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NOX2 Complex–Derived ROS as Immune Regulators

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Abstract

Reactive oxygen species (ROS) are a heterogeneous group of highly reactive molecules that oxidize targets in a biologic system. During steady-state conditions, ROS are constantly produced in the electron-transport chain during cellular respiration and by various constitutively active oxidases. ROS production can also be induced by activation of the phagocyte NADPH oxidase 2 (NOX2) complex in a process generally referred to as an oxidative burst. The induced ROS have long been considered proinflammatory, causing cell and tissue destruction. Recent findings have challenged this inflammatory role of ROS, and today, ROS are also known to regulate immune responses and cell proliferation and to determine T-cell autoreactivity. NOX2-derived ROS have been shown to suppress antigen-dependent T-cell reactivity and remarkably to reduce the severity of experimental arthritis in both rats and mice. In this review, we discuss the role of ROS and the NOX2 complex as suppressors of autoimmunity, inflammation, and arthritis. *Antioxid. Redox Signal.* 15, 2197–2208.

Introduction

Oxidative stress is considered as one of the pathologic arms of the immune system at sites of tissue destruction in inflammatory diseases such as rheumatoid arthritis (RA) (2, 104), atherosclerosis (37), multiple sclerosis (MS) (30, 63), and inflammatory bowel disease (IBD) (84). NADPH oxidase 2 (NOX2) complex–derived ROS are necessary for eradication of invading pathogens, as mutations in any of the genes coding for NOX2 complex proteins can result in chronic granulomatous disease (CGD) (17, 61), characterized by severe and recurrent life-threatening infections. During pathogen killing, ROS are produced into the phagosome to activate proteolytic enzymes (81), and during this process, ROS are believed to "leak out" and cause collateral damage in the surrounding tissue.

Surprisingly, and in contrast to the general idea that ROS promote inflammation, we and others have shown that low ROS production due to impaired phagocyte NOX2 complex function mediates the enhanced severity of autoimmune diseases in rodent models of arthritis and MS (29, 42, 70). In addition, leukocytes from patients with severe MS produce less superoxide than do leukocytes from patients with milder disease (65). Similarly, patients with severe Guillain–Barré syndrome, another inflammatory demyelinating autoimmune disorder, have lower oxygen radical production in peripheral blood leukocytes compared with that in patients with milder disease (66). Defective ROS production by neutrophils has been associated with hyperinflammatory conditions (23), and CGD patients with defective oxidative bursts exhibit a hyperinflammatory immunologic status resembling idiopathic inflammatory diseases (8). These observations support the regulatory role of ROS in autoimmune diseases. In this review, we discuss the inflammation-limiting role of NOX2 complex–derived ROS and mechanisms underlying the protection.

The NOX2 Complex Responsible for Phagocyte ROS Production

The NOX family of NADPH oxidases consists of transmembrane enzymes that oxidize intracellular NADPH/NADH, leading to electron transportation across the membrane and reduction of molecular oxygen into superoxide. The seven members in the NOX family include the NOX complexes 1 through 5 and DUOX 1 and 2. The enzymes are isoforms that share the capability of transporting electrons through biologic membranes and producing superoxide, but differ in their expression pattern and the accessory components needed for the enzymatic activity. For reviews, see (6, 75).

The NADPH oxidase complex with NOX2 ($GP91^{phox}$) as the transmembrane oxidase, the NOX2 complex, expressed mainly in phagocytes, is responsible for the oxidative burst

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required to kill ingested pathogens, and is thus considered to be the main source of ROS in inflamed tissues (53, 80).

The transmembrane catalytic core of the NOX2 complex is flavocytochrome b558, a heterodimer that contains a large glycoprotein GP91^{phox} (alias NOX2/CYBB) and the smaller protein P22^{phox} (alias CYBA) (Fig. 1). The other components of the complex are P47^{phox}(alias NCF1), P67^{phox} (alias NCF2), P40^{phox} (alias NCF4), and RAC, which all are regulatory cytosolic proteins. The NOX2 complex is inactive in unstimulated cells but can be readily activated by various stimuli (69, 107). Not only the transmembrane enzymatic core but also the cytosolic regulatory subunits P47^{phox}, P67^{phox}, and P40^{phox} are essential for NOX2 activation and functionality *in vivo*, as mutations in any of the genes encoding those cytosolic regulatory components of NOX2 complex have been described in CGD patients (17, 61). The small GTPase RAC is also a cytosolic regulatory component of the NOX2 complex and exists in two isoforms; RAC1 predominates in monocytes, and RAC2, in neutrophils (69, 107).

NOX2 was first identified and characterized in phagocytes. Phagocytes express high amounts of *gp91phox, p22phox,* and all the cytosolic regulatory components (*p40phox, p47phox, p67phox, Rac*) of the complex. In addition to phagocytes, NOX2 complex components also have been detected in a variety of nonphagocytic cells, such as cardiomyocytes, endothelial cells, and muscle cells (6). Within the group of phagocytic cells, neutrophils, macrophages, and dendritic cells express relatively high levels of *gp91phox* (6, 96). B cells, mast cells, eosinophils, and natural killer (NK) cells also are reported to express NOX2 complex components, but at a lower level (6, 96, 106). In addition, T-cell expression of NOX2 complex proteins has been reported (43), but is considered to be very low (28, 82, 102). Interestingly, all constitutively MHC class II (MHC II)-expressing antigen-presenting cells (dendritic cells, monocytes/macrophages, and B cells) express the NOX2 complex and thus produce ROS on activation.

A wide variety of tissues are reported to contain cells expressing NOX2 complex components. In blood, neutrophils, monocytes, and B cells are cell populations that are capable of generating significant amounts of ROS by the NOX2 complex (6). Spleen and lymph node cells have a relatively high content of NOX2 complex–expressing cells (6). These secondary immunologic organs have an important role in antigen presentation and contain mature phagocytic antigen-presenting cells. GP91^{phox} is also expressed in bone marrow (103) and in the thymus (14), indicating a possible role for NOX2-derived ROS in B- and T-cell development, maturation, and education. At a tissue level, the expression profile of P47^{phox}, one of the essential components of the NOX2 complex, resembles that of the enzymatic core membrane components, with expression in both central and peripheral immune organs (Fig. 2).

NOX2 Activation

The NOX2 complex exists in three different activation states: resting, primed, and activated. Tight control of the complex activation efficiently prevents inadvertent superoxide production and, together with potent and abundant antioxidant systems, efficiently protects cellular structures against oxidative damage (22).

In resting cells, the regulatory subunits reside in the cytosol. P67^{phox}, P40^{phox}, and P47^{phox} are associated together (54);

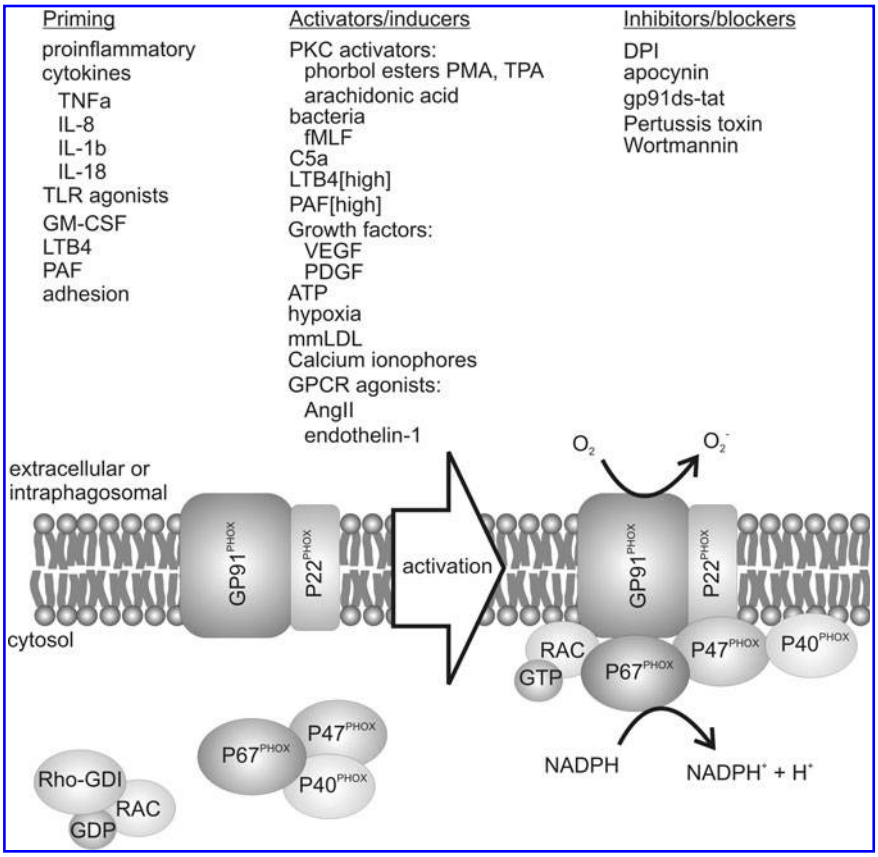

FIG. 1. The phagocyte NADPH oxidase complex generates superoxide (O_2^-) into the phagosome or into the extracellular space. The transmembrane catalytic core of the complex consists of glycoprotein GP91^{phox} (alias NOX2) and P22^{phox}. The cytosolic components regulating the activity of the enzyme are P40^{phox} (alias NCF4), P47^{phox} (NCF1), P67^{phox} (NCF2), and GTP-bound RAC. Agents that have been reported to prime or activate NOX2 complex to produce ROS are shown, in addition to agents that inhibit ROS production by NOX2. DPI, diphenylene iodonium; fMLF, formyl-methionyl-leucyl-phenylalanine; GM-CSF, granulocyte-macrophage colony-stimulating factor; GPCR, G protein–coupled receptor; LTB4, leukotriene B4; mmLDL, minimally oxidized LDL; PAF, platelet-activating factor; PDGF, platelet-derived growth factor; PKC, protein kinase C; PMA, phorbol-12-myristate-13-acetate; TPA, 4ß-12-*O*-tetradecanoylphorbol-13-acetate; VEGF, vascular endothelial factor.

