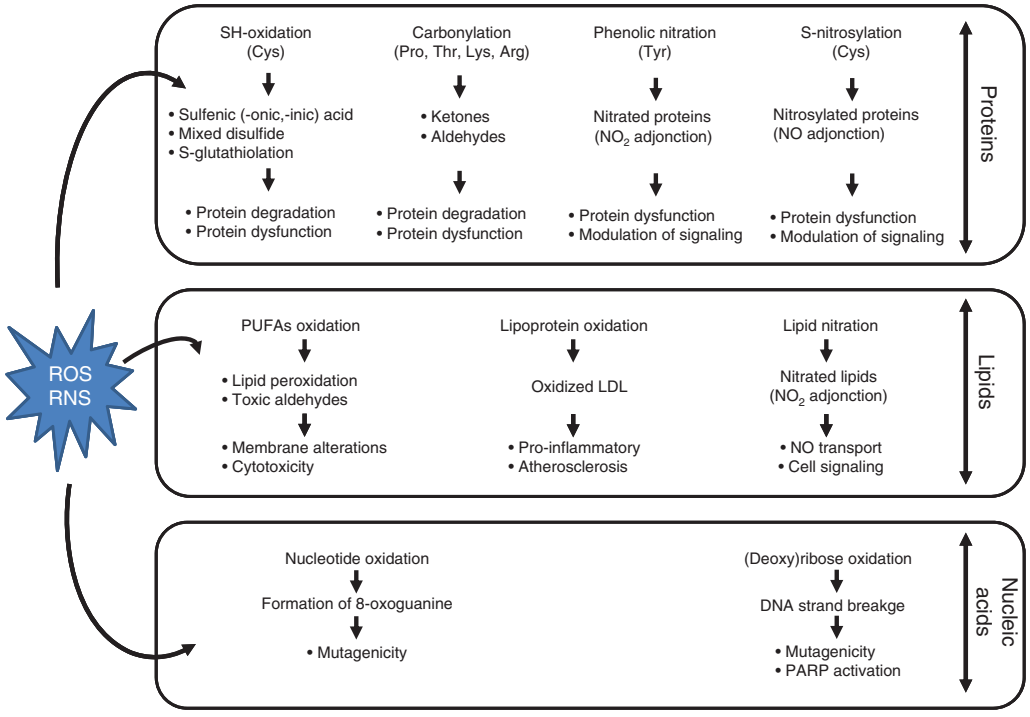

Figure 2 Major molecular targets and biological consequences of oxidative stress.
ROS/RNS attack proteins, lipids, and nucleic acids, promoting various molecular modifications responsible for disturbed biological functions. Abbreviations: Cys, cysteine; Pro, proline; Thr, threonine; Lys, lysine; Tyr, tyrosine; PUFAs, poly-unsaturated fatty acids; LDL, low-density lipoproteins; PARP, poly(ADP-ribose) polymerase.

act as endogenous donors of ·NO and as signaling molecules (Baker et al., 2009; Rubbo et al., 2009).

Several oxidative modifications of proteins may be introduced by ROS-RNS. The thiol (-SH) group of cysteine residues is a particularly sensitive target of oxidant species, forming sulfenic acid, mixed disulfide, *S*-glutathiolated derivatives, as well as sulfinic and sulfonic acid (Shao et al., 2012). Cysteine-bound thiol may also be nitrosylated through the addition of an NO group. Such *S*-nitrosylation is a reversible modification playing essential roles in modulating the function of a great number of cellular proteins (Murray and Van Eyk, 2012; Haldar and Stamler, 2013). Tyrosine residues may be affected by peroxynitrite-mediated nitration, that is, the addition of an NO_2 group to the phenolic ring of tyrosine (Castro et al., 2011). A further oxidative modification of proteins is the addition of a carbonyl group such as ketone or aldehyde groups (carbonylation) to the side chain of amino acids, mainly proline, threonine, lysine, and arginine (Dalle-Donne et al., 2003). Oxidized proteins may be subject to accelerated degradation and loss of function, with potentially significant cytotoxic consequences (Dalle-Donne et al., 2003). For instance, oxidative alterations of mitochondrial proteins (mainly induced by peroxynitrite) may precipitate apoptotic and necrotic cell death by triggering bioenergetic failure (Brown, 2007) and opening of the permeability transition pore (Radi et al., 2002). An important aspect of redox modifications within proteins relies in the modulation of multiple cell signal transduction pathways (Burgoyne et al., 2012). While physiological variations of the cellular redox state play a crucial role in cellular homeostasis (concept of redox signaling), oxidative stress may result in pathological alterations of cell signaling, which may notably confer an inflammatory phenotype to the cell (Liaudet et al., 2009).

In DNA, oxidants can damage nucleobases, especially guanine, resulting in the formation of 8-oxoguanine, with potential mutagenic and carcinogenic consequences (Valko et al., 2007). Oxidants can also abstract hydrogen atoms from the sugar phosphate backbone of DNA, promoting the generation of DNA strand breaks (Cadet et al., 2012). A striking consequence is the activation of the nuclear enzyme poly(ADP-ribose) polymerase (PARP). The latter is a family of 17 enzymes (primarily PARP-1 and PARP-2), which sense DNA strand breaks to initiate a program of DNA repair through the poly(ADP-ribosylation) of multiple nuclear protein substrates (Bai and Virag, 2012; Burkle and Virag, 2013). The catalytic activity of PARP consumes substantial amounts of cellular NAD^+, initiating a secondary depletion of ATP. This may precipitate cell death through the necrotic pathway in conditions of overt oxidative stress and PARP overactivation (Bai and Canto, 2012). In turn, necrotic cells release various intracellular components within the extracellular milieu, which trigger inflammatory responses through the activation of specific innate immune receptors (Kono and Rock, 2008). It is also noteworthy that PARP enzymes recently emerged as important modulators of inflammatory diseases, by affecting immune cell maturation and differentiation and by regulating the expression of multiple inflammatory mediators, as extensively reviewed recently (Bai and Virag, 2012). These roles of PARP activation in promoting necrotic cell death and inflammation have prompted the development of a series of pharmacological inhibitors with significant therapeutic potential in many disease processes (Virag and Szabo, 2002).

Besides PARP, it is also worth to mention the role of another DNA repair enzyme in the regulation of inflammation, namely 8-oxoguanine DNA glycosylase (OGG-1). The latter is a base excision repair enzyme that initiates the repair of oxidatively modified guanine (Hajas et al., 2013). Recent findings have provided evidence that this process is paralleled by enhanced immune responses (Mabley et al., 2005), as demonstrated by significant reduction of inflammation in OGG-1 knockout mice in conditions as diverse as endotoxic shock, diabetes, contact hypersensitivity (Mabley et al., 2005), and airway allergy (Li et al., 2012; Bacsi et al., 2013). The generation of DNA single-strand breaks during OGG-1-mediated DNA repair could represent the major underlying mechanism, by triggering the activation of inflammatory transcription factors and the expression of multiple cytokines (Li et al., 2012; Bacsi et al., 2013).

Inflammation in diseases: the role of innate immune mechanisms

Inflammation represents an adaptive response to any condition perceived as potentially dangerous to the host, and which aims at the removal of the danger, the induction of tissue repair, and the restoration of tissue homeostasis (Okin and Medzhitov, 2012). Conceptually, inflammation can be viewed as a four-stage process, including a triggering system (the danger), a sensor mechanism (danger receptors), the transmission of the signal, and the production of mediators, and the activation of cellular effectors (Medzhitov, 2008), which may all be affected, at various degrees, by reactive oxidants (Figure 3), as will be detailed in the next sections. The prototypical acute inflammatory response, characterized by local vasodilation, extravasation of leukocytes, and release of

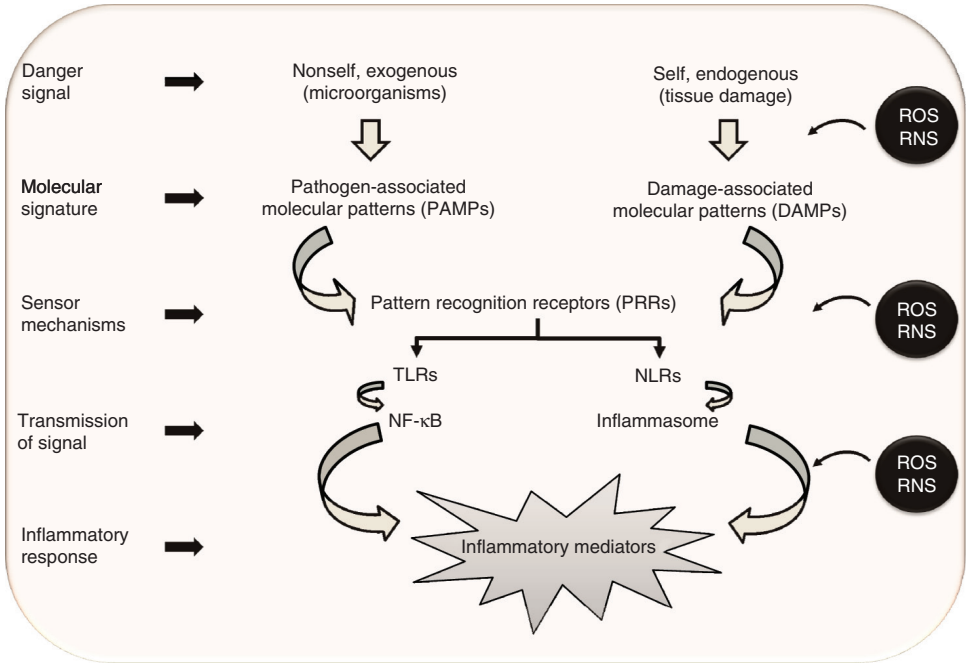

Figure 3 Molecular mechanisms of innate immune response.
The presence of danger signals (either exogenous or endogenous) alert the immune system through PRRs belonging to the Toll-like (TLRs) and the NOD-like (NLRs) families, triggering intracellular signaling through the transcription factor NF-κB and the molecular platform termed the inflammasome, fostering the production of multiple inflammatory mediators. Oxidative stress affects these processes at several levels, including the release of danger molecules, their sensing by PRRs, and their downstream signal transduction systems.

multiple plasma components, has been particularly well worked up in the field of invasion by microorganisms. In such infectious conditions, signature molecules released by the microbes, termed pathogen-associated molecular patterns (PAMPs), activate innate immune defenses through the activation of the so-called pattern recognition receptors (PRRs) and downstream cellular pro-inflammatory signaling, with the ultimate goal of eradicating the pathogen (Barton, 2008; Kono and Rock, 2008; Medzhitov, 2010b). Over the past decade, evidence has been obtained that a comparable scenario is set in motion by a wide variety of non-infectious stimuli, from exogenous or endogenous origin. Thus, the innate immune system operates not only to sense 'self' vs. 'non-self' and to safeguard against invading pathogens, but more generally, to identify and provide an adapted response to any form of danger (Matzinger, 2002) (Figure 3).

