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SPECIAL ARTICLE

Reactive oxygen homeostasis – the balance for preventing autoimmunity

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Being mainly known for their role in the antimicrobial defense and collateral damage they cause in tissues as agents of oxidative stress, reactive oxygen species were considered ''the bad guys'' for decades. However, in the last years it was shown that the absence of reactive oxygen species can lead to the development of immune-mediated inflammatory diseases. Animal models of lupus, arthritis and psoriasis revealed reactive oxygen species-deficiency as a potent driver of pathogenesis. On the contrary, in chronic stages oxidative stress can still contribute to progression of inflammation. It seems that a neatly adjusted redox balance is necessary to sustain an immune state that both prevents the development of overt autoimmunity and attenuates chronic stages of disease. *Lupus* (2016) **25,** 943–954.

Key words: Reactive oxygen species (ROS); autoimmune diseases; systemic lupus erythematosus (SLE); NAPDH oxidase (NOX); oxidative stress; rheumatoid arthritis (RA)

Introduction

In the traditional view, production of reactive oxygen species (ROS) during the oxidative burst has been connected with promotion of inflammation and tissue damage, but in recent years 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.[1–14] In this review we want to illustrate how ROS are produced, what targets they modify, and how they influence various immune cell types. We further want to elaborate on how the ROS (im)balance influences the initiation, onset, and pathogenesis of several autoimmune diseases.

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Reactive oxygen species (ROS)

ROS are permanently created in the human body and often (wrongly) perceived to be associated with ''danger signals'' or it is assumed that their sole production occurs within phagocytes killing infectious agents. However, not only are ROS involved in many physiological cellular functions, but a dysbalanced ROS homeostasis is linked to a vast number of diseases – often with contradictory results and mechanisms that are still elusive. The term ''reactive oxygen species'' comprises oxygen based radicals (molecules with an unpaired electron) and their highly reactive oxidizing derivates, namely: superoxide (O_2^-), singlet oxygen ($^1O_2$), ozone (O_3), hydrogen peroxide (H_2O_2), hypohalous acids (HOI, HOBr, HOCl), hydroxyl radicals ($OH^\bullet$) and organic peroxides (e.g. lipid peroxides) (reviewed in Nathan and Cunningham-Bussel[15]) (Figure 1).

Production and sources

ROS in the human body originate from exogenous agents or endogenous production. Exogenous ROS appear for example upon contact with air pollutants, smoking, UV- or ionizing radiation and certain drugs.[15] Endogenous ROS production is