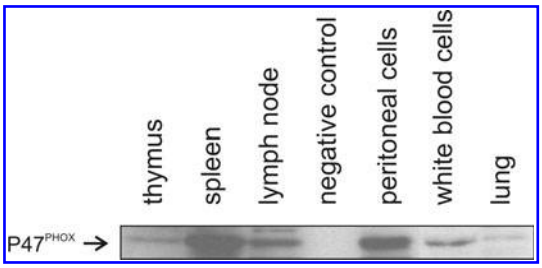

FIG. 2. P47^{phox} (NCF1) protein expression in selected tissues in B10.Q mice.

GDP-bound RAC is in complex with its inhibitor GDI, but does not interact with the other cytosolic components. In the resting state, only a minority of GP91^{phox}/P22^{phox} heterodimers are believed to reside in the plasma membrane (22).

Cell activation can prime the NOX2 complex and prepare it for ROS production. The priming stimuli do not initiate the oxidative burst by themselves but can initiate only a very weak oxidative response. Priming of the NOX2 complex [reviewed in (22)] can be induced by different stimuli, including proinflammatory cytokines (*e.g.*, TNF-α, IL-1*β*), TLR agonists (*e.g.*, LPS, lipoarabinomannans flagellin), peroxynitrite, and proteases (see Fig. 1). The neutrophil adhesion to the endothelium and extracellular matrix during migration can prime the NOX2 complex. Priming of the NOX2 complex is characterized by changes in the subcellular localization or conformation or both of GP91^{phox}/P22^{pho}, and P47^{phox}. During priming, P47^{phox} is partially phosphorylated, causing relaxation of its autoinhibitory conformation. Primed P47^{phox} translocates to the plasma membrane to interact with the enzymatic core proteins (GP91^{phox} and P22^{phox}). It has been proposed that Ser^{345} phosphorylation on P47^{phox} is a critical event in the priming of ROS production by neutrophils (9, 16, 22).

Primed NOX2 complex requires additional activation to initiate substantial ROS production. PMA, a potent PKC activator, and fMLF, an *N*-formylated peptide that acts via the formylpeptide receptor, are the best-known NOX2 complex activators that can induce oxidative burst with or without preceding priming. Some of the activators act as priming agents at low concentrations but can induce ROS production at higher concentrations.

Activation of the NOX2 complex is characterized by protein phosphorylation and translocation. Cell activation induces the recruitment of the GP91^{phox}/P22^{phox} complex from granule membranes to the lipid rafts of the plasma membrane (22, 34, 93), which can then form endosomes and phagosomes. After priming, cell activation further increases the phosphorylation of the cytosolic subunits and induces translocation of P67^{phox}, P40^{phox}, and RAC to interact with membrane-associated P47^{phox} and the transmembrane enzymatic core, thus initiating superoxide production (22).

NADPH oxidase–driven superoxide production is known to be inhibited by the plant phenol apocynin (also known as acetovanillone) (94), *Aspergillus fumigatus* toxin (95), and neopterin, a catabolic product of GTP (51). Superoxide production by NADPH oxidases is also inhibited by agents that inhibit the signaling cascades involved in enzyme activation. These include protein kinase C inhibitors, PI3K inhibitors (*e.g.*, wortmannin) and G protein–coupled receptor inhibitors (*e.g.*, Pertussis toxin) (57), and formyl peptide receptor antagonists. Immunosuppressant drugs (*e.g.*, cyclosporin and mycophenolic acid) (56) downregulate NADPH oxidase activity in addition to their overall immune suppressant function. Flavoenzyme inhibitor diphenylene iodonium (DPI) is a widely used NOX2 complex inhibitor, but even more specific pharmacologic inhibitors such as gp91ds-tat (44, 83) have been engineered to block NOX2 complex activation. Research aiming at dissecting the role of the specific NOX isoforms and other ROS-generating enzymes has largely been based on the use of enzyme inhibitors. The lack of inhibitor specificity has made it difficult to investigate the source of ROS or to assess the role of specific NOX complexes (44, 52). This problem was recently illustrated by van Bruggen and co-workers (99) when they reported how the use of DPI and shRNA introduced technical artifacts that radically compromised the inflammatory response, thus misleadingly implying that NOX enzymes activate the inflammasome (99).

Introduction to ROS

ROS are a heterogeneous group of oxygen radicals and other strongly oxidizing molecules (Fig. 3). After generation, ROS are further converted into other oxidative species or neutralized by enzymatic and nonenzymatic reactions inside and outside of the cell [reviewed in (105)].

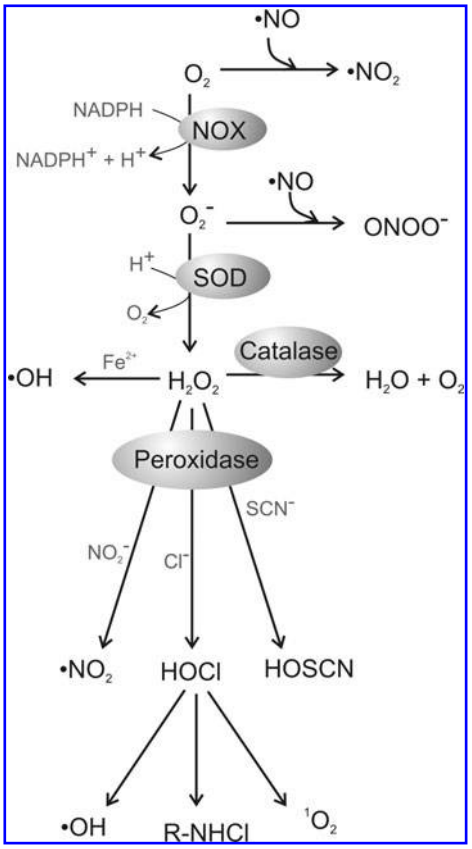

FIG. 3. Reactive oxygen species and their reactions. (HOCl, hypochlorous acid; HOSCN, hypothiocyanous acid; •NO, nitric oxide; •NO_2 nitrogen dioxide; $^1O_2$, singlet oxygen; •OH, hydroxyl radical; $ONOO^-$, peroxynitrite; R-NHCl, chloramines). Figure modified from (80, 105).

NOX2 catalyzes a reaction in which molecular oxygen is converted into superoxide anion (O_2^-), which can either spontaneously or by the action of one of the three superoxide dismutases (SODs) be further converted into hydrogen peroxide (H_2O_2). Additionally, superoxide can react with nitric oxide to form peroxynitrite ($ONOO^-$), converging reactive nitrogen and oxygen species metabolism. Superoxide is highly reactive and cannot cross the cell membrane, whereas hydrogen peroxide is more long lived, and its diffusion is not believed to be hindered by lipid bilayers. In the Fenton reaction (catalyzed by Fe^{2+}), highly reactive hydroxyl radical ($HO^\bullet$) is formed from hydrogen peroxide, and hydrogen peroxide can be neutralized into water and molecular oxygen by catalase. In addition, hydrogen peroxide can be metabolized by various peroxidases. Hypochlorous acid (HOCl) is another extremely reactive ROS that is formed when hydrogen peroxide is oxidized by myeloperoxidase. Hypochlorous acid together with hypobromous acid (HOBr) and hypothiocyanous acid (HOSCN) are involved in antimicrobial defense by neutrophils (50, 105).

Most reagents that are used to measure ROS are not specific for a particular oxygen radical but rather react with a set of radicals (105). Lack of specificity of the dyes has led to development of methods that use different ROS scavengers, enzyme inhibitors, and antioxidant enzymes to differentiate between different radical species (67, 105). Unfortunately, by means of today's technology, it is still not possible to dissect the roles of specific radicals in *in vivo* systems, which would be critical for understanding their qualitative and quantitative role in different tissue compartments.