Innate immune defense and inflammation: the danger model

A multiplicity of danger signals can be sensed by the host. Exogenous signals include primarily microbial molecules, for example, lipopolysaccharide (LPS) and flagellin from gram-negative bacteria, lipoteichoic acid from gram-positive bacteria, and viral RNA (Kawai and Akira, 2006), as well as non-infectious molecules, including allergens (Holgate, 2012) and airborne pollutants such as silica and asbestos (Dostert et al., 2008). Multiple endogenous signals are also able to elicit an inflammatory response through the activation of innate immune defense mechanisms. These are collectively termed 'damage-associated molecular patterns' or DAMPS (Kono and Rock, 2008), sometimes also referred as to 'alarmins' (Chan et al., 2012). DAMPs are released in conditions of tissue injury, and originate either from dying (necrotic) cells or from the breakdown of the extracellular matrix (Medzhitov, 2010a). Examples of intracellular DAMPs include heat shock proteins, S-100 proteins, high mobility group box-1 (HMGB1), ATP, and DNA (Kono and Rock, 2008). A specific subgroup of DAMPs originating from mitochondria (mito-DAMPs, MTDs) has been also recently described, comprising formyl peptides and mitochondrial DNA (Zhang et al., 2010). Extracellular DAMPs comprise fibronectin, hyaluronic acid, as well as peptides derived from collagen or elastin (Kono and Rock, 2008). Finally, various crystals acting as endogenous danger signals can promote strong inflammatory response, including monosodium urate (Martinon et al., 2009), calcium pyrophosphate dihydrate

(Martinon et al., 2009), and cholesterol ester (Duewell et al., 2010). The main endogenous DAMPs and their receptors are presented in Table 1.

The appropriate recognition of the danger by the host is primordial for the elaboration of proper antimicrobial and adaptive responses. Sensing of PAMPs and DAMPs is ensured by a complex set-up of PRRs, which include primarily the Toll-like receptors (TLRs) (Trinchieri and Sher, 2007) and the nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) (Martinon et al., 2009). Other PRRs include C-type lectins, the receptor for advanced glycation end-products (RAGE), the retinoid acid-inducible gene I-like receptors, and the AIM2-like receptors (see Kingeter and Lin, 2012; Miyake and Yamasaki, 2012; Tang et al., 2012b for extensive recent reviews). PRR activation triggers a wealth of intracellular signaling pathways, including kinases [for instance, mitogen-activated protein kinases (MAPKs), PI3 kinase], adaptors [such as myeloid differentiation primary response protein 88 (MyD88)], transcription factors [mainly nuclear factor-κB (NF-κB), activator protein-1 (AP-1), and interferon regulatory factors], as well as the inflammasome in the case of NLR activation (see below). Such signaling cascades foster the expression of cytokines, chemokines, enzymes, growth factors, and additional molecules that are required for antimicrobial resistance and tissue repair (Medzhitov and Horng, 2009; Tang et al., 2012b).

Table 1 Major endogenous activators of TLRs and NLRP3.

TLR activators
Cellular proteins
– HMGB1: TLR4 (Yang et al., 2013)
– S-100 proteins: TLR4 (Donato et al., 2013)
– Heat shock proteins: TLR2, TLR4 (Tamura et al., 2012)
– Myosine: TLR2, TLR8 (Zhang et al., 2009)
– Neutrophil elastase: TLR4 (Benabid et al., 2012)
– Lactoferrin: TLR4 (Ando et al., 2010)
– Serum amyloid A: TLR2, TLR4 (Eklund et al., 2012)
Lipids and lipoproteins
– Saturated fatty acids: TLR4 (Holland et al., 2011)
– Oxidized phospholipids: TLR4 (Greig et al., 2012)
– Oxidized low-density lipoproteins: TLR4 (Miller et al., 2009)
Components of the extracellular matrix, extracellular proteins
– Biglycan, Decorin: TLR2, TLR4 (Frey et al., 2013)
– Versican: TLR4/TLR6 heterodimer (Frey et al., 2013)
– Low molecular weight hyaluronan: TLR2, TLR4 (Frey et al., 2013)
– Fibronectin extra domain A: TLR4 (Lefebvre et al., 2011),
– Tenascin-C: TLR4 (Midwood et al., 2009)
– Fibrinogen fragments: TLR4 (Millien et al., 2013)
– Surfactant protein A: TLR2, TLR4 (Kuroki et al., 2007)
Nucleic acids
– Mitochondrial DNA: TLR9 (Holm et al., 2013)
– mRNA: TLR3 (Kariko et al., 2004)
– Small nuclear ribonucleoprotein particles (snRNPs): TLR7, TLR8 (Vollmer et al., 2005)
NLRP3 activators
– Crystals: monosodium urate, calcium pyrophosphate, calcium oxalate, cholesterol (Davis et al., 2011)
– Extracellular ATP (Davis et al., 2011)
– Amyloid β (Heneka et al., 2013)
– Oxidized mitochondrial DNA (Shimada et al., 2012)
– Hyaluronan (Yamasaki et al., 2009)
– Pancreatic islet-derived amyloid polypeptide (Masters et al., 2010)
– High glucose (Tack et al., 2012)
– Fatty acids: palmitate, ceramide (Tack et al., 2012)

As stated previously, inflammation represents an essential adaptive response aimed at eradicating invading pathogens and repairing tissue damage elicited by noxious stimuli. Appropriate regulation of the mechanisms involved in such adaptation is essential to confine the inflammatory response in a localized compartment and to promote the switch between inflammation and repair, thereby allowing, *in fine*, the restoration of tissue homeostasis (Medzhitov, 2010a). However, there are situations in which such restoration may not adequately occur, resulting in persistent cellular stress perpetuating and amplifying the inflammatory response. In these conditions, the process becomes maladaptive, leading to significant alterations of tissue functions, with systemic and persistent derangements of homeostasis (Okin and Medzhitov, 2012). Diabetes, atherosclerosis, chronic heart failure, neurodegenerative diseases, and cancer are typical examples of pathological processes associated with such chronic inflammatory changes (Pacher et al., 2007).

It is particularly noticeable that the release of ROS has long been recognized as a typical consequence of immune cell stimulation *in vitro* (Meier et al., 1989, 1990), and that both acute and chronic inflammatory states *in vivo* are coupled with significant alterations of redox equilibrium, due to the associated enhancement of oxidant generation (Pacher et al., 2007; Roberts et al., 2010; Li et al., 2013; Rochette et al., 2013). Accordingly, mitigating oxidative stress by the use of antioxidants has been evaluated as a potentially useful anti-inflammatory strategy in such conditions, as recently reviewed (Spychalowicz et al., 2012). Several distinct approaches to reduce oxidative stress have been used for this purpose, including natural (e.g., vitamin C, thiol compounds, flavonoids, and polyphenols) or synthetic (e.g., Trolox, dihydropyridines, edaravone) free radical scavengers and antioxidants (Augustyniak et al., 2010; Rahman and MacNee, 2012; Carocho and Ferreira, 2013), enzymatic inhibitors targeting NADPH oxidase (Drummond et al., 2011), NO synthase (Feihl et al., 2004), xanthine oxidase (Pacher et al., 2006), or PARP (Virag and Szabo, 2002), as well as catalytic antioxidants, including

superoxide dismutase mimetics (Muscoli et al., 2003) and peroxynitrite decomposition catalysts (Szabo et al., 2007). Overall, the results of these innumerable studies have clearly pointed out the strong association between oxidative stress and inflammation. Notwithstanding, the molecular mechanisms underlying the connection of these two fundamental biological processes have often not been precisely examined. In the next sections of this review, we will therefore attempt to fill this gap by presenting the current evidence supporting a mechanistic link binding redox stress, innate immunity, and inflammation, by focusing primarily on sterile (non-infectious) causes of inflammation.

Role of oxidative stress in inflammation

Oxidative stress and inflammatory danger signals: the illustrative case of HMGB1

As previously discussed, conditions promoting significant oxidative stress may precipitate cellular death and extracellular matrix breakdown, due to biomolecular damage exceeding any capacity of repair. Necrotic cells and damaged ECM in turn release various intracellular and extracellular molecules, which act as 'alarmins' triggering inflammatory cascades through recognition by PRRs (Chan et al., 2012). Furthermore, oxidative stress conditions may induce various modifications within lipids and proteins, generating the so-called oxidation-specific epitopes, which act as potent DAMPs able to trigger innate immune responses through binding to multiple PRRs. Examples of such oxidation-specific epitopes include oxidized phospholipids such as oxidized 1-palmytoyl-2-arachidonyl-*sn*-glycero-3-phosphocholine (Imai et al., 2008; Kampfrath et al., 2011), oxidized cholesteryl esters (Choi et al., 2009), and oxidized low-density lipoporoteins (Lahoute et al., 2011), which are increasingly recognized as key mediators of the inflammation associated with the process of atherogenesis, as detailed in extensive recent reviews (Miller et al., 2011; Greig et al., 2012). As a detailed description of the multiple identified DAMPs is beyond the scope of this review, we will focus in the next section on the protein HMGB1 to illustrate the topics of the relations linking oxidative stress, DAMPs, and the development of sterile inflammation (Figure 4).