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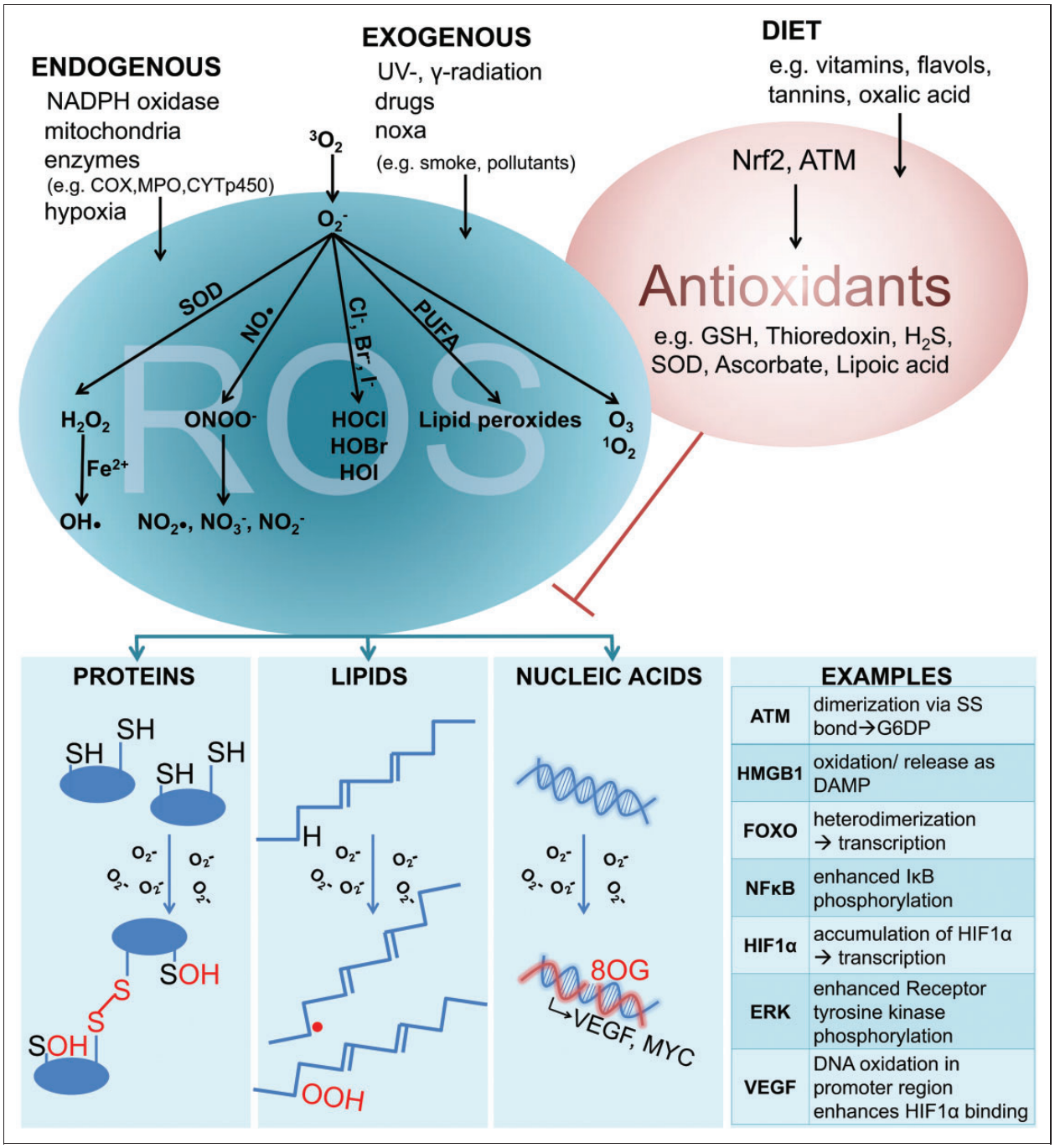

Figure 1 Sources of ROS and its cellular targets.
ROS not only occur in the human body via exogenous sources like UV radiation but are also produced endogenously by a number of enzymes such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidases. The formed superoxide (O_2^-), arising from molecular oxygen ($^3O_2$), is further converted into other ROS. Cellular targets for ROS are proteins, lipids or nucleic acids. *Protein* oxidation via ROS mainly occurs on cysteyl residues (P-SH) leading to sulfenyl groups (P-SOH) favoring further reaction with other factors such as phosphate, nitrates/nitrites and sulfides or disulfide bond formation. *Lipid* peroxidation of poly-unsaturated fatty acids (PUFAs) results in formation of fatty acid radicals in a chain reaction ultimately creating carcinogenic aldehydes, such as 4-hydroxy-2-nonenal (HNE). *Nucleic acid* bases are susceptible to reaction with ROS. In particular, the oxidation of guanine to 8-hydroxy-2′-deoxyguanosine (8-OH-dG) leads to differential binding of transcription factors or DNA damage. Together these modifications enable ROS to be mediators of redox signaling. The human body counteracts these highly reactive molecules with a tightly regulated, continuously available system of antioxidants. Many of these antioxidants utilize vitamins as cosubstrates. The general pool of antioxidants is, for the most part, replenished by dietary means.