From antioxidants to prooxidants

ROS are constantly produced and consumed as a part of normal physiology, whereas oxidative stress arises when ROS production and ROS removal by antioxidant systems is in disequilibrium. Antioxidants prevent or slow the oxidation of other molecules by being oxidized themselves or by catalyzing enzymatic conversion of the oxidant, thus eliminating ROS. As a result, antioxidants both regulate ROS-dependent signaling and give protection against excess oxidant load. The most important physiologic enzymatic antioxidant systems include, in addition to the previously mentioned SOD enzymes and catalase, the thioredoxin, glutathione peroxidase, and peroxiredoxin systems. Dietary tocopherols (vitamin E) and ascorbic acid (vitamin C) are low-molecular-mass antioxidants that can neutralize ROS by directly reducing them. Melatonin can also reduce ROS, but unlike tocopherols, ascorbic acid, and glutathione, it does not participate in redox cycling (*i.e.*, once it is oxidized, it cannot be reduced back to its original state). Oxidative stress is suggested to increase inflammation and damage in chronic inflammatory diseases, such as rheumatoid arthritis (11, 20, 21, 72, 92), but some studies have failed to show significant association (38).

Even though evidence exists for a proinflammatory role of ROS during oxidative stress, little evidence supports the antiinflammatory role of antioxidants. Antioxidants are suggested to ameliorate inflammation, but experimental treatment of autoimmune joint inflammation with antioxidants has resulted in inconsistent results in both human and rodent setups [reviewed in (26)]. Even if epidemiologic evidence supports the protective role of dietary antioxidants in autoimmune arthritis (73), interventional studies of antioxidant supplementation have not proven successful in ameliorating rheumatoid arthritis (5, 46); [for summary, see (10, 73)]. In similarity to arthritis, little evidence exists of the efficacy of antioxidant treatment in multiple sclerosis (63). Additionally, antioxidant vitamins have failed to show a benefit in atherosclerosis (97) [reviewed in (37)]. Antioxidant treatment has even been reported to prolong inflammation when administered during the acute phase of kidney inflammation (47), and a study on vitamin E supplementation has shown damaging effects in patients with cardiovascular disease (18).

Prooxidants shift the redox balance toward more oxidized either by increasing ROS production or by inhibiting antioxidant systems. Phytol is a prooxidant that increases superoxide production by activating the NOX2 complex, and it has been shown to prevent and even ameliorate ongoing inflammation in animal models of arthritis (41). Interestingly, phytol is a precursor to the antioxidant vitamin E.

It is important to remember that ROS are important regulators of biologic functions under normal conditions (6), and not all ROS production leads to oxidative stress. Experimental work with prooxidants (41) challenges the widespread idea of the harmfulness of ROS and suggests that increased oxidation could make a promising new therapy approach for RA.

The biologic role of ROS

Oxygen radicals exert a wide array of different biologic functions ranging from harmful and nonspecific events, such as DNA laddering and lipid peroxidation, to regulation of specific and fine-tuned steps in cell signaling. Although oxidative stress is induced by means of uncontrolled, massive production of superoxide, ROS that act as signaling molecules are produced under tight regulation. ROS signaling takes place within a defined compartment into which ROS are rapidly produced and subsequently removed to avoid sustained signaling and oxidative damage (36). Traditionally, ROS are considered as enhancers of inflammation, but today it is evident that ROS signaling also potently downregulates inflammatory processes.

ROS are important for phagosome function

NOX2-derived ROS play a significant role in host defense against invading pathogens. Phagocytic cells engulf invading microorganisms and generate ROS into the phagolysosome, thus elevating intraphagosomal pH and activating proteolytic enzymes that destroy the invaders and any other ingested material. ROS production is critical for the clearance of pathogens, which is well demonstrated in CGD patients, in whom mutated NOX2 complex subunits impair ROS production, leading to severe and recurrent bacterial and fungal infections (87).

Phagocytes produce ROS inside the phagolysosome, and when radicals escape from the vacuole, they are quickly removed by antioxidant systems abundantly present in the cytoplasm. Local oxidative stress and tissue damage arise when the regulation fails and radicals are produced excessively and inadvertently released into cellular compartments. In inflamed tissues (*e.g.*, rheumatoid joints), NOX2 complex is chronically activated, and increased production of ROS overloads the antioxidant defense, giving rise to local oxidative stress (76).

Many *in vitro* studies on ROS imitate this scenario with pathologically high ROS concentrations and thus fail to gain understanding of more fine-tuned regulatory effects of ROS.

Importance of ROS in NLRP3 inflammasome activation

In addition to their role in bacterial killing, ROS are suggested to initiate and potentiate inflammation by inducing the activation of NLRP3 (also called NALP3) inflammasome. NLRP3 inflammasome is a protein complex that activates caspase-1 and thus controls maturation and secretion of the proinflammatory cytokine IL-1β. NLRP3 inflammasome can be activated by a wide range of innate stimuli, including whole pathogens, environmental insults, endogenous danger signals, and particulate adjuvants (alum, oil emulsions, silica, and urate crystals) that are commonly used to potentiate immune reactions.

NLRP3 inflammasome activation does not require the presence of functional NOX2 complex, although ROS might partially regulate some of the suggested activating pathways (90, 99, 101). Primary cells from CGD patients with mutations in $p47^{phox}$, $p22^{phox}$, or $gp91^{phox}$ were shown to respond normally to inflammasome activators and to secrete IL-1β in levels comparable to those in healthy controls (99, 101). Conversely, NOX inhibition by an unspecific chemical inhibitor DPI or by shRNA technique introduced artifacts that inhibited NLRP3 inflammasome activation and subsequent IL-1β secretion (99). Similarly, macrophages from *sod1* knockout mice with elevated ROS levels were shown to secrete less IL-1β (62) and also peritoneal cells and bone marrow–derived DC from $gp91^{phox}$ knockout mice produced normal or even elevated levels of IL-1β (29, 39).

Taken together, these findings confirm that the NLPR3 inflammasome can be activated independent of NOX enzymes and suggest that *in vitro* data using chemical inhibitors or shRNA introduce artifacts that severely compromise the experimental readout.

Nonfunctional NOX2 and autoimmunity

Both mice and rats with nonfunctional NOX2 complex and reduced or abrogated superoxide production develop more severe experimental arthritis than do littermates with functional ROS production (29, 42, 70, 100). Despite evident inflammation, no radical production can be measured in the inflamed ears or paws of $p47^{phox}$ mutated mice by *in vivo* imaging with L-012 (Fig. 7B) (48), indicating that the mutation is not compensated by other ROS-producing systems and, more important, that massive ROS production is not a prerequisite for fulminant joint inflammation.

Enhanced disease susceptibility in rodents deficient in functional NOX2 complex is accompanied by an increased number of reduced thiol groups on T-cell membrane proteins, resulting in a more activated and highly arthritogenic T-cell phenotype (27). Results from an adoptive transfer model of arthritis also support the importance of T cells as the most important disease-enhancing cells in the absence of functional NOX2 complex. Primed $CD4^+$ T cells from rats with low ROS production were able to induce arthritis when transferred to rats with normal ROS levels, whereas $CD4^+$ T cells from rats with normal ROS production failed to induce inflammation in naïve rats with low ROS production (70) (Fig. 4). Adoptive transfer of arthritis was also used to show that treatment of the transferred arthritogenic splenocytes with GSSG (oxidized glutathione) reduced arthritis severity in recipient rats (27), further strengthening the idea that the NOX2 complex controls arthritis development by modulating the T-cell phenotype. These experiments do not, however, reveal whether membrane oxidation is affected through the altered redox status of plasma or if direct cell to cell contact with another NOX2-expressing cell is needed.

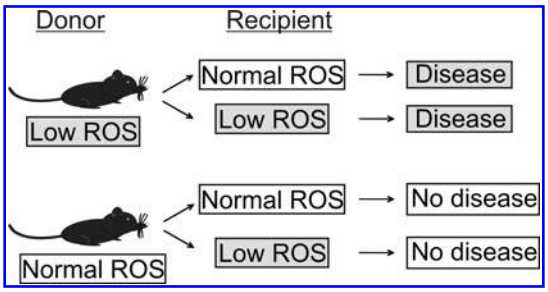

FIG. 4. Primed T cells are transferred from rats with low or normal ROS levels to naïve rats. T cells from rats producing low amounts of ROS are able to induce disease in the recipient rats, irrespective of the recipients' capability to produce ROS, but cells from rats with normal ROS production do not transfer the disease, highlighting the role of ROS in T-cell education/priming (70).

Monocyte-derived ROS modulate T-cell reactivity

The phagocyte NOX2 complex is expressed mainly on granulocytes and antigen-presenting cells. When looking for the responsible cell affecting the T-cell phenotype, we focused on antigen-presenting cells, as they directly interact with T cells during T-cell education and priming.

Among antigen-presenting cells, macrophages were identified as the most potent ROS producers, although ROS production was also detected from B cells and DC, but at a much lower level. Thus, we constructed a transgenic mouse, which expresses functional $p47^{phox}$ on monocytes/macrophages by taking advantage of the human CD68 promoter. The transgenic mouse had significantly reduced severity and incidence of collagen-induced arthritis, confirming the role of macrophage-derived ROS as potent immune regulators. *In vitro* experiments showed that the transgene efficiently suppressed antigen-dependent T-cell proliferation and proinflammatory cytokine production, irrespective of T-cell origin, strongly suggesting that monocytes downregulate T-cell activation by producing superoxide during antigen presentation (28).