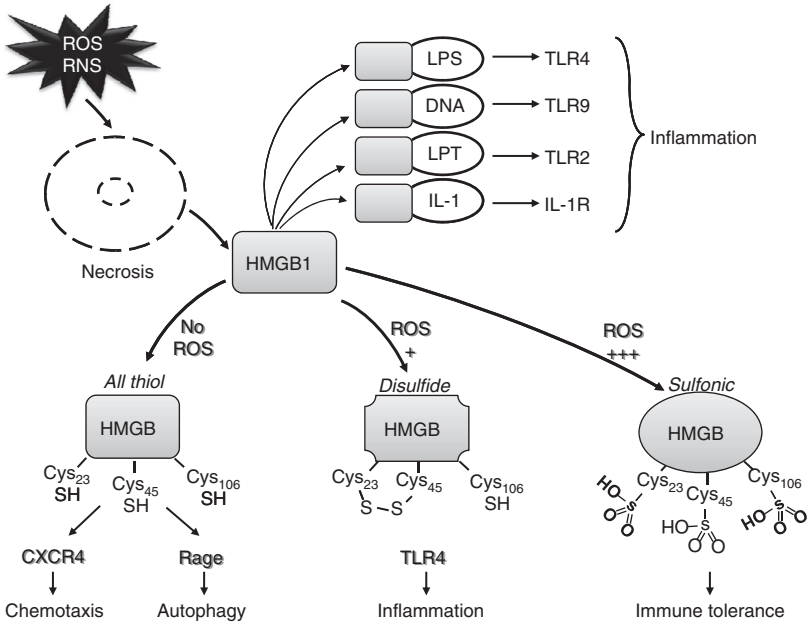

Figure 4 Role of oxidative stress on the biology of HMGB1.
Excess ROS/RNS promote cell necrosis, leading to the passive release of intracellular HMGB1 into the extracellular milieu. HMGB1 can interact with multiple molecules, including LPS, DNA, lipoteichoic acid (LPT), and IL-1, to elicit inflammatory responses through distinct TLRs. Alternatively, HMGB1 can undergo various cysteine modifications depending on the local concentrations of oxidants. In the absence of ROS, HMGB1 is in the all thiol conformation, owing to reduced forms of Cys23, Cys45, and Cys106. All thiol HMGB1 interacts with the receptor for advanced glycation end-product (RAGE) to promote autophagic responses, and with the chemokine receptor CXCR4, triggering chemotactic responses. In the presence of increasing amounts of ROS, HMGB1 occurs either in the disulfide conformation (oxidation of Cys23 and Cys45), which targets TLR4 to initiate inflammatory responses, or in the fully oxidized conformation, due to oxidation to sulfonic acid of its three redox active cysteines. This latter form cannot activate inflammatory cells and thus promotes immune tolerance.

HMGB1 has been the first DAMP to be characterized (Scaffidi et al., 2002) and has been the subject of extensive literature over the past decade (Harris et al., 2012). HMGB1 is a ubiquitous nuclear non-histone protein that binds to DNA and participates in DNA replication, transcription, and repair (see Andersson and Tracey, 2011 for recent review). HMGB1 can be released extracellularly either through active secretion by activated monocytes (Wang et al., 1999) or by passive release by dying cells (Scaffidi et al., 2002), where it triggers a plethora of actions, including inflammation, chemotaxis, maturation of dendritic cells, and endothelial cell activation to name a few (Andersson and Tracey, 2011). These effects are mediated following interactions of HMGB1 with several PRRs, primarily TLR4 and RAGE (Janko et al., 2013). HMGB1 also forms various complexes with many different molecules in the extracellular milieu, promoting additional pro-inflammatory activities through interaction with TLR2, TLR3, TLR9, and the IL-1 receptor (Bianchi, 2009; Janko et al., 2013). HMGB1 plays significant roles in the pathophysiology of sterile inflammation (Andersson and Tracey, 2011) associated with diverse acute and chronic conditions, including myocardial infarction (Loukili et al., 2011), hepatic ischemia (Tsung et al., 2005), stroke (Schulze et al., 2013), circulatory shock and trauma (Andersson and Tracey, 2011), diabetes (Nogueira-Machado et al., 2011), cancer (Krysko et al., 2012), and arthritis (Harris et al., 2012), and it also acts as a key pro-inflammatory cytokine involved in the pathogenesis of sepsis (Yang et al., 2004). Thus, HMGB1 emerges as a central acting mediator at the intersection between sterile and infectious inflammation (Andersson and Tracey, 2011).

Several lines of experimental evidence support a key role played by oxidative stress in the process of HMGB1 release in the extracellular milieu. In a study using isolated cardiomyocytes *in vitro*, we reported that exposure of the cells to toxic concentrations of peroxynitrite induced necrotic cell death and was associated with the release of copious amounts of HMGB1 (Loukili et al., 2011). Similarly, Tang et al. (2007, 2011) reported that treatment of various mouse and human cell lines with H_2O_2 promoted significant extracellular HMGB1 release, whereas Tsung et al. (2007) showed that oxidants promoted the active release of HMGB1 by cultured hepatocytes through calmodulin-dependent kinase signaling. *In vivo*, using a rat model of myocardial infarction, we reported that a significant formation of peroxynitrite occurred in the necrotic myocardium, together with HMGB1 accumulation. The elimination of peroxynitrite using peroxynitrite decomposition catalysts reduced myocardial infarct size and suppressed the build-up of HMGB1, providing direct evidence that peroxynitrite-mediated cell death was the key trigger of cardiac HMGB1 accumulation during myocardial ischemia (Loukili et al., 2011). Thus, HMGB1 release represents a common response to the cellular stress imposed by free radicals and oxidants *in vitro* and *in vivo*, thereby representing an important mechanism linking redox stress and inflammation.

It is particularly noteworthy that HMGB1, while being released by oxidatively damaged cells, is also extremely redox sensitive, due to the presence of three critical cysteine residues. HMGB1 consists of two DNA binding motifs, box A and box B, and an acidic C-terminal tail. Box A possesses two vicinal cysteines (Cys23 and Cys45), whereas box B bears one single cysteine in position 106 (Cys106) (Tang et al., 2012a). Depending on the particular redox environment, three distinct redox forms of HMGB1 can therefore be present: all thiol-HMGB1 (three cysteines in reduced SH form), disulfide HMGB1 (presence of a disulfide bridge between Cys23 and Cys45 in box A), and fully oxidized HMGB1 (oxidation to sulfonate of Cys23, Cys45, and Cys106) (Tang et al., 2012a) (Figure 4). According to recent investigations, the recognition of the different redox conformations of HMGB1 may help devise specific pharmacological inhibitors, with potential therapeutic activity against HMGB1-dependent inflammation (Gero et al., 2013).

Among these distinct redox forms, only disulfide HMGB1 has the ability to bind TLR4 and to promote innate immune responses (Tang et al., 2012a), as long as Cys106 is, at the same time, in a reduced conformation (Yang et al., 2013). In contrast, all thiol HMGB1 cannot bind to TLR4, but has been associated with the induction of autophagic responses in target cells through binding to the RAGE receptor (Kang et al., 2011). In addition, all thiol HMGB1 forms a complex with the chemokine CXCL12, to promote strong chemotactic responses on leukocytes through binding to the receptor CXCR4 (Venereau et al., 2012). These two distinct redox forms of HMGB1 are mutually exclusive, which implies that, depending on the particular microenvironment, HMGB1 functions either as a chemoattractant or as an inducer of cytokine release (Tang et al., 2012a; Venereau et al., 2012). In conditions of tissue injury *in vivo*, both redox forms of HMGB1 have been shown to be sequentially present (all thiol followed by disulfide), indicating that HMGB1 successively orchestrate leukocyte recruitment and the induction of cytokine secretion by adopting distinct redox conformations (Tang et al., 2012a; Venereau et al., 2012). Finally, fully oxidized HMGB1 loses both chemoattractant and pro-inflammatory activities, but instead triggers immunologic tolerance by preventing the activation of dendritic cells, which might explain the lack of inflammation associated with

apoptotic cell death (Kazama et al., 2008; Peter, 2008). Indeed, during apoptosis, mitochondrial production of ROS appears sufficient to promote the full oxidation and complete inhibition of HMGB1 (Kazama et al., 2008; Peter, 2008).

In addition to oxidative modifications, HMGB1 can also undergo acetylation of key lysine residues located within its nuclear localization sequence. This promotes the accumulation of HMGB1 within the cytoplasm, by preventing its nuclear re-entry, resulting in the active release of HMGB1 by monocytes/macrophages, particularly under conditions of inflammasome activation (Lu et al., 2012). The various posttranslational modifications of HMGB1 (oxidation and acetylation) have been the subject of an outstanding recent review by Yang et al. (2013).

To complete the picture of HMGB1-oxidant stress interactions, it is worth mentioning that HMGB1 itself may induce significant redox modifications by fostering the cellular generation of ROS and RNS (Janko et al., 2013). HMGB1-dependent activation of TLR4 triggers the upregulation of multiple genes, among which the genes encoding NOX and iNOS. Fan et al. (2007) showed that stimulation of ploymorphonuclear neutrophils (PMNs) *in vitro* with recombinant HMGB1 caused TLR4-dependent activation of NOX and subsequent increased ROS production. Using an experimental model of hemorrhagic shock in mice, these authors then reported that hemorrhagic shock was associated with significant increases of HMGB1 levels in most organs, together with a marked induction of NOX in PMNs, which could be abrogated both by TLR4 suppression and by neutralizing antibodies to HMGB1 (Fan et al., 2007). In another study by Sappington et al. (2002), incubation of Caco-2 human enterocytic monolayers with recombinant human HMGB1 resulted in enhanced expression of iNOS, increased generation of NO and peroxynitrite, together with an increased epithelial permeability, which could be abrogated by strategies removing NO and peroxynitrite (Sappington et al., 2002). Thus, it appears that oxidative stress represents both a cause and a consequence of HMGB1 release in multiple situations, and one may envision that such crosstalk might promote a vicious cycle of progressive inflammatory amplification.

