Free Radical Biology and Medicine xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Review article

The dual role of ROS in autoimmune and inflammatory diseases: Evidence from preclinical models

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ARTICLE INFO

Keywords:
Reactive oxygen species
NADPH oxidase 2
Autoimmunity
Inflammation
Neutrophil extracellular traps
T cells
Oxidative stress
Glutathione
Dendritic cells
Immune Synapse

ABSTRACT

Reactive oxygen species (ROS) are created in cells during oxidative phosphorylation by the respiratory chain in the mitochondria or by the family of NADPH oxidase (NOX) complexes. The first discovered and most studied of these complexes, NOX2, mediates the oxidative burst in phagocytes. ROS generated by NOX2 are dreadful weapons: while being essential to kill ingested pathogens they can also cause degenerative changes on tissue if production and release are not balanced by sufficient detoxification. In the last fifteen years evidence has been accumulating that ROS are also integral signaling molecules and are important for regulating autoimmunity and immune-mediated inflammatory diseases. It seems that an accurate redox balance is necessary to sustain an immune state that both prevents the development of overt autoimmunity (the bright side of ROS) and minimizes collateral tissue damage (the dark side of ROS). Herein, we review studies from rodent models of arthritis, lupus, and neurodegenerative diseases that show that low NOX2-derived ROS production is linked to disease and elaborate on the underlying cellular and molecular mechanisms and the translation of these results to disease in humans.

1. The dual role of ROS in autoimmune and autoinflammatory diseases

Molecular oxygen is the driving force behind oxidative phosphorylation (OXPHOS), the process by which mitochondria use multi-subunit enzyme complexes to oxidize nutrients and use the released energy for the formation of adenosine triphosphate (ATP). The fact that this absolutely vital function is inevitably connected to the creation of highly reactive and toxic side products, reactive oxygen species (ROS), has been coined the molecular oxygen paradox [1]. However, it has become evident that ROS are not mere by-products but can also function as secondary messengers. Thus, mitochondrial ROS are not just the price we pay for breathing, but essential to regulate mitochondrial function together with changes in physiology.

ROS are not only produced during oxidative phosphorylation, but also, and in even higher amounts by NADPH oxidase complexes (NOX). The best described and most potent of these complexes is NOX2, which is predominantly expressed in phagocytic cells such as neutrophils, monocytes and macrophages, or dendritic cells (DC) and is the dominant ROS-generating complex in mammals [2]. ROS-production via NOX2 is regulated by the cytosolic adaptors p47phox (also known as Neutrophil cytosolic factor 1, Ncf1) and p40phox (Ncf4) that bridge interactions of NOX activators with the enzymatic flavocytochrome components embedded in phagosomal and plasma membranes. NOX2-dependent ROS production is found both extracellularly and intracellularly.

While ROS production by phagocytes during the oxidative burst is essential to kill off pathogens, phagocyte-derived ROS has in the traditional view also been connected with promotion of inflammation and tissue damage. However, in the last decade it has also been implicated in regulation of inflammation and protection from autoimmunity. Evidence for the latter comes from association of ROS-deficiency with severe chronic inflammation in animal models and human patients in an ever growing number of pathologic conditions [3–13].

In this review we will discuss the evidence for pro-inflammatory and regulatory roles of ROS that has been emerging from animal models during the last years. We will elaborate on the underlying cellular and molecular mechanisms, focusing on the roles of innate immune cells, and on the relevance of these mechanisms for auto-inflammatory and auto-immune diseases in humans.

2. ROS requirement for bactericidal and anti-fungal activity

One of the hallmarks associated with the antimicrobial and

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https://doi.org/10.1016/j.freeradbiomed.2018.03.016
Received 7 December 2017; Received in revised form 5 March 2018; Accepted 11 March 2018

inflammatory actions of phagocytes is the activation of a powerful oxidative burst, during which large amounts of oxygen are consumed and converted to the primary radical superoxide (O_2^-), which then dismutates to build the membrane-permeable hydrogen peroxide. Via the activity of myeloperoxidase (MPO) that is mainly expressed in neutrophils a variety of other reactive products are generated. These include non-radical oxidants, the main ones being hypochlorous and hypothiocyanous acid, as well as radicals formed from organic and inorganic substrates [14]. All these products together are commonly referred to as ROS.

There is an associated acidification of the phagosomal compartment by H^+ produced by NOX2. The mechanisms that control phagosomal pH differ between phagocytes: in macrophages, rapid acidification has been reported whereas the pH remains neutral for several minutes in neutrophils. This is achieved by activation of the Hv1 voltage channel [15]. In DC, the phagosomes may become alkaline and this may promote antigen cross-presentation. In contrast to intracellular antigens produced by viruses which associate with cytosolic major histocompatibility complex (MHC) I, extracellular antigens that are phagocytosed are processed in endosomes, then associate with MHCII after cathepsin cleavage and traffic to the membrane. ROS production may also inhibit cathepsin activity and so increase the likelihood of cross-presentation.

During the oxidative burst NOX2 is strongly activated by phagocytosis of particulate matter, but also by soluble stimulants such as phorbol-12-myristate-13-acetate (PMA) or the bacterial peptide fMLP. Different stimuli induce different localization and timing of the ROS production: whereas upon phagocytosis NOX2 activity is comparable short and mainly occurs at the phagosomal membrane, thus directing ROS into the phagosomal space onto the ingested particle, the soluble agonists induce prolonged NOX2 activity and ROS release mostly on the plasma membrane, directing superoxide into the pericellular space [16–18].