Monocytes/macrophages are a heterogeneous population, and classifying them into different lineages and activational subcategories is a challenging, if not impossible, task. Monocytes differentiate to macrophages but show extreme phenotypic plasticity, and even at maturity, some reports suggest that monocytes even have the potential to differentiate into different vascular elements in both blood and lymph vessels, including endothelial cells in lymph vessels, myofibroblasts, and smooth muscle cells (25, 60, 64). Traditionally, inflammatory macrophages are divided into classically activated type 1 (inflammatory) and alternatively activated type 2 (wound-healing phenotype) macrophages (31). This classification works well with *in vitro* polarized macrophages,

whereas their respective roles and functions *in vivo* remain inconclusive.

Classic activation can be induced by *in vitro* culture with IFN-γ and TLR ligands (*e.g.*, LPS), resulting in a phenotype with increased production of proinflammatory cytokines, ROS, iNOS, and higher expression of MHC II (31). This subtype of macrophages is also suggested to contain the previously discussed NLRP3 inflammasome (74).

Wound healing (alternatively activated) macrophages are characterized by enhanced tissue repair and phagocytosis. They express mannose receptor MR (CD206) and dectin-1 on their plasma membrane and are generally considered antiinflammatory macrophages. Macrophage phenotypes are highly dependent on the surrounding microenvironment, and *in vitro* experiments support the idea that a given cell may participate in both induction and resolution of the inflammatory process (78).

CD11b and Gr-1 double-positive monocytes are an interesting cell population. Classically, immunologists denote them as inflammatory monocytes because they have been shown to produce high levels of TNF-α IL-1β, ROS, and NOS, as well as to express high levels of MHC II, CD80, and CD86. Conversely, cells with exactly the same phenotype are found in tumors, and, when isolated from tumors, they are called myeloid-derived suppressor cells (MDSCs) and are shown to support Treg function and to promote tumor growth (91). The immune-suppressive function of MDSCs was shown to be mediated by NOX2 complex–derived ROS (15). The same CD11b and Gr-1–positive monocyte subset is also shown to suppress experimental autoimmune encephalomyelitis, a commonly used animal model of multiple sclerosis (108), further strengthening the immune-suppressive role of monocyte-derived ROS.

Antigen-presenting cells express MHC II and can activate T cells in an antigen-specific manner. MHC II expression and antigen presentation leading to priming of T cells is classically associated with dendritic cells, a subset of mononuclear cells. However, macrophages also express MHC II and present antigens, and traditionally these are classified as proinflammatory monocytes. In light of recent data from our laboratory (28) and others' work with tumor-associated macrophages (15), this is now challenged. In the transgenic mouse expressing functional P47^{phox} on macrophages, no oxidative burst or P47^{phox} expression was detected on DC, pointing to a macrophage-driven and antigen-dependent protection against excessive T-cell activation and inflammation (28).

To summarize the main functions of inflammatory macrophages, we suggest a division into three functional phenotypes that largely depend on the surrounding tissue homeostasis and are at least to some extent interconvertible (Fig. 5). Inflammatory macrophages can trigger an early inflammatory response and mediate immune reactions against exogenous pathogens (*i.e.,* infectious organisms) or endogenous dangers (trauma, tissue degradation, abnormal cell behavior), whereas healing macrophages are needed in limiting immune-driven inflammatory responses and in the restoration of tissue homeostasis. Antigen-presenting regulatory macrophages regulate immune responses antigen specifically and thus provide the immune system with a fine-tuned and specifically targeted regulatory mechanism (31, 59, 74). Importantly, the capacity to make an induced ROS response

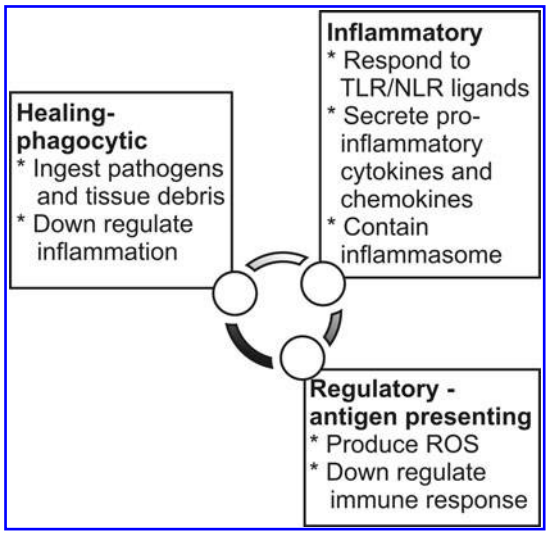

FIG. 5. Macrophages are a heterogeneous group of leukocytes capable of modifying immune responses in multiple ways and show extreme phenotypic plasticity.

during antigen presentation is an important tool for regulating T-cell activity (28), and the fine-tuned details of this system remain to be investigated.

How do the ROS affect T cells?

During antigen presentation, a closed compartment, the so-called immunologic synapse, is formed between the T cell and the antigen-presenting cell (24). Within the immunologic synapse, the T-cell receptor (TCR) recognizes the antigen that is presented by the antigen-presenting cell on the MHC II complex, and the T cell is activated. NOX2 complex is assembled on antigen-presenting cell membranes in lipid rafts and subsequently transported to the immunologic synapse (4). NOX2 complex–produced superoxide is quickly dismutated either by SOD enzymes or by spontaneous reaction into longer-lived hydrogen peroxide, which presumably more readily penetrates lipid bilayers and thus more likely can affect intracellular events. The most well-known redox-sensitive elements in the TCR signaling cascade are different phosphatases. In light of the biologic chemistry, physiologic concentrations of ROS do not easily reach concentrations high enough to modify the redox-sensitive active-site cysteines, unless the reaction is restricted to a limited compartment, such as the immunologic synapse [for review, see (105)], making it a good candidate for ROS-mediated down-regulation of T-cell activation.

As already mentioned, cysteine-based phosphatases, which include protein tyrosine phosphatases (PTPs), dual-specificity phosphatases, low-molecular-weight PTPs, and the lipid phosphatase PTEN, all have a redox-regulated cysteine group in their enzymatically active catalytic site (89). Lymphoid tyrosine phosphatase (LYP) (encoded by the *PTPN22* gene, called PEP in mice) is a tyrosine phosphatase located downstream the TCR (Fig. 6), and it is reproducibly associated with several autoimmune diseases, including RA, type I diabetes, and thyroiditis (7, 19). In addition to the redox-regulated cysteine in the catalytic site, structural and mutational data suggest that LYP phosphatase activity is also

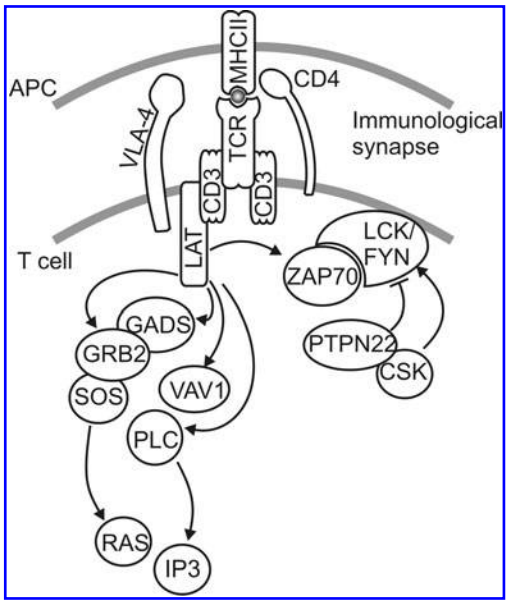

FIG. 6. A simplified picture of antigen presentation by an antigen-presenting cell to a T cell, emphasizing the molecules subjected to redox regulation or related to autoimmunity (see text for details). The antigen–MHC class II molecule complex is recognized by T-cell receptor (TCR). Other players in the immunologic synapse are CD3 and CD4, in addition to redox-regulated VLA-4 integrin. The proteins involved in signal transduction downstream of TCR activation include linker for activation of T cells (LAT), leukocyte-specific protein tyrosine kinase (LCK), the Src family kinase FYN; the Syk protein kinase ZAP70, protein tyrosine phosphatase, nonreceptor type 22 (PTPN22), C-src tyrosine kinase (CSK), GRB2-related adaptor protein GADS, VAV1 guanine nucleotide exchange factor, phospholipase C (PLC), inositol 1,4,5-trisphosphate (IP3), and RAS GTPase [modified from (1)].

regulated by other, noncatalytic redox-sensitive cysteines around the catalytically active center (98).

T lymphocytes from RA patients' synovium are subjected to chronic oxidative stress, exhibit an activated phenotype, but fail to respond to TCR stimuli *ex vivo*. This hyporesponsiveness was shown to depend on the localization of linker for activation of T cells (LAT). Oxidative stress induced the translocation of LAT from the plasma membrane to cytosol, thus impairing TCR signaling (32, 33). This creates a direct link from oxidation-dependent TCR signaling regulation and T-cell function in human RA.