Role of oxidative stress in the biology of PRRs

Toll-like receptors

TLRs are key components of the innate immune system (Figure 5), whose primary function is to sense danger signals released from pathogens (PAMPs). They are type I transmembrane proteins composed of an extracytoplasmic, leucine-rich repeat domain for ligand recognition, a short transmembrane domain, and a cytoplasmic Toll/IL-1 receptor (TIR) homology domain for signal transduction (O'Neill and Bowie, 2007). Ten TLRs are expressed in humans, among which only TLR10 has no identified ligand thus far. TLR1, TLR2, TLR4, TLR5, and TLR6 are present in the plasma membrane to sense surface components of microbes. TLR2, which forms homodimers and heterodimers with TLR1 and TLR6, and which uses various coreceptors such as CD14, CD36, and Dectin-1, recognizes bacterial lipopetides and peptidoglycans as well as mannans and glucans from fungal origin. TLR4, with the coreceptor MD-2, senses the complex formed by bacterial LPS and CD14, and TLR5 detects bacterial flagellin. TLR3, TLR7, TLR8, and TLR9 are expressed in intracellular compartments (ER, endosomes, lysosomes, and endolysosomes) where they sense viral and bacterial nucleic acids (Lee et al., 2012; Song and Lee, 2012).

As previously discussed, TLRs also detect endogenous DAMPs released from damaged cells and extracellular matrix, comprising proteins, fatty acids, lipoproteins, proteoglycans, and glycosaminoglycans (mainly detected by TLR4, and, to a lesser extent, TLR2), as well as nucleic acids and protein-nucleic acid complexes (detected by TLR3, TLR7, TLR8, and TLR9) (see Piccinini and Midwood, 2010 for extensive review). Several differences in the recognition of PAMPs and DAMPs (different binding sites, different coreceptors and accessory molecules), and the engagement of distinct regulatory pathways may help discriminate exogenous from endogenous dangers. Most notably, DAMP recognition triggers negative feedback mechanisms to limit the potential damage to the host, and recent findings support an essential role for sialoside-based pattern recognition by members of the Siglec family in such negative regulation (Chen et al., 2009; Liu et al., 2011).

All TLRs, with the exception of TLR3, interact with the adaptor MyD88. The endosomal TLR7, TLR8, TLR9 and the cell surface TLR5 directly link MyD88, whereas TLR1, TLR2, TLR4, and TLR5 present on cell surface additionally recruit the linker protein TIR domain-containing adaptor protein (TIRAP also known as MAL) that connects TLRs and MyD88 TIR domains. Upon ligand binding, TLR3 and TLR4 recruit the protein TIR domain-containing adaptor inducing interferon-β (TRIF) either directly for TLR3 or by the intermediate of TRIF-related adaptor molecule (TRAM) for TLR4 (Gay and Gangloff, 2007; Song and Lee, 2012). MyD88 activates the IRAK-TRAF6-TAK1 axis that turns on inhibitor of κB (IκB) kinase (IKK) and MAPKs, which lead

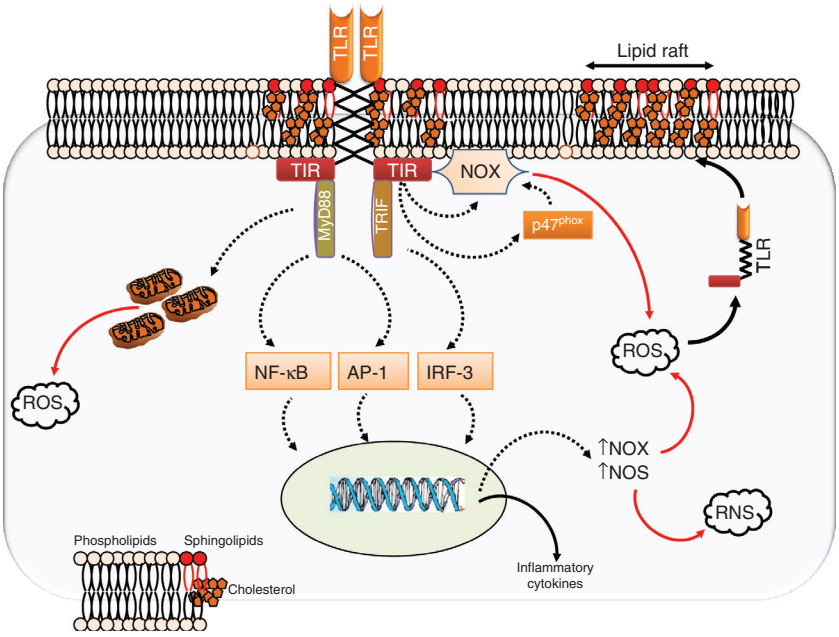

Figure 5 Interactions between oxidative stress and Toll-like receptors.
TLRs form active dimers within specific lipid rafts containing various amounts of phospholipids, cholesterol, and sphingolipids in the cell membrane. The extracellular domain connects through a transmembrane domain to the cytoplasmic TIR domain, which interacts with adaptor molecules such as MyD88 and TRIF to activate downstream signaling through NF-κB, AP-1, and interferon-regulatory factor-3 (IRF-3). Consequently, the expression of inflammatory mediators is upregulated, comprising notably pro-oxidant enzymes such as NOX and iNOS, producing high levels of ROS. TLR engagement also facilitates the generation of ROS within mitochondria, and promotes activation of NOX through direct interaction at the cell membrane or through enhanced phosphorylation of its $p47^{phox}$ subunit within the cytoplasm. The resulting increase in intracellular ROS favor the mobilization and dimerization of TLRs within lipid rafts, creating a cycle of progressive amplification of the TLR response.

to the activation of NF-κB and AP-1 transcription factors, respectively (Kawai and Akira, 2010; Song and Lee, 2012).

Oxidative stress and Toll-like receptors: role of oxidants in TLR activation

It has been known for a long time that conditions associated with significant oxidative stress trigger enhanced responsiveness of cells from the innate immune system to pro-inflammatory stimuli (Botha et al., 1995; Fan et al., 1998). The underlying mechanism has remained elusive until recently, when it was proposed that oxidants may prime immune cells ('reprogramming') by upregulating TLR-dependent signaling (Figure 5). In a milestone study by Powers and coworkers (2006), exposure of rodent macrophages to oxidants, either *in vivo* (using an experimental model of hemorrhagic shock and resuscitation) or *in vitro* (using direct macrophage activation with H_2O_2), induced a significant increase in the surface levels of TLR4, as well as an increased responsiveness of cells to LPS. This effect was dependent on exocytosis of TLR4 from cytoplasmic compartments, as it could be suppressed by disruption of the cytoskeleton. Most important, the translocation of TLR4 into the cell membrane colocalized with the lipid raft marker ganglioside GM1, implying the redistribution of TLR4 within lipid rafts (which are 'floating' microdomains within cell membranes, serving as signaling platforms). Finally, preventing TLR4 movement to lipid rafts using methyl-β-cyclodextrin suppressed the increased cellular responsiveness to LPS exposure to oxidants (Powers et al., 2006).

The study by Powers et al. emphasizes the crucial importance of receptor trafficking in the regulation of the ability of TLRs to sense their ligands, an issue that has been reviewed recently by McGettrick and O'Neill (2010). The essential role of oxidants in such process, as demonstrated in the above-discussed study, has been further confirmed by Nakahira et al. (2006), who showed that the trafficking of TLR4 to lipid rafts in response to LPS was entirely dependent on ROS produced secondary to the activation of NOX in RAW 264.7 murine macrophages. Similarly, Wong et al. (2009) demonstrated that a NOX-dependent generation of ROS was necessary for TLR4 recruitment and dimerization within membrane lipid rafts of cultured macrophages exposed to LPS, and additional studies have suggested comparable effects of oxidants in

the membrane localization of TLR2 (Frantz et al., 2001; Dasu et al., 2008; Paul-Clark et al., 2009). The precise mechanisms of oxidant-mediated lipid raft modifications remain only partially elucidated. They involve, at least partly, alterations in lipid raft annexin VI content, activation of calcium-dependent kinases, and the generation of ceramide. In turn, such modifications may result in modifications of lipid raft density and protein composition, with subsequent stimulation of TLR complex assembly (Cuschieri and Maier, 2007; de la Haba et al., 2013).

Role of TLR activation in the generation of oxidants

The previous paragraphs have highlighted the importance of oxidants in the activation of TLRs, by promoting their trafficking to the cell membrane. We will now focus on the opposite mechanism, that is, the induction of oxidant production in response to TLR activation (Figure 5). Increasing evidence is indeed accumulating showing that the formation of ROS represents an essential pathway of TLR-dependent signaling in cells from immune and non-immune origin, which occurs mainly through the activation of various NOX isoforms (Tsung et al., 2007; Ogier-Denis et al., 2008; Gill et al., 2010). In addition, the pro-inflammatory signaling cascades triggered by TLR engagement enhance the expression of iNOS, and thus promotes the generation of NO (Lewis et al., 2011; Shweash et al., 2011). In turn, the concomitant generation of $O_2^{\cdot-}$ (by NOXs) and $NO^{\cdot}$ (by iNOS) results in the formation of peroxynitrite and other toxic RNS, implying that TLR activation may result both into oxidative and nitroxidative stress (Jozsef et al., 2006; Lucas and Maes, 2013).

While TLR-dependent oxidant formation is important to promote killing of invading pathogens (West et al., 2011), it may also result in significant cytotoxicity and collateral tissue injury in conditions of sterile inflammation. Additionally, oxidants may facilitate further TLR activation, resulting in a cycle of progressive amplification of the initial inflammatory response. This process, which has been designed as the 'TLR-radical cycle', may represent an important mechanism in the maintenance of chronic inflammation in multiple human diseases, including cardiovascular diseases, diabetes, or neurodegenerative processes, as reviewed recently (Lucas and Maes, 2013).