Although the production of O_2^- is localised and efficient, the high local ROS concentration can cause rapid inactivation of enzymes e.g., NOX2 itself via adducts formed with the lipid peroxidation product 4-hydroxynon-2-enal (HNE) [19]. To maintain ROS production in DCs after phagosomal uptake of antigen, cytochrome b558 is trafficked via Soluble NSF Attachment Receptor (SNARE) 23 from lysosomes to enable ROS production to continue [20]. This fail-safe mechanism will enable bacteria to be killed efficiently through sufficient local ROS but also maybe important for regulation of autoimmunity [21]; in the absence of Ncf4 to traffic NOX2 to endosomes, autoimmune responses are promoted without effect on bacterial killing. Increased NOX2 activity leads to an increase in lipid peroxidation and disruption of intracellular membrane integrity with leakage of antigens into the cytosol for association with MHC class I [22]. Similarly, cross-presentation of the intracellular pathogen *Salmonella typhimurum* via MHCI is promoted by the presence of ROS [23]. When ROS production is impaired, e.g. in chronic granulomatous disease (CGD), there is an inefficient leakage of antigens from endosomes for cross-presentation to CD8 T cells. Taking these lines of evidence together, it is clear that efficient ROS production is essential for bacterial killing using the innate arm of the immune system and also for the start of the pathway to activation of an adaptive immune response involving antigen presentation via MHC class I to $CD8^+$ cells [24].

ROS contribute to the maturation of DC and the processing and presentation of antigens by DC. However, there is conflicting evidence in support and against a role for NOX2-derived ROS. On the one hand, it has been reported that on receiving an inflammatory stimulus, DC mature and a ROS-dependent increase expression of MHC class II antigens is observed [25]. However, functional analysis of the role of NOX2 in human myeloid DC has shown that NOX2-dependent O_2^- production does not play a role in DC differentiation, maturation, cytokine production, despite being essential for intracellular bacterial killing [26]. Similarly, in a recent study using $gp91^{-/-}$ mice and the cytochrome inhibitor apocynin, NOX2-dependent ROS formation was not required for lipopolysaccharide (LPS)-mediated maturation of bone marrow derived DC [27].

NOX2 activity also has an inhibitory effect on peptide presentation. Nevertheless, ROS-dependent oxidation of cathepsin cysteinyl thiols in the early phagosome prevents excessive protein degradation and so enables controlled antigen presentation by MHCII at a later stage within the endosomal lumen [28]. The reasons for these discrepancies are unclear but may relate to local changes in the redox environment.

Although not required for killing of some microorganisms, ROS production is essential for broad spectrum antimicrobial defense. The strongest evidence for this comes from the persistent bacterial and fungal infections associated granuloma formation in CGD [29], an inherited immunodeficiency in which NOX2 activity is lacking. Typical clinical manifestations of CGD are pneumonia, suppurative adenitis, subcutaneous and/or hepatic abscesses, osteomyelitis, and sepsis [30]. CGD is caused by mutations in one of the genes encoding for the subunits of NOX2 that lead to a defective activation of the complex and therefore cause a strongly diminished ROS production [31]. Most of the susceptibility of CGD patients to microbial infections appears to be due to impaired phagolysosomal killing of pathogens by ROS. Interestingly, hypochlorous acid that requires peroxidation by MPO is the fastest acting and most potent antimicrobial oxidant, while superoxide and hydrogen peroxide have more limited antimicrobial activity (reviewed in [18]). Nevertheless, MPO deficiency is much more common than CGD and only rarely associated with serious infections [32]. It is therefore possible that there are NOX2-dependent backup mechanisms that are sufficient under most circumstances.

One of those might be the formation of neutrophil extracellular traps (NETs), characterized by externalization of chromatin decorated with antimicrobial peptides and proteases [33]. In these NETs, bacteria are trapped and degraded. The canonical process of NET formation in response to microbes is dependent on a functional oxidative burst, because ROS are essential for the release of neutrophil elastase and myeloperoxidase from azurophilic neutrophil granules from where they migrate to the nucleus and mediate degradation of histones [34,35]. The antimicrobial activity of NETs has been confirmed in many studies [36]. However, individuals with Papillon-Lefèvre syndrome (PLS), a rare disease characterized by persistent periodontal disease, are also not able to form NETs but do not exhibit impaired killing of bacteria [37,38]. Thus, the phenotypes associated with CGD and PLS might arise, at least in part, from a dysfunctional regulation of the inflammatory response.

3. The bright side - ROS requirement for resolution of inflammation and regulation of autoimmunity

Interestingly, a functional deficiency of ROS production via NOX2 is associated with dysregulated inflammation and autoimmune diseases in humans and rodents. Genome-wide gene expression analysis of blood of CGD patients and a mouse model of CGD revealed a pronounced type I IFN signature, comparable to the one in patients with systemic lupus erythematosus (SLE) [8]. In line with that, there are reports of co-occurrence of CGD and SLE and other autoimmune manifestations, such as antiphospholipid syndrome, juvenile idiopathic arthritis, IgG nephropathy, and Crohn-like inflammatory bowel disease [39–45].

These findings are bolstered by genetic association of lower or absent NOX2-derived ROS production in humans with developing SLE, Sjögren´s syndrome, and rheumatoid arthritis (RA) [46–48]. For a more detailed review on the implications of genetic deficiencies for disease mechanisms see the review written by Holmdahl *et al.* an in this issue.

The genetic association of a defective NOX2 with human disease is corroborated in a broad spectrum of animal models of inflammation and autoimmunity. In a persistent and painstaking effort, Olofsson and colleagues positionally identified a polymorphism of *Ncf1*, one of the cytosolic subunits of NOX2, that confers diminished production of