Co-stimulatory molecules are essential regulators of TCR–MHC interaction. Integrin VLA-4, which works as a costimulatory molecule in the immunologic synapse, is functionally regulated by cellular redox balance, making VLA-4 one potential target molecule of redox-sensitive signaling events critical for T-cell activation (49, 55). In synovial fluid T cells, ROS affect TCR signaling by modifying the structure of the protein tyrosine kinase LCK (85). In addition, it has been reported that oxidation may contribute to loss of TCR function by structural modifications in the C-terminal domain of TCR zeta and p56 LCK (12).

The hyperinflammatory state observed in $p47^{phox}$ knockout mice was recently described to be a consequence of nonfunctional L-kynurenine pathway (86). ROS serves as cofactor for indoleamine 2,3-dioxygenase (IDO), an enzyme responsible for cleavage of tryptophan to an intermediate that deformulates to L-kynurenine. L-Kynurenine favors T-cell tolerance, reduces IL-17 production, and limits inflammation (77). In the absence of ROS, L-kynurenine production was reported to be inhibited, and T cells become hyperreactive (57, 86). We have not yet been able to observe a differential effect on the L-kynurenine pathway in the $p47^{phox}$ mutated B10.Q mice (unpublished observations), and further experiments and confirmation are thus warranted.

Lack of NOX2-derived ROS, due to $p47^{phox}$ mutation, has a profound effect on T-cell regulation, as it can break tolerance against autologous type II collagen in transgenic mice expressing mutated collagen, mimicking rat/human collagen, used in arthritis immunization (40). ROS-mediated protection against arthritis has been shown to be accompanied by lowered expression of IFN-γ, TNF-α (28), IL-17 (29), and IL-5 (35). The versatility of inflammatory pathways suppressed by ROS argues for a profound T-helper cell polarization–independent role for ROS in disease suppression.

From latent to active TGF-β1: ROS activate resolution of inflammation

During early infection, phagocytes produce radicals to destroy ingested pathogens and locally accelerate the immune system to neutralize the invaders. Later, ROS mediate extinction of the immune reaction, thereby preventing inflammation from becoming chronic.

Transforming growth factor-beta1 (TGF-β1) is an important cytokine involved in wound healing and immune suppression. TGF-β1 is also an important factor maintaining the suppressor function and Foxp3 expression of $CD4^+CD25^+$ regulatory T cells (58). Superoxide has been shown to increase the release of TGF-β1 from cultured fibroblasts (79), and interestingly, ROS also can activate latent TGF-β1 by cleaving it free from latency-associated peptide (LAP) (45). Subsets of Tregs express LAP on their cell surface, implying that ROS can at least in part mediate the suppressive function of these cells (13, 68).

Concluding Remarks

The traditional view of ROS as disease-promoting factors is being reevaluated as new evidence of their protecting and regulating role is accumulating (Fig. 7A, C). Our knowledge of the biologic role of ROS largely relies on *in vitro* studies that provide us with valuable information on molecular mechanisms of ROS-mediated effects but unfortunately fail to address questions on the role of ROS *in vivo*. Clearly, ROS-mediated immune regulation and protection against autoimmunity is context dependent and must be studied *in vivo*. Genetically and environmentally controlled mouse and rat models are good tools to dissect ROS-mediated physiological mechanisms *in vivo* (3).

However, it is not straightforward to translate the findings from one species to another. For example, *Ncf1* is polymorphic in laboratory and wild rats, whereas no such polymorphism exists in laboratory mice. To make it even more complicated, the human genome carries the *NCF1* gene in several variable duplicates. Because ROS-dependent immune regulation is likely to be evolutionary conserved, we believe that in both

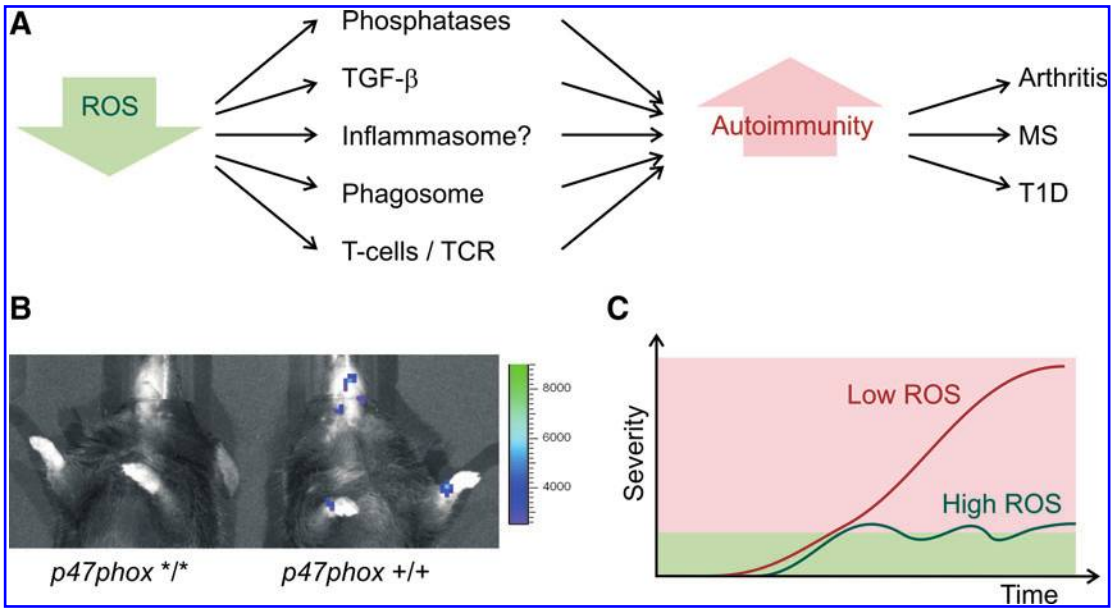

FIG. 7. NOX2-derived ROS in autoimmune diseases. (A) NOX2-derived ROS may regulate several targets, which potentially alter the severity of or susceptibility to autoimmune diseases via various routes discussed in this article. (MS, multiple sclerosis; T1D, type 1 diabetes). **(B)** $p47^{phox}$ mutated and wild-type animals with similar disease severity were injected intraperitoneally with L-012, which is a luminol derivative that, on contact with ROS, produces luminescent signal that can be detected with Ivis *in vivo* imaging system. Signal is not detectable in the $p47^{phox}$ mutated mouse, whereas inflammation in the wild-type control induces clear inflammation-dependent luminescence signal. Luminescence counts are presented as p/sec/cm^2/sr. **(C)** A schematic example of the protective role of NOX2-derived ROS in relation to autoimmune disease severity. The green area represents subclinical autoimmunity without any clinical symptoms, whereas the red shading represents the presence of clinical symptoms. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

mice and humans, other genetic polymorphisms are responsible for regulation of ROS-dependent immunomodulation. In humans, many possible candidates operating upstream or downstream of *NCF1* (*NCF4*, *PTPN22*, and *PRKC*) are already identified (7, 71, 88).

It is clear that much more research is needed to elucidate the mechanisms by which ROS regulate immunity. Undeniably, the paradigm of ROS as harmful disease mediators is obsolete, and ROS must be considered important immune regulators that can potently prevent autoimmune inflammation.

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Author Disclosure Statement

R.H. is one of the founders of, and M.H. is employed by, the company Redoxis AB, which is developing treatment for autoimmune conditions by modulating ROS production.

References

1. Abraham RT and Weiss A. Jurkat T cells and development of the T-cell receptor signalling paradigm. *Nat Rev Immunol* 4: 301–308, 2004.
2. Afonso V, Champy R, Mitrovic D, Collin P, and Lomri A. Reactive oxygen species and superoxide dismutases: role in joint diseases. *Joint Bone Spine* 74: 324–329, 2007.
3. Ahlqvist E, Hultqvist M, and Holmdahl R. The value of animal models in predicting genetic susceptibility to complex diseases such as rheumatoid arthritis. *Arthritis Res Ther* 11: 226, 2009.
4. Babior BM. NADPH oxidase: an update. *Blood* 93: 1464–1476, 1999.
5. Bae SC, Jung WJ, Lee EJ, Yu R, and Sung MK. Effects of antioxidant supplements intervention on the level of plasma inflammatory molecules and disease severity of rheumatoid arthritis patients. *J Am Coll Nutr* 28: 56–62, 2009.
6. Bedard K and Krause KH. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiol Rev* 87: 245–313, 2007.
7. Begovich AB, Carlton VE, Honigberg LA, Schrodi SJ, Chokkalingam AP, Alexander HC, Ardlie KG, Huang Q, Smith AM, Spoerke JM, Conn MT, Chang M, Chang SY, Saiki RK, Catanese JJ, Leong DU, Garcia VE, McAllister LB, Jeffery DA, Lee AT, Batliwalla F, Remmers E, Criswell LA, Seldin MF, Kastner DL, Amos CI, Sninsky JJ, and Gregersen PK. A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. *Am J Hum Genet* 75: 330–337, 2004.
8. Björkman L, Dahlgren C, Karlsson A, Brown KL, and Bylund J. Phagocyte-derived reactive oxygen species as suppressors of inflammatory disease. *Arthritis Rheum* 58: 2931–2935, 2008.
9. Brown GE, Stewart MQ, Bissonnette SA, Elia AE, Wilker E, and Yaffe MB. Distinct ligand-dependent roles for p38 MAPK in priming and activation of the neutrophil NADPH oxidase. *J Biol Chem* 279: 27059–27068, 2004.