The activation of NOX enzymes appears as a key process linking TLRs with secondary ROS generation. Such activation of NOX appears to result from several mechanisms, including (1) increased NOX protein expression, (2) stimulated assembly of the NOX subunits, and (3) direct interactions between NOX and the TIR domain of TLRs. Increased NOX expression has been notably well documented for NOX1 present in cells from gastrointestinal origin. NOX1 upregulation occurred in gastric epithelial cells in response to TLR4 activation by LPS from pathogenic *Helicobacter pylori* strains, and was shown to promote the induction of TNFα or cyclooxygenase 2 mRNA expression. This implies that NOX1 could be significantly involved in the pathogenesis of chronic gastric inflammation induced by *H. pylori* infection (Kawahara et al., 2005). Induction of NOX1 has also been evidenced in multiple colon cancer cell lines, including T84 (Kawahara et al., 2004), SW480, SW620, and CT-26 cells (O'Leary et al., 2012), as a result of TLR4 (O'Leary et al., 2012) and TLR5 (Kawahara et al., 2004) activation by LPS and flagellin, respectively. NOX1-dependent ROS production in these conditions promoted the release of the major chemokine IL-8 (Kawahara et al., 2004), and also greatly facilitated the adhesion of colon cancer cells to collagen I (O'Leary et al., 2012), implying a possible role in the establishment of intestinal inflammatory processes, as well as in increasing the metastatic potential of colon cancer cells.

The second important mechanism of TLR-mediated NOX activation is represented by stimulated assembly of the NOX subunits, particularly well documented in the case of NOX2 expressed by phagocytes. Various agonists of TLR2 (Huang et al., 2009), TLR4 (Bae et al., 2009; Kampfrath et al., 2011), and TLR7/8 (Makni-Maalej et al., 2012) promoted NOX2 activation by triggering phosphorylation of its $p47^{phox}$ subunit. Deciphering the underlying signaling mechanisms revealed a crucial role of MyD88 (Laroux et al., 2005), IRAK4 (Pacquelet et al., 2007; Picard et al., 2007), and phospho-p38 (Yang et al., 2008a; Makni-Maalej et al., 2012) signaling pathway in the upregulated $p47^{phox}$ phosphorylation. Finally, activation of NOX may also result from direct interactions between TLRs and NOX, as indicated in a study by Park et al. (2004). Using yeast two-hybrid and GST pull-down assays, these authors showed that the carboxy-terminal region of NOX4 directly interacted with the cytoplasmic tail of TLR4 in HEK293T cells. Stimulation of TLR4 with LPS in this system induced ROS generation followed by NF-κB activation, pointing to direct interaction between TLR4 and NOX4 as a critical mechanism regulating TLR-dependent innate immune responses (Park et al., 2004).

Generation of ROS secondary to TLR engagement does not only rely on the activation of NOX but also depends on the mitochondria (Figure 5). West and coworkers (2011) reported that the engagement of various TLRs (TLR1, TLR2, and TLR4) augmented mitochondrial ROS production by inducing the recruitment of mitochondria to macrophage phagosomes. This response occurred through the translocation of the adaptor TRAF6 to mitochondria,

leading to ubiquitination and enrichment at the mitochondrial periphery of ECSIT (evolutionarily conserved signaling intermediate in Toll pathways), a protein implicated in mitochondrial respiratory chain assembly, with subsequent mitochondrial generation of ROS. The importance of this process in antibacterial defense was critical, in view of the significant impaired capacity of ECSIT- and TRAF6-depleted macrophages to kill intracellular bacteria (West et al., 2011). However, it remains thus far unknown whether a similar mechanism accounts for TLR-dependent oxidative stress under sterile conditions.

To sum up, significant evidence has been obtained in recent years that TLR-ROS/RNS interactions are instrumental in the induction and maintenance of innate immune responses. One may argue that targeting this 'TLR-radical cycle' might prove useful to prevent or treat many chronic inflammatory diseases (Lucas and Maes, 2013). Still, given the critical importance of TLR-dependent ROS generation in the elimination of invading pathogens, impaired infectious control might well represent an important drawback of such strategy. Many issues need to be addressed to pinpoint how the organism discriminates between exogenous from endogenous danger signals and whether distinct signaling pathways modulate TLR-mediated responses during infectious and sterile inflammation, in order to manipulate safely the TLR machinery for the therapy of chronic inflammatory diseases.

NOD-like receptors and inflammasomes

NLRs are located in the cytosol and sense a wide range of PAMPs and DAMPs. NLRs possess a common structure characterized by C-terminal leucine-rich repeat domain for ligand sensing, a central nucleotide-binding and oligomerization domain for activation, and an N-terminal domain for downstream signaling whose structure defines different subfamilies of NLRs (NLRA, NLRB, NLRC, NLRP, and NLRX) (Kersse et al., 2011). Upon activation, NLRs of the NLRP or NLRC families form multiprotein complexes termed inflammasomes, following assembly with the adapter protein ASC (apoptosis associated speck-like containing a CARD domain) and caspase-1. Once activated, the inflammasome platform promotes caspase-1-mediated cleavage and maturation of pro-IL-1β and pro-IL-18 into mature IL-1β and IL-18, which are critical to the development of inflammation (Martinon et al., 2009).

The NLRP3 inflammasome (also termed cryopirin or NALP3) has been thus far the best-studied member of this wide family (Figure 6). Two signals are required for full NLRP3 activation, including, first, a TLR-dependent activation of NF-κB, resulting in the upregulation of NLRP3 and pro-IL-1β expression ('priming'), and, second, an NLRP3 activating signal, which comprises multiple foreign and endogenous molecules (Franchi et al., 2012). Foreign signals comprise multiple pathogens and PAMPs that enter the cytosol, including bacteria (notably *Staphylococcus*, *Listeria*, *Clostridium*, and *Escherichia coli* species), fungi (mainly *Candida* and *Aspergillus* species), and viruses (for instance, adenovirus and influenza virus) (Davis et al., 2011). The activation of NLRP3 in response to invading pathogens mainly depends on the release of pore-forming toxins by bacteria (such as hemolysin from *Staphylococcus aureus* and toxin A from *Clostridium difficile*) (Koizumi et al., 2012), the release of double-stranded RNA by viruses (Yu and Levine, 2011), and the release of hyphal fragments and the activation of the Syk tyrosine kinase by fungi (Said-Sadier et al., 2010). Besides microbes, several non-infectious foreign molecules are robust activators of NLRP3, notably crystalline structures responsible for occupational lung inflammatory diseases (silica and asbestos) and particulate structures such as Co-Cr-Mo alloy metal particles used in prosthetic orthopedic material (Caicedo et al., 2009) and metal nanoparticles such as titanium dioxide, used as a pigment in paint and cosmetics (Yazdi et al., 2010).

Multiple endogenous signals derived from the host itself have the ability to trigger NLRP3 activation, leading to a variety of acute and chronic inflammatory processes. Diverse crystals, including monosodium urate, calcium pyrophosphate dehydrate, and calcium oxalate, potently activate NLRP3 to induce joint inflammation in crystal-induced arthritis such as gout and pseudogout (Busso and Ea, 2012), as well as renal damage in crystalline nephropathy (nephrocalcinosis) (Mulay et al., 2013). Cholesterol crystals can also be sensed by NLRP3, promoting IL-1β-dependent inflammation in the vascular wall, a critical process involved in atherogenesis (Duewell et al., 2010). In the central nervous system, NLRP3 activation in the microglia due to sensing of extracellular insoluble β-amyloid peptide aggregates represents a key mechanism of neuroinflammation involved in the pathogenesis of Alzheimer disease (Heneka et al., 2013). NLRP3 is also activated during tissue injury, mainly by hyaluronan released from damaged ECM (Yamasaki et al., 2009) and by ATP released by dying cells (Iyer et al., 2009; Silverman et al., 2009; Riteau et al., 2010; Davis et al., 2011), fostering the development of sterile inflammation in conditions as various as ischemia (Sandanger et al., 2013), cancer (Zitvogel et al., 2012), circulatory shock (Xu et al., 2013), and toxic organ injury, e.g., acetaminophen-induced hepatotoxicity (Imaeda et al., 2009). Recent findings also indicated that

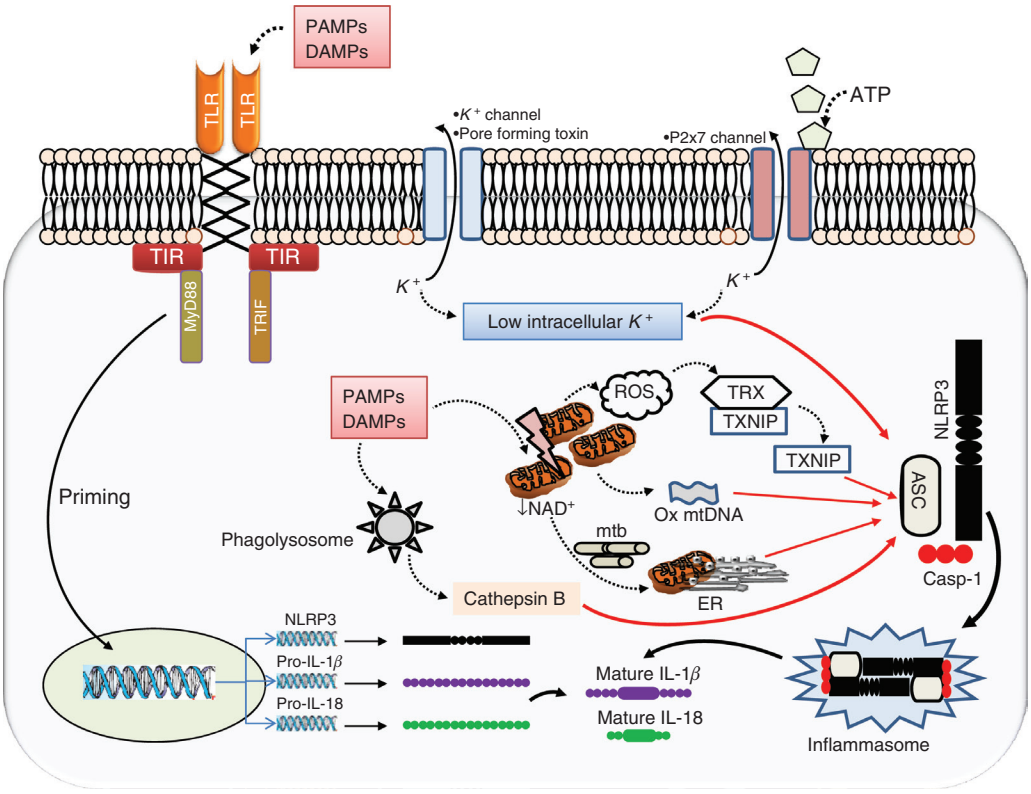

Figure 6 Role of oxidative stress on the process of NLRP3 activation.
The activation of TLRs by pathogen- or damage-associated molecular patterns (PAMPs, DAMPs) results in an increased expression of NLRP3, pro-IL-1β, and pro-IL-18 (priming stage). Activation of NLRP3 occurs through three distinct mechanisms: (A) K^+ efflux in response to the opening of K^+ channels, the presence of bacterial pore-forming toxins, or the activation of the purinergic receptor channel P2X7 by extracellular adenosine. (B) The release of cathepsin B by activated phagolysosomes. (C) Mitochondrial damage and ROS production. ROS can either separate thioredoxin-interacting protein (TXNIP) from its inhibitor thioredoxin (TRX), and TXNIP directly activates NLRP3. Damaged mitochondria also release oxidized DNA (Ox mtDNA) that activates NLRP3, or may be transported by microtubules (mtb) to the ER due to NAD^+ shortage, bringing in close proximity NLRP3 with the adaptor ASC (apoptosis associated speck-like containing a CARD domain). Ultimately, activated NLRP3 associates with ASC and caspase-1 in a multiprotein platform termed the inflammasome, which converts pro-IL-1β and pro-IL-18 into mature cytokines.

NLRP3 contributes to a large extent to the development of tissue fibrosis during chronic inflammatory processes (Xu et al., 2012; Negash et al., 2013), at least in part by augmenting TGF-β signaling (Wang et al., 2013). Furthermore, inflammasome activation and IL-1β production also play an important role in the development and perpetuation of inflammation during metabolic syndrome, insulin resistance, and type 2 diabetes (De Nardo and Latz, 2011; Wen et al., 2012). Here, high glucose concentration, lipids (palmitate, ceramide), and islet-derived amyloid polypeptide represent the main triggers for NLRP3 activation (Menu and Vince, 2011; Zambetti et al., 2012).

Activation of NLRP3: the role of oxidative stress

The large number of known activators of NLRP3 makes it highly unlikely that NLRP3 directly senses all these different triggers. Instead, it is now generally acknowledged that NLRP3 activation occurs mainly as a consequence of a common form of cellular stress elicited by the different stimuli (Lamkanfi and Dixit, 2012). Three main putative mechanisms are currently considered for such activation (Figure 6). The first one, termed the channel model, is related to K^+ efflux from the cell (Tschopp and Schroder, 2010). In this model, K^+ efflux is consecutive to the activation of the purinergic $P2X_7$ receptor by extracellular ATP and the subsequent formation of pores by the recruitment of pannexin-1 (Pelegrin and Surprenant, 2006), or is elicited by microbial pore-forming toxins such as nigericin and hemolysin (Lamkanfi and Dixit, 2012). The second one, termed the lysosome rupture model (Tschopp and Schroder, 2010), depends on the destabilization and the rupture of the phagolysosome compartment, especially following the digestion of particulate material, and which results in the release of the lysosomal protein cathepsin B as an activator of NLRP3 (Hornung et al., 2008). Recent

data have indicated that a similar mechanism triggers NLRP3 activation in myeloid cells exposed to chemotherapeutic agents such as gemcitabine and 5-fluorouracil (Bruchard et al., 2013).

The third model, termed the ROS model (Tschopp and Schroder, 2010), implicates the formation of ROS, deregulation of cellular redox status, and mitochondrial stress as key mechanisms in the process of NLRP3 activation (Rubartelli et al., 2011; Zhou et al., 2011; Rubartelli, 2012). Two main arguments support this model. First, it is particularly noteworthy that all NLRP3 activators are capable of inducing intracellular ROS generation, and second, treatment with various ROS scavengers can block NLRP3 activation by multiple agonists (Tschopp and Schroder, 2010). This later argument remains, however, controversial, owing to the fact that ROS inhibitors also inhibit the upregulation of NLRP3 and pro-IL-1β (mechanism of 'priming'), which may occur independently from the mechanism of NLRP3 activation (Bauernfeind et al., 2011). A possible molecular link connecting oxidative stress with NLRP3 activation has been proposed by Zhou et al. (2010) to be a protein termed TXNIP (thioredoxin-interacting protein). TXNIP is a member of the α-arrestin protein superfamily, which binds to the antioxidant protein thioredoxin (TRX), acting as a negative regulator of the TRX reductase activity (Yoshioka et al., 2012). In their study, Zhou et al. (2010) first showed that TXNIP has the ability to bind to NLRP3 in human embryonic kidney T cells. They then showed that various NLRP3 agonists, including monosodium urate (MSU) and the imidazoquinoline imiquimod (R-837), promoted the association of NLRP3 with TXNIP, followed by inflammasome assembly and secretion of IL-1β by THP-1 cells. The interaction between TXNIP and NLRP3 was dependent on the secondary generation of ROS induced by MSU and R-837, which prompted the dissociation of TXNIP from TRX and its subsequent binding to NLRP3 (Zhou et al., 2010). This role of TXNIP has been debated, as it could not be reproduced in a further study by Masters et al. (2010), who used islet amyloid polypeptide and ATP as NLRP3 activators in mouse bone marrow-derived macrophages (BMDMs). When comparing IL-1β secretion activation by BMDMs from TXNIP- or NLRP3-deficient mice, they found a decrease IL-1β in *Nlrp3*$^{-/-}$, but not in *TXNIP*$^{-/-}$ cells, ruling out a significant role of TXNIP, at least in their experimental model (Masters et al., 2010).

Verification of the hypothesis that ROS act as secondary mediators responsible for NLRP3 activation requires the identification of the source of ROS responsible for such role. In a report by Dostert et al. (2008), stimulation of THP-1 cells with asbestos, MSU, and ATP resulted in a ROS-dependent activation of NLRP3 and IL-1β production, which was inhibited by NOX inhibitors and by knock down of its p22^{phox} subunit. The possible role of NOX-derived ROS in NLRP3 activation has, however, not been uniformly verified. Thus, in a study using macrophages obtained from mice lacking gp91^{phox} (NOX2), IL-1β response to multiple NLRP3 agonists was not modified (Hornung et al., 2008). Furthermore, a study by van Bruggen et al. (2010) reported that primary peripheral blood mononuclear cells obtained from patients with chronic granulomatous disease, who lack the expression of p22^{phox} and thus disclose impaired NOX activity, were able to secrete normal amounts of IL-1β.

Recent observations have implicated mitochondria as the primary source of ROS ultimately responsible for the activation of NLRP3 (Zhou et al., 2011; Martinon, 2012). Many activators of NLRP3 have been shown to disrupt the inner mitochondrial membrane potential, possibly as a consequence of cellular potassium efflux and resulting alterations of mitochondrial matrix volume (Martinon, 2012). The ensuing mitochondrial dysfunction promotes the accelerated generation of ROS from the electron transport chain, leading to the oxidation of mitochondrial DNA (mtDNA) and its release into the cytosol (Figure 6), where it binds to NLRP3 to trigger its activation, as recently demonstrated by three independent groups (Nakahira et al., 2011; Zhou et al., 2011; Shimada et al., 2012). Oxidized mtDNA is thus emerging as a key 'secondary' danger molecule linking many forms of cellular stress to inflammasome activation (Martinon, 2012). The crucial role of mitochondria in this scheme of event has been further reinforced by a recent report by Misawa et al. (2013), who showed that inducers of NLRP3 promoted the relocalization of mitochondria near the ER, allowing the apposition of the adaptor protein ASC (on mitochondria) to NLRP3 (on the ER) with consecutive assembly of the inflammasome. These processes were driven by a series of integrated signals involving a decrease of mitochondrial NAD^+, followed by the accumulation of acetylated microtubules with subsequent, dynein-dependent, spatial rearrangement of mitochondria (Misawa et al., 2013).

The latest developments in the molecular biology of NLRP3 provide essential information to understand the mechanisms governing the initiation and perpetuation of inflammation in many pathological conditions. Mitochondria are vulnerable targets of multiple cellular stressors, and as such are particularly well positioned to perceive and signal the presence of noxious stimuli. These stimuli influence normal mitochondrial metabolism, resulting in a decrease of cellular NAD^+, the production of ROS, and the release of oxidized mtDNA. Whereas such mechanisms have long been recognized as the key triggers of cell

death (Yu et al., 2012), they also emerge as fundamental players in the activation of inflammatory pathways by fostering the activation of NLRP3. Therefore, beyond their role in governing cell death processes, mitochondria also represent master regulators of innate immune processes (Arnoult et al., 2011; Galluzzi et al., 2012), which explains the frequent association of mitochondrial damage with acute and chronic inflammatory diseases.

At variance with the oxidative stress model of NLRP3 activation, it must be mentioned that a limited number of observations have suggested that ROS could instead inhibit the inflammasome, through a mechanism involving the oxidation and glutathionylation of critical cysteine residues (Cys397 and Cys362) within caspase-1 (Meissner et al., 2008). These results raise the possibility that NLRP3, instead of being purely responsive to oxidative stress, might function, at least in some cell types, as a sensor of the global redox status of the cell, governed by a highly dynamic balance between oxidant production and the redox buffering capacities, as well developed by Rubartelli in two recent articles (Rubartelli et al., 2011; Rubartelli, 2012).