10. Canter PH, Wider B, and Ernst E. The antioxidant vitamins A, C, E and selenium in the treatment of arthritis: a systematic review of randomized clinical trials. *Rheumatology (Oxford)* 46: 1223–1233, 2007.
11. Cedergren J, Forslund T, Sundqvist T, and Skogh T. Intracellular oxidative activation in synovial fluid neutrophils from patients with rheumatoid arthritis but not from other arthritis patients. *J Rheumatol* 34: 2162–2170, 2007.
12. Cemerski S, van Meerwijk JP, and Romagnoli P. Oxidative-stress-induced T lymphocyte hyporesponsiveness is caused by structural modification rather than proteasomal degradation of crucial TCR signaling molecules. *Eur J Immunol* 33: 2178–2185, 2003.
13. Chen ML, Yan BS, Bando Y, Kuchroo VK, and Weiner HL. Latency-associated peptide identifies a novel CD4+CD25+ regulatory T cell subset with TGFbeta-mediated function and enhanced suppression of experimental autoimmune encephalomyelitis. *J Immunol* 180: 7327–7337, 2008.
14. Cheng G, Cao Z, Xu X, van Meir EG, and Lambeth JD. Homologs of gp91phox: cloning and tissue expression of Nox3, Nox4, and Nox5. *Gene* 269: 131–140, 2001.
15. Corzo CA, Cotter MJ, Cheng P, Cheng F, Kusmartsev S, Sotomayor E, Padhya T, McCaffrey TV, McCaffrey JC, and Gabrilovich DI. Mechanism regulating reactive oxygen species in tumor-induced myeloid-derived suppressor cells. *J Immunol* 182: 5693–5701, 2009.
16. DeLeo FR, Renee J, McCormick S, Nakamura M, Apicella M, Weiss JP, and Nauseef WM. Neutrophils exposed to bacterial lipopolysaccharide upregulate NADPH oxidase assembly. *J Clin Invest* 101: 455–463, 1998.
17. Dinauer MC and Orkin SH. Chronic granulomatous disease. *Annu Rev Med* 43: 117–124, 1992.
18. Dotan Y, Pinchuk I, Lichtenberg D, and Leshno M. Decision analysis supports the paradigm that indiscriminate supplementation of vitamin E does more harm than good. *Arterioscler Thromb Vasc Biol* 29: 1304–1390, 2009.
19. Dultz G, Matheis N, Dittmar M, Rohrig B, Bender K, and Kahaly GJ. The protein tyrosine phosphatase non-receptor type 22 C1858T polymorphism is a joint susceptibility locus for immunothyroiditis and autoimmune diabetes. *Thyroid* 19: 143–148, 2009.
20. Eggleton P, Wang L, Penhallow J, Crawford N, and Brown KA. Differences in oxidative response of subpopulations of neutrophils from healthy subjects and patients with rheumatoid arthritis. *Ann Rheum Dis* 54: 916–923, 1995.
21. El Benna J, Hayem G, Dang PM, Fay M, Chollet-Martin S, Elbim C, Meyer O, and Gougerot-Pocidalo MA. NADPH oxidase priming and p47phox phosphorylation in neutrophils from synovial fluid of patients with rheumatoid arthritis and spondylarthropathy. *Inflammation* 26: 273–278, 2002.
22. El-Benna J, Dang P, and Gougerot-Pocidalo M. Priming of the neutrophil NADPH oxidase activation: role of p47phox phosphorylation and NOX2 mobilization to the plasma membrane. *Semin Immunopathol* 30: 279–289, 2008.
23. Ferguson PJ, Lokuta MA, El-Shanti HI, Muhle L, Bing X, and Huttenlocher A. Neutrophil dysfunction in a family with a SAPHO syndrome-like phenotype. *Arthritis Rheum* 58: 3264–3269, 2008.
24. Fooksman DR, Vardhana S, Vasiliver-Shamis G, Liese J, Blair DA, Waite J, Sacristan C, Victora GD, Zanin-Zhorov A, and Dustin ML. Functional anatomy of T cell activation and synapse formation. *Annu Rev Immunol* 28: 79–105, 2010.
25. Gao Z, McAlister VC, and Williams GM. Repopulation of liver endothelium by bone-marrow-derived cells. *Lancet* 357: 932–933, 2001.
26. Gelderman KA, Hultqvist M, Olsson LM, Bauer K, Pizzolla A, Olofsson P, and Holmdahl R. Rheumatoid arthritis: the role of reactive oxygen species in disease development and therapeutic strategies. *Antioxid Redox Signal* 9: 1541–1567, 2007.
27. Gelderman KA, Hultqvist M, Holmberg J, Olofsson P, and Holmdahl R. T cell surface redox levels determine T cell reactivity and arthritis susceptibility. *Proc Natl Acad Sci U S A* 103: 12831–12836, 2006.
28. Gelderman KA, Hultqvist M, Pizzolla A, Zhao M, Nandakumar KS, Mattsson R, and Holmdahl R. Macrophages suppress T cell responses and arthritis development in mice by producing reactive oxygen species. *J Clin Invest* 117: 3020–3028, 2007.
29. George-Chandy A, Nordström I, Nygren E, Jonsson I, Postigo J, Collins LV, and Eriksson K. Th17 development and autoimmune arthritis in the absence of reactive oxygen species. *Eur J Immunol* 38: 1118–1126, 2008.
30. Gilgun-Sherki Y, Melamed E, and Offen D. The role of oxidative stress in the pathogenesis of multiple sclerosis: the need for effective antioxidant therapy. *J Neurol* 251: 261–268, 2004.
31. Gordon S and Taylor PR. Monocyte and macrophage heterogeneity. *Nat Rev Immunol* 5: 953–964, 2005.
32. Gringhuis SI, Papendrecht-van der Voort EA, Leow A, Nivine Levarht EW, Breedveld FC, and Verweij CL. Effect of redox balance alterations on cellular localization of LAT and downstream T-cell receptor signaling pathways. *Mol Cell Biol* 22: 400–411, 2002.
33. Gringhuis SI, Leow A, Papendrecht-Van Der Voort EA, Remans PH, Breedveld FC, and Verweij CL. Displacement of linker for activation of T cells from the plasma membrane due to redox balance alterations results in hyporesponsiveness of synovial fluid T lymphocytes in rheumatoid arthritis. *J Immunol* 164: 2170–2179, 2000.
34. Guichard C, Pedruzzi E, Dewas C, Fay M, Pouzet C, Bens M, Vandewalle A, Ogier-Denis E, Gougerot-Pocidalo M, and Elbim C. Interleukin-8-induced priming of neutrophil oxidative burst requires sequential recruitment of NADPH oxidase components into lipid rafts. *J Biol Chem* 280: 37021–37032, 2005.
35. Hagenow K, Gelderman KA, Hultqvist M, Merky P, Bäcklund J, Frey O, Kamradt T, and Holmdahl R. Ncf1-associated reduced oxidative burst promotes IL-33R+ T cell-mediated adjuvant-free arthritis in mice. *J Immunol* 183: 874–881, 2009.
36. Hancock JT. The role of redox mechanisms in cell signalling. *Mol Biotechnol* 43: 162–166, 2009.
37. Harrison D, Griendling KK, Landmesser U, Hornig B, and Drexler H. Role of oxidative stress in atherosclerosis. *Am J Cardiol* 91: 7–11, 2003.
38. Heliövaara M, Knekt P, Aho K, Aaran RK, Alfthan G, and Aromaa A. Serum antioxidants and risk of rheumatoid arthritis. *Ann Rheum Dis* 53: 51–53, 1994.
39. Hornung V, Bauernfeind F, Halle A, Samstad EO, Kono H, Rock KL, Fitzgerald KA, and Latz E. Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization. *Nat Immunol* 9: 847–856, 2008.
40. Hultqvist M, Bäcklund J, Bauer K, Gelderman KA, and Holmdahl R. Lack of reactive oxygen species breaks T cell