Oxidative stress and pro-inflammatory signaling pathways: the role of NF-κB

As presented in the previous sections, the inflammatory response mounted upon sensing of danger signals involves both inflammasome- and TLR-dependent processes. Responses triggered by TLRs are conveyed primarily by the activation of the transcription factor NF-κB, and, to a lesser extent, by MAPKs and other transcription factors, AP-1 and IRFs. NF-κB is heavily redox sensitive, and as such has a strategic position at the crossroad between oxidative stress and inflammation, which will be discussed here.

Molecular biology of NF-κB

NF-κB is a master regulator of inflammation, controlling the expression of hundreds of genes implicated in innate immune responses, and is also a key transcription factor regulating many antiapoptotic genes. It encompasses a family of dimeric proteins, which comprise NF-κB1 (p50 and its precursor p105), NF-κB2 (p52 and its precursor p100), p65 (RelA), RelB, and c-Rel. These proteins bear a conserved domain of ~300 amino acids termed the Rel homology domain, responsible for dimerization into multiple homodimers or heterodimers with various abilities to activate target genes. The most common dimeric form of NF-κB is the heterodimer p50/p65. Of note, only RelA, RelB, and c-Rel have potent transcriptional activation domains, whereas Rel proteins lacking these domains, e.g., p50 homodimers, function as transcriptional repressors (Bonizzi and Karin, 2004; Napetschnig and Wu, 2013). Under resting conditions, NF-κB is held inactive in the cytoplasm through binding to an inhibitory protein named IκB (IκBα, IκBβ, and IκBε), which masks the nuclear localization sequence of NF-κB subunits (Senftleben and Karin, 2002). Additional IκBs (IκBξ, Bcl-3, and IκBNS) are found in the nucleus, where they bind specifically to p50 homodimers and modulate their activity (Napetschnig and Wu, 2013).

The activation of NF-κB proceeds through two distinct pathways, termed the canonical and non-canonical pathways. The 'canonical' (or classic) pathway, which is responsible for activation of innate immune and antiapoptotic responses, relies on the induced phosphorylation of IκB at two conserved serine residues (S32 and S36 in IκBα), followed by its polyubiquitination and degradation by the 26S proteasome (Figure 7). IκB degradation unmasks the nuclear localization sequence of NF-κB, which can then freely translocate to the nucleus and activate target genes. Phosphorylation of IκB is secondary to the activation of a protein kinase complex, IKK, composed of a heterodimer of two catalytic subunits, IKKα and IKKβ, associated with a dimer of a regulatory subunit, IKKγ, in response to the engagement of TLRs and the IL-1 receptor. The 'non-canonical' (or alternative) pathway, which is essential for the maturation of B cells and secondary lymphoid organ development, is specifically dependent on IKKα. The latter is activated by an upstream kinase termed NIK (NF-κB inducing kinase), consecutively to the engagement of various members of the TNF receptor family. In turn, activated IKKα promotes the phosphorylation of p100 and its processing into mature p52, resulting in its translocation to the nucleus and the induction of p52-dependent target genes (see Karin et al., 2004; Vallabhapurapu and Karin, 2009; DiDonato et al., 2012 for extensive reviews). The last level of NF-κB regulation occurs in the nucleus, and includes chromatin remodeling and p65 serine phosphorylation. Chromatin remodeling, regulated by acetylation-deacetylation processes of lysine residues within histones by histone acetyltransferases and histone deacetylases, is required to allow DNA uncoiling and accessibility of NF-κB. Phosphorylation of p65 represents a final step essential for full transcriptional activity of NF-κB, the most important being Ser276 phosphorylation mediated by protein kinase A (Gloire and Piette, 2009).

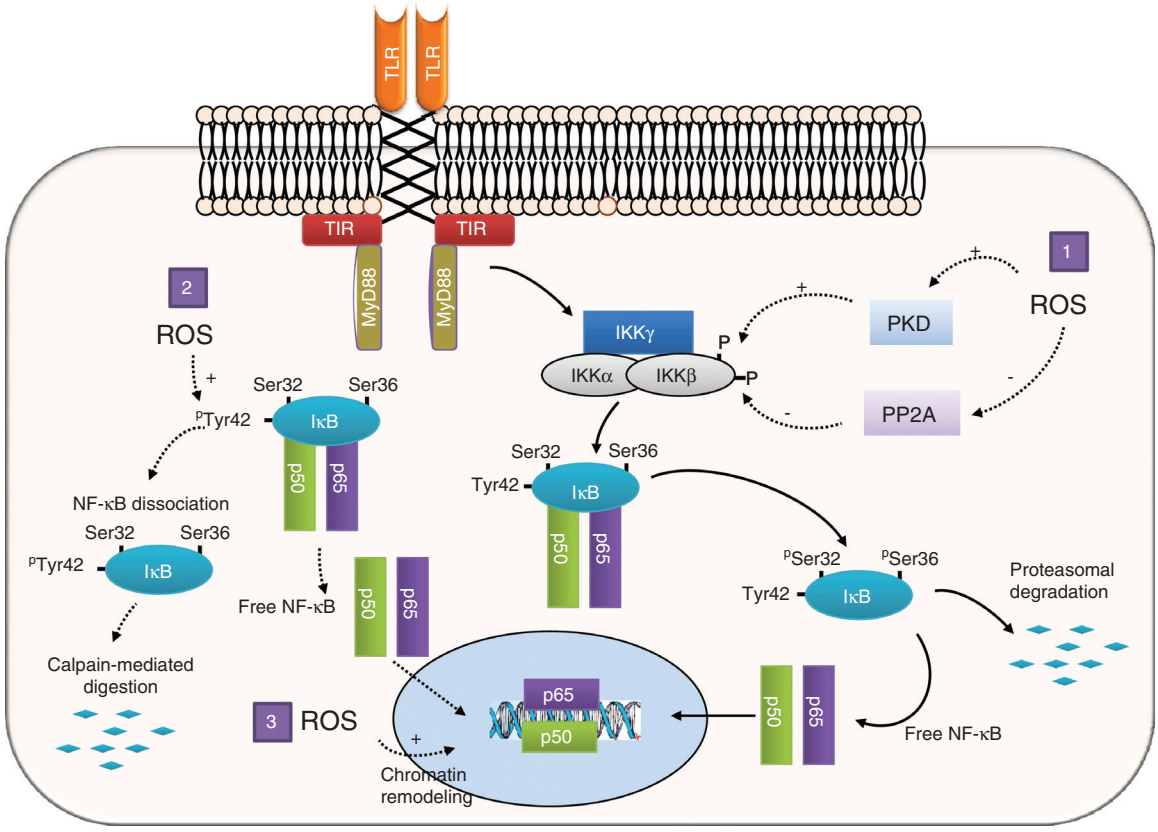

Figure 7 Positive regulation of NF-κB by oxidative stress.
Upon TLR engagement, the IKK complex becomes phosphorylated and activated. Activated IKK in turn phosphorylates the inhibitor IκB on serine 32 and 36, targeting IκB to proteasomal degradation. Free NF-κB dimers (mainly p50/p65 heterodimers) can then freely translocate to the nucleus and activate target genes. ROS promote NF-κB activation at three distinct levels: (1) ROS enhance IKK phosphorylation by activating protein kinase D (PKD) or by inhibiting PP2A, the phosphatase responsible for IKK dephosphorylation. (2) ROS trigger the phosphorylation of tyrosine 42 within IκB, resulting either in its dissociation from the NF-κB dimer or its degradation by calpain-mediated digestion. (3) ROS can initiate various intranuclear changes inducing chromatin remodeling, which foster DNA binding of the NF-κB dimer.

Modulation of NF-κB by oxidative stress

The first evidence of the redox sensitivity of NF-κB was presented by Schreck et al. (1991), who showed that H_2O_2 potently and rapidly activated NF-κB in a human T-cell line, whereas it was inhibited by antioxidants such as *N*-acetylcysteine and other thiol compounds. This concept was further extended by a series of experimental studies reporting on the direct activation of NF-κB by various oxidants and its inhibition by chemical antioxidants or by the upregulation of endogenous antioxidant defenses (reviewed in Li and Karin, 1999). It was then proposed that ROS might represent key secondary mediators responsible for the activation of NF-κB in response to multiple stimuli, including LPS and pro-inflammatory cytokines (Gloire et al., 2006). This notion was, however, soon debated, as it became evident that NF-κB activation by exogenous ROS was highly cell specific (Li and Karin, 1999), and that, in multiple cell lines, endogenously produced ROS did not mediate such activation (Hayakawa et al., 2003). The controversy gained further momentum with the publication of several reports showing that, in some cell types, ROS triggered an inhibition, instead of an activation, of this transcription factor (Zahler et al., 2000; Korn et al., 2001; Torrie et al., 2001). Therefore, the role of ROS in the regulation of NF-κB has evolved over the years from inducer to modulator, as presented in detail in a recent review by Oliveira-Marques et al. (2009).

Redox regulation occurs at multiple levels of the NF-κB pathway, both in the cytoplasm and in the nucleus (Gloire and Piette, 2009). In cells disclosing NF-κB activation in response to ROS, two distinct triggering mechanisms have been identified in the cytoplasm (Figure 7). The first one includes the classic pathway of IκB serine phosphorylation and degradation in response to IKK activation. In this scenario, oxidants induce the upstream activation of protein kinase D, both through tyrosine phosphorylation (at Tyr463) by the tyrosine kinase Abl, and serine

phosphorylation (at Ser738/Ser742) by protein kinase Cδ. In turn, PKD activates IKKβ, resulting in IκB degradation and transcriptional activation of NF-κB (Storz and Toker, 2003; Storz et al., 2004). Besides this PKD-dependent activation of IKK, a mechanism of IKK phosphorylation related to oxidant-dependent inhibition of phosphatases (especially PP2A) has also been postulated (Li et al., 2006; Witt et al., 2009; Loukili et al., 2010).