tolerance to collagen type II and allows development of arthritis in mice. *J Immunol* 179: 1431–1437, 2007.
41. Hultqvist M, Olofsson P, Gelderman KA, Holmberg J, and Holmdahl R. A new arthritis therapy with oxidative burst inducers. *PLoS Med* 3: e348, 2006.
42. Hultqvist M, Olofsson P, Holmberg J, Bäckström BT, Tordsson J, and Holmdahl R. Enhanced autoimmunity, arthritis, and encephalomyelitis in mice with a reduced oxidative burst due to a mutation in the Ncf1 gene. *Proc Natl Acad Sci U S A* 101: 12646–12651, 2004.
43. Jackson SH, Devadas S, Kwon J, Pinto LA, and Williams MS. T cells express a phagocyte-type NADPH oxidase that is activated after T cell receptor stimulation. *Nat Immunol* 5: 818–827, 2004.
44. Jaquet V, Scapozza L, Clark RA, Krause KH, and Lambeth JD. Small-molecule NOX inhibitors: ROS-generating NADPH oxidases as therapeutic targets. *Antioxid Redox Signal* 11: 2535–2552, 2009.
45. Jobling MF, Mott JD, Finnegan MT, Jurukovski V, Erickson AC, Walian PJ, Taylor SE, Ledbetter S, Lawrence CM, Rifkin DB, and Barcellos-Hoff MH. Isoform-specific activation of latent transforming growth factor beta (LTGF-beta) by reactive oxygen species. *Radiat Res* 166: 839–848, 2006.
46. Karlson EW, Shadick NA, Cook NR, Buring JE, and Lee IM. Vitamin E in the primary prevention of rheumatoid arthritis: the Women's Health Study. *Arthritis Rheum* 59: 1589–1595, 2008.
47. Khodr B and Khalil Z. Modulation of inflammation by reactive oxygen species: implications for aging and tissue repair. *Free Radic Biol Med* 30: 1–8, 2001.
48. Kielland A, Blom T, Nandakumar KS, Holmdahl R, Blomhoff R, and Carlsen H. In vivo imaging of reactive oxygen and nitrogen species in inflammation using the luminescent probe L-012. *Free Radic Biol Med* 47: 760–766, 2009.
49. Kim TK, Billard MJ, Wieder ED, McIntyre BW, and Komanduri KV. Co-engagement of alpha(4)beta(1) integrin (VLA-4) and CD4 or CD8 is necessary to induce maximal Erk1/2 phosphorylation and cytokine production in human T cells. *Hum Immunol* 71: 23–28, 2010.
50. Klebanoff SJ. Myeloperoxidase: friend and foe. *J Leukoc Biol* 77: 598–625, 2005.
51. Kojima S, Nomura T, Icho T, Kajiwara Y, Kitabatake K, and Kubota K. Inhibitory effect of neopterin on NADPH-dependent superoxide-generating oxidase of rat peritoneal macrophages. *FEBS Lett* 329: 125–128, 1993.
52. Lambeth JD, Krause KH, and Clark RA. NOX enzymes as novel targets for drug development. *Semin Immunopathol* 30: 339–363, 2008.
53. Lambeth JD. NOX enzymes and the biology of reactive oxygen. *Nat Rev Immunol* 4: 181–189, 2004.
54. Lapouge K, Smith SJM, Groemping Y, and Rittinger K. Architecture of the p40-p47-p67phox complex in the resting state of the NADPH oxidase. *J Biol Chem* 277: 10121–10128, 2002.
55. Laragione T, Bonetto V, Casoni F, Massignan T, Bianchi G, Gianazza E, and Ghezzi P. Redox regulation of surface protein thiols: identification of integrin alpha-4 as a molecular target by using redox proteomics. *Proc Natl Acad Sci U S A* 100: 14737–14741, 2003.
56. Lassegue B and Griendling KK. Mycophenolic acid is a new Nox2 inhibitor. *Hypertension* 49: 25–26, 2007.
57. Lindsay MA, Perkins RS, Barnes PJ, and Giembycz MA. Leukotriene B4 activates the NADPH oxidase in eosinophils by a pertussis toxin-sensitive mechanism that is largely independent of arachidonic acid mobilization. *J Immunol* 160: 4526–4534, 1998.
58. Marie JC, Letterio JJ, Gavin M, and Rudensky AY. TGF-beta1 maintains suppressor function and Foxp3 expression in CD4+CD25+ regulatory T cells. *J Exp Med* 201: 1061–1067, 2005.
59. Martinez FO, Sica A, Mantovani A, and Locati M. Macrophage activation and polarization. *Front Biosci* 13: 453–461, 2008.
60. Maruyama K, Ii M, Cursiefen C, Jackson DG, Keino H, Tomita M, Van Rooijen N, Takenaka H, D'Amore PA, Stein-Streilein J, Losordo DW, and Streilein JW. Inflammation-induced lymphangiogenesis in the cornea arises from CD11b-positive macrophages. *J Clin Invest* 115: 2363–2372, 2005.
61. Matute JD, Arias AA, Wright NAM, Wrobel I, Waterhouse CCM, Li XJ, Marchal CC, Stull ND, Lewis DB, Steele M, Kellner JD, Yu W, Meroueh SO, Nauseef WM, and Dinauer MC. A new genetic subgroup of chronic granulomatous disease with autosomal recessive mutations in p40phox and selective defects in neutrophil NADPH oxidase activity. *Blood* 114: 3309–3315, 2009.
62. Meissner F, Molawi K, and Zychlinsky A. Superoxide dismutase 1 regulates caspase-1 and endotoxic shock. *Nat Immunol* 9: 866–872, 2008.
63. Mirshafiey A and Mohsenzadegan M. Antioxidant therapy in multiple sclerosis. *Immunopharmacol Immunotoxicol* 31: 13–29, 2009.
64. Mooney JE, Rolfe BE, Osborne GW, Sester DP, van Rooijen N, Campbell GR, Hume DA, and Campbell JH. Cellular plasticity of inflammatory myeloid cells in the peritoneal foreign body response. *Am J Pathol* 176: 369–380, 2010.
65. Mossberg N, Movitz C, Hellstrand K, Bergström T, Nilsson S, and Andersen O. Oxygen radical production in leukocytes and disease severity in multiple sclerosis. *J Neuroimmunol* 213: 131–134, 2009.
66. Mossberg N, Andersen O, Nilsson S, Dahlgren C, Hellstrand K, Lindh M, Svedhem Å, Bergström T, and Movitz C. Oxygen radical production and severity of the Guillain–Barré syndrome. *J Neuroimmunol* 192: 186–191, 2007.
67. Nagano T. Bioimaging probes for reactive oxygen species and reactive nitrogen species. *J Clin Biochem Nutr* 45: 111–124, 2009.
68. Nakamura K, Kitani A, and Strober W. Cell contact-dependent immunosuppression by CD4(+)CD25(+) regulatory T cells is mediated by cell surface-bound transforming growth factor beta. *J Exp Med* 194: 629–644, 2001.
69. Nauseef WM. Nox enzymes in immune cells. *Semin Immunopathol* 30: 195–208, 2008.
70. Olofsson P, Holmberg J, Tordsson J, Lu S, Åkerström B, and Holmdahl R. Positional identification of Ncf1 as a gene that regulates arthritis severity in rats. *Nat Genet* 33: 25, 2003.
71. Olsson L, Lindqvist A, Kallberg H, Padyukov L, Burkhardt H, Alfredsson L, Klareskog L, and Holmdahl R. A case-control study of rheumatoid arthritis identifies an associated single nucleotide polymorphism in the NCF4 gene, supporting a role for the NADPH-oxidase complex in autoimmunity. *Arthritis Res Ther* 9: R98, 2007.
72. Ostrakhovitch EA and Afanas'ev IB. Oxidative stress in rheumatoid arthritis leukocytes: suppression by rutin and other antioxidants and chelators. *Biochem Pharmacol* 62: 743–746, 2001.