The second one involves an atypical mechanism implicating tyrosine phosphorylation (Tyr42) on IκB (Figure 7), instead of the classic, IKK-dependent, IκB serine phosphorylation (Schoonbroodt et al., 2000). The mechanism may implicate here various kinases, including the tyrosine kinases Syk (Takada et al., 2003), p56 LCK and ZAP-70 (Livolsi et al., 2001), as well as casein kinase II (Romieu-Mourez et al., 2001). In response to Tyr42 phosphorylation, IκB can either be degraded, by a mechanism independent from the proteasome but dependent on calpain-mediated digestion (Schoonbroodt et al., 2000), or it simply dissociates from NF-κB without degradation in a process implicating the kinases PI3K and c-Src (Beraud et al., 1999; Fan et al., 2003). In either case, the release of NF-κB from its inhibitor allows it to translocate to the nucleus and activate target genes (Gloire et al., 2006; Oliveira-Marques et al., 2009). Besides these cytoplasmic mechanisms, several redox-based processes occurring in the nucleus have also been reported to promote NF-κB activation by favoring chromatin remodeling, such as the reduction of histone deacetylase activity, as well as the induction of lysine acetylation and serine phosphorylation of histone H3 (Yang et al., 2006, 2008b).

At variance with the above-described ROS-dependent NF-κB activation, evidence exists that, in multiple conditions, ROS serve instead as potent inhibitors of NF-κB (Figure 8). Three main underlying mechanisms have been postulated. The first one involves the ROS-dependent prevention of IKK phosphorylation, related to various redox-based modifications of a critical cysteine residue (Cys179) in IKKβ (extensively reviewed in Pantano et al., 2006). The second mechanism involves a direct inhibition of the proteasome by oxidants, resulting

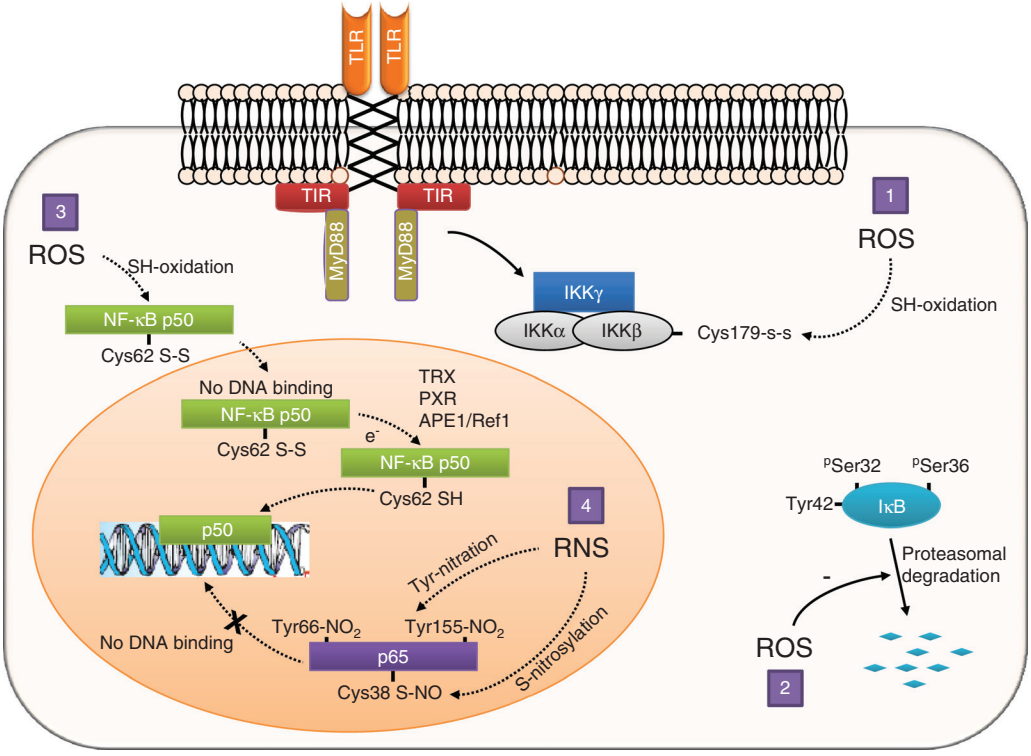

Figure 8 Negative regulation of NF-κB by oxidative stress.
Oxidative stress can downregulate NF-κB signaling by acting at four distinct levels: (1) ROS oxidize cysteine 179 within IKKβ, preventing its phosphorylation and activation. (2) ROS inhibit the 26S proteasome, thereby preventing IκB degradation and release of free NF-κB. (3) Cytoplasmic ROS oxidize cysteine 62 within the p50 subunit of NF-κB. Oxidized p50 cannot bind to DNA. Binding is restored following reduction of oxidized p50 within the nucleus by the concerted actions of three antioxidant enzymes, thioredoxin (TRX), peroxiredoxin (PRX), and the DNA repair enzyme APE1/Ref1. 4. RNS can modify p65 by *S*-nitrosylation of cysteine 38, or by nitration of tyrosine 66 and tyrosine 155, preventing p65-DNA binding and favoring p65 nuclear export.

in the prevention of IκB degradation, as notably demonstrated in airway epithelial cells (Jaspers et al., 2001) and polymorphonuclear cells (Zmijewski et al., 2007). The third mechanism involves direct redox modifications of NF-κB subunits affecting either the p50 or the p65 subunit. The p50 subunit possesses a highly redox active cysteine within its Rel homology domain (Cys62), which is oxidized within the cytoplasm in conditions of oxidative stress. Upon translocation to the nucleus, oxidized Cys62 impairs DNA binding, thereby preventing NF-κB to activate target genes (Kabe et al., 2005). DNA binding is restored through the reduction of Cys62 within the nucleus, following the concerted activity of several antioxidant enzymes, including thioredoxin (Hirota et al., 1999), peroxiredoxin-1 (Hansen et al., 2007), and the DNA repair enzyme APE1/Ref-1 (Nishi et al., 2002). The compartmentalization of Cys62 oxidation/reduction illustrates the important aspect of spatial specificity in the process of NF-κB redox regulation, as brilliantly reviewed by Gloire and Piette (2009) in a recent article. A further redox modification of Cys62 in p50 is NO-mediated *S*-nitrosylation, which prevents DNA binding of NF-κB (Sha and Marshall, 2012). Besides p50, p65 may also be affected by two distinct redox modifications, the first one being *S*-nitrosylation of Cys38 (Sha and Marshall, 2012) and the second one being nitration of two tyrosine residues (Tyr66/Tyr152) by peroxynitrite (Park et al., 2005). Both modifications inhibit NF-κB transcriptional activity, by inducing its unbinding from DNA and its nuclear export (Gloire and Piette, 2009).

In summary, ROS and RNS definitely play a central role in the regulation of NF-κB, although this role remains incompletely understood, in view of the contrasted outcome (activation vs. inhibition) reported. The underlying reasons remain thus far unclear, but it is likely that the particular experimental conditions explain, at least partly, these contrasted results: these include notably the specific cell type studied, the particular ROS or RNS studied, the concentration of the oxidants used, as well as the concomitant stimulation of cells with NF-κB activators such as cytokines or LPS together with oxidants (Gloire and Piette, 2009; Oliveira-Marques et al., 2009; Loukili et al., 2010). It is also probable that the redox regulation of NF-κB may dynamically evolve over time and that distinct mechanisms are set in motion at different stages of the inflammatory response. One possibility might be that ROS could promote NF-κB responses in the early phases of the process, while they would instead inhibit these responses at later stages, thereby favoring the induction of tissue repair. In support of such hypothesis, it has been proposed that the modifications introduced by NO and peroxynitrite on p50 and p65 (as described above) could represent important negative feedback mechanisms downregulating inflammation under conditions of elevated NO production mediated by the inducible isoform of NO synthase (Gloire and Piette, 2009).

The NF-κB signaling pathway has been by far the most widely studied in terms of redox regulation. It is, however, worth to mention here that additional signal transduction cascades linked with inflammation also disclose redox-sensitive characteristics. These include primarily proteins from the MAPK family (ERK, JNK, and p38) and the transcription factor AP-1, which have been the matter of several recent reviews to which we refer the interested reader for further information (McCubrey et al., 2006; Liaudet et al., 2009; Brigelius-Flohe and Flohe, 2011; Ray et al., 2012).

Conclusions, future perspectives

Since its early clinical description by Celsus about 2000 years ago (*rubor*, *tumor*, *calor*, *et dolor*: redness, swelling, heat, and pain), inflammation had been essentially considered an internal response to an external stressor, primarily infection and injury. Recent discoveries in the field of innate immune response have dramatically changed such vision, and inflammation is now recognized as an adaptive response to any form of danger arising both from the outside and from the inside. At various degrees, inflammation is therefore present in virtually every acute or chronic human malady, and a precise understanding of its pathophysiological mechanisms might, *de facto*, help devise novel therapeutics for these diseases. Of particular relevance, these same conditions are also typically associated with significant alterations of redox balance, related to the upregulated production of multiple oxygen- and nitrogen-centered reactive chemical species, promoting the development of oxidative stress. Evidence accumulated over the past two decades has pointed to significant connections between inflammation and oxidative stress, both processes contributing to fuel the other one, thereby establishing a vicious cycle able to perpetuate and propagate the inflammatory response. Still, only the tip of the iceberg regarding this complex crosstalk has been thus far illuminated, and many questions remain unanswered. For instance, the precise sources and the particular form of redox species involved in a given situation, the spatial and temporal resolution relevant to specific molecular interactions, the influence of the peculiar chemical environment on redox-based biological reactions, or the role of oxidants

pertaining to anti-inflammatory processes represent a few issues that deserve further in-depth exploration. Unraveling these various aspects of the inflammatory-oxidative connection will undoubtedly foster the development of efficient strategies to treat a wide range of debilitating human illnesses.

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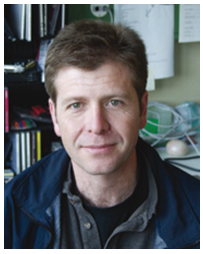

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