73. Pattison DJ and Winyard PG. Dietary antioxidants in inflammatory arthritis: do they have any role in etiology or therapy? *Nat Clin Pract Rheumatol* 4: 590–596, 2008.
74. Pelegrin P and Surprenant A. Dynamics of macrophage polarization reveal new mechanism to inhibit IL-1beta release through pyrophosphates. *EMBO J* 28: 2114–2127, 2009.
75. Petry A, Weitnauer M, and Görlach A. Receptor activation of NADPH oxidases. *Antioxid Redox Signal* 13: 467–487, 2010.
76. Phillips DC, Dias HK, Kitas GD, and Griffiths HR. Aberrant reactive oxygen and nitrogen species generation in rheumatoid arthritis (RA): causes and consequences for immune function, cell survival, and therapeutic intervention. *Antioxid Redox Signal* 12: 743–785, 2010.
77. Platten M, Ho PP, Youssef S, Fontoura P, Garren H, Hur EM, Gupta R, Lee LY, Kidd BA, Robinson WH, Sobel RA, Selley ML, and Steinman L. Treatment of autoimmune neuroinflammation with a synthetic tryptophan metabolite. *Science* 310: 850–855, 2005.
78. Porcheray F, Viaud S, Rimaniol AC, Leone C, Samah B, Dereuddre-Bosquet N, Dormont D, and Gras G. Macrophage activation switching: an asset for the resolution of inflammation. *Clin Exp Immunol* 142: 481–4789, 2005.
79. Qi S, den Hartog GJ, and Bast A. Superoxide radicals increase transforming growth factor-beta1 and collagen release from human lung fibroblasts via cellular influx through chloride channels. *Toxicol Appl Pharmacol* 237: 111–118, 2009.
80. Rada B and Leto TL. Oxidative innate immune defenses by Nox/Duox family NADPH oxidases. *Contrib Microbiol* 15: 164–187, 2008.
81. Reeves EP, Lu H, Jacobs HL, Messina CG, Bolsover S, Gabella G, Potma EO, Warley A, Roes J, and Segal AW. Killing activity of neutrophils is mediated through activation of proteases by K+ flux. *Nature* 416: 291–297, 2002.
82. Reth M. Hydrogen peroxide as second messenger in lymphocyte activation. *Nat Immunol* 3: 1129–1134, 2002.
83. Rey FE, Cifuentes ME, Kiarash A, Quinn MT, and Pagano PJ. Novel competitive inhibitor of NAD(P)H oxidase assembly attenuates vascular O_2- and systolic blood pressure in mice. *Circ Res* 89: 408–414, 2001.
84. Roessner A, Kuester D, Malfertheiner P, and Schneider-Stock R. Oxidative stress in ulcerative colitis-associated carcinogenesis. *Pathol Res Pract* 204: 511–524, 2008.
85. Romagnoli P, Strahan D, Pelosi M, Cantagrel A, and van Meerwijk JP. A potential role for protein tyrosine kinase p56(lck) in rheumatoid arthritis synovial fluid T lymphocyte hyporesponsiveness. *Int Immunol* 13: 305–312, 2001.
86. Romani L, Fallarino F, De Luca A, Montagnoli C, D'Angelo C, Zelante T, Vacca C, Bistoni F, Fioretti MC, Grohmann U, Segal BH, and Puccetti P. Defective tryptophan catabolism underlies inflammation in mouse chronic granulomatous disease. *Nature* 451: 211–215, 2008.
87. Rosenzweig SD. Inflammatory manifestations in chronic granulomatous disease (CGD). *J Clin Immunol* 28 suppl 1: S67–S72, 2008.
88. Saarela J, Kallio SP, Chen D, Montpetit A, Jokiaho A, Choi E, Asselta R, Bronnikov D, Lincoln MR, Sadovnick AD, Tienari PJ, Koivisto K, Palotie A, Ebers GC, Hudson TJ, and Peltonen L. PRKCA and multiple sclerosis: association in two independent populations. *PLoS Genet* 2: e42, 2006.
89. Salmeen A and Barford D. Functions and mechanisms of redox regulation of cysteine-based phosphatases. *Antioxid Redox Signal* 7: 560–577, 2005.
90. Schroder K and Tschopp J. The inflammasomes. *Cell* 140: 821–832, 2010.
91. Serafini P, Mgebroff S, Noonan K, and Borrello I. Myeloid-derived suppressor cells promote cross-tolerance in B-cell lymphoma by expanding regulatory T cells. *Cancer Res* 68: 5439–5449, 2008.
92. Seven A, Guzel S, Aslan M, and Hamuryudan V. Lipid, protein, DNA oxidation and antioxidant status in rheumatoid arthritis. *Clin Biochem* 41: 538–543, 2008.
93. Sheppard FR, Kelher MR, Moore EE, McLaughlin NJD, Banerjee A, and Silliman CC. Structural organization of the neutrophil NADPH oxidase: phosphorylation and translocation during priming and activation. *J Leukoc Biol* 78: 1025–1042, 2005.
94. Simons JM, Hart BA, Ip Vai Ching TR, Van Dijk H, and Labadie RP. Metabolic activation of natural phenols into selective oxidative burst agonists by activated human neutrophils. *Free Radic Biol Med* 8: 251–258, 1990.
95. Slight J, Nicholson WJ, Mitchell CG, Pouilly N, Beswick PH, Seaton A, and Donaldson K. Inhibition of the alveolar macrophage oxidative burst by a diffusible component from the surface of the spores of the fungus *Aspergillus fumigatus*. *Thorax* 51: 389–396, 1996.
96. Sorescu D, Weiss D, Lassegue B, Clempus RE, Szocs K, Sorescu GP, Valppu L, Quinn MT, Lambeth JD, Vega JD, Taylor WR, and Griendling KK. Superoxide production and expression of nox family proteins in human atherosclerosis. *Circulation* 105: 1429–1435, 2002.
97. Tardif J, McMurray JJ, Klug E, Small R, Schumi J, Choi J, Cooper J, Scott R, Lewis EF, L'Allier PL, and Pfeffer MA. Effects of succinobucol (AGI-1067) after an acute coronary syndrome: a randomised, double-blind, placebo-controlled trial. *Lancet* 371: 1761–1768, 2008.
98. Tsai SJ, Sen U, Zhao L, Greenleaf WB, Dasgupta J, Fiorillo E, Orru V, Bottini N, and Chen XS. Crystal structure of the human lymphoid tyrosine phosphatase catalytic domain: insights into redox regulation. *Biochemistry* 48: 4838–4845, 2009.
99. van Bruggen R, Koker MY, Jansen M, van Houdt M, Roos D, Kuijpers TW, and van den Berg TK. Human NLRP3 inflammasome activation is Nox1-4 independent. *Blood* 2010.
100. van de Loo FA, Bennink MB, Arntz OJ, Smeets RL, Lubberts E, Joosten LA, van Lent PL, Coenen-de Roo CJ, Cuzzocrea S, Segal BH, Holland SM, and van den Berg WB. Deficiency of NADPH oxidase components p47phox and gp91phox caused granulomatous synovitis and increased connective tissue destruction in experimental arthritis models. *Am J Pathol* 163: 1525–1537, 2003.
101. van de Veerdonk FL, Smeekens SP, Joosten LA, Kullberg BJ, Dinarello CA, van der Meer JW, and Netea MG. Reactive oxygen species-independent activation of the IL-1{beta} inflammasome in cells from patients with chronic granulomatous disease. *Proc Natl Acad Sci U S A* 2010.
102. van Reyk DM, King NJ, Dinauer MC, and Hunt NH. The intracellular oxidation of 2′,7′-dichlorofluorescin in murine T lymphocytes. *Free Radic Biol Med* 30: 82–88, 2001.
103. Verbeek W, Lekstrom-Himes J, Park DJ, Dang PM, Vuong PT, Kawano S, Babior BM, Xanthopoulos K, and Koeffler HP. Myeloid transcription factor C/EBPvarepsilon is involved in the positive regulation of lactoferrin gene expression in neutrophils. *Blood* 94: 3141–3150, 1999.
104. Weissmann G. Pathogenesis of rheumatoid arthritis. *J Clin Rheumatol* 10: S26–S31, 2004.
105. Winterbourn CC. Reconciling the chemistry and biology of reactive oxygen species. *Nat Chem Biol* 4: 278–286, 2008.
106. Yamashita T, Someya A, and Hara E. Response of superoxide anion production by guinea pig eosinophils to

various soluble stimuli: comparison to neutrophils. *Arch Biochem Biophys* 241: 447–452, 1985.
107. Zhao X, Carnevale KA, and Cathcart MK. Human monocytes use Rac1, not Rac2, in the NADPH oxidase complex. *J Biol Chem* 278: 40788–40792, 2003.
108. Zhu B, Bando Y, Xiao S, Yang K, Anderson AC, Kuchroo VK, and Khoury SJ. CD11b+Ly-6C(hi) suppressive monocytes in experimental autoimmune encephalomyelitis. *J Immunol* 179: 5228–5237, 2007.

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Abbreviations Used

CD = cluster of differentiation
CGD = chronic granulomatous disease
DC = dendritic cell
DUOX = dual oxidase
fMLF = formyl-methionyl-leucyl phenylalanine
IBD = inflammatory bowel disease
IFN = interferon
IL = interleukin
MHC II = major histocompatibility complex II
MS = multiple sclerosis
NOX2 = phagocytic NADPH oxidase
PMA = phorbol 12-myristate 13-acetate
RA = rheumatoid arthritis
ROS = reactive oxygen species
SOD = superoxide dismutase
TCR = T-cell receptor
TGF = transforming growth factor

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3. Michael J. Surace, Michelle L. Block. 2012. Targeting microglia-mediated neurotoxicity: the potential of NOX2 inhibitors. *Cellular and Molecular Life Sciences* **69**:14, 2409-2427. [CrossRef]
4. Sebastian Altenhöfer, Pamela W. M. Kleikers, Kim A. Radermacher, Peter Scheurer, J. J. Rob Hermans, Paul Schiffers, Heidi Ho, Kirstin Wingler, Harald H. H. W. Schmidt. 2012. The NOX toolbox: validating the role of NADPH oxidases in physiology and disease. *Cellular and Molecular Life Sciences* **69**:14, 2327-2343. [CrossRef]
5. Barry Halliwell. 2012. Free radicals and antioxidants: updating a personal view. *Nutrition Reviews* **70**:5, 257-265. [CrossRef]
6. Helen R. Griffiths, Christopher R. Dunston, Stuart J. Bennett, Melissa M. Grant, Darren C. Phillips, George D. Kitas. 2011. Free radicals and redox signalling in T-cells during chronic inflammation and ageing. *Biochemical Society Transactions* **39**:5, 1273-1278. [CrossRef]