mediated via the mitochondrial respiratory chain as well as a number of other enzymes specifically allocated for ROS creation, ROS release as a side product or reaction with metal centers.[16,17] ROS-generating enzymes include the seven isoforms of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX), Cyclooxygenases, xanthine oxidase, lipoxygenases, myeloperoxidase, ERO1, cytochrome P450 and others.[15,18] NOXs are the professional producers of ROS and are mainly expressed in phagocytic cells catalyzing the oxidative burst. Upon activation, the regulatory subunits of the multiprotein NOX complexes translocate to the catalytic transmembrane flavocytochrome domain, transferring electrons from cytosolic NADPH to extracellular O_2 forming O_2^-, which is the precursor of most other ROS.[19] The resulting O_2^- dismutation leads to the formation of the strong oxidizing agent H_2O_2, which can be partially reduced to $OH^\bullet$ or react further forming hypohalous acids (e.g. HOCl) and others. In addition, O_2^- may react with other radicals including nitric oxide ($NO^\bullet$) in a reaction controlled only by the rate of diffusion. The product, peroxynitrite (ONOO-), is also a very powerful oxidant which potently damages cells[20,21] (Figure 1).

Scavenging and antioxidants

Constant formation of highly reactive ROS warrants a thorough anti-oxidative system within the body. This system is constantly in place and based on superoxide dismutases (SODs) that convert O_2^- into H_2O_2, catalases (detoxification of H_2O_2), the glutathione redox cycle (Glutathione oxidation to Glutathionine), thioredoxins, thioredoxin reductases,[22] peroxiredoxins and methionine sulphoxide reductases and others.[15] In addition to ROS catabolizing enzymes, many small molecules react with ROS non-enzymatically. Amongst these are pyruvate, α-ketoglutarate and oxaloacetate as well as ascorbate or Vitamin E,[23] melatonin and uric acid. Additionally, the gasotransmitter H_2S was shown to scavenge ROS, especially peroxynitrite, effectively and therefore prevents apoptosis.[24,25]

An example for adaptation to rising ROS levels and cellular antioxidative protection is the nuclear factor (erythroid-derived 2)-like 2 (Nrf2). In the resting state, Nrf2 is degraded rapidly within the cytoplasm. Upon contact with ROS, Nrf2 is translocated to the nucleus where it stimulates expression of antioxidant proteins upon binding to antioxidant response elements (AREs). These proteins include gluathione-S-transferase, NAD(P)H: quinone oxidoreductase (NQO) 1, thioredoxin, thioredoxin reductases and others[26] (Figure 1).

Molecular functions of ROS

ROS are not only influencing apoptosis and microbicidal properties of phagocytes but are involved in a multitude of other cellular functions. An example is the release of ROS in platelets, which not only activates them,[27,28] but triggers the recruitment of additional platelets to the site of injury,[29,30] enables the binding of leukocytes and serves as an ROS donor for surrounding cells.[31–35] But how do ROS elicit these functions? Here, we discuss some molecular mechanisms dependent on the level of ROS a cell is challenged with as an aspect of redox signaling.

Proteins

Many proteins were shown to be modified by ROS, including tyrosine and serine/threonine phosphatases (e.g. PTEN), tyrosine and serine/threonine kinases (e.g. EGFR), zinc fingers and other transcription factors (e.g. Nrf2, see above), caspases, signal-regulating binding proteins (e.g. heat shock proteins) and others (reviewed in Nathan and Cunningham-Bussel[15]).

Modification of proteins via ROS can occur for example as transient oxidation of the cysteyl thiols (sulfenylation: P-SH → P-SOH) often found in the active site of enzymes. This transient modification promotes the reaction with other molecules (phosphorylation, nitrosylation: P-SOH → P-SNO or sulfhydration: P-SOH → P-SSH) that would otherwise not have been chemically favorable.[36] Upon elevation of ROS levels, the formation of disulfide bonds can be increased as it happens in Ataxia-Telangiectasia mutated (ATM) kinase. The formation of the disulfide bond between 2 ATM molecules promotes glucose flux through the pentose phosphate shunt, increasing the levels of NADPH being the reductant for oxidized glutathione and thioredoxin, hence enabling the restoration of redox homeostasis[37,38] (Figure 1).

High-mobility group box protein 1 (HMGB1) acts as a danger molecule with properties of a proinflammatory cytokine. Its leakage can be promoted by superoxide and peroxynitrite.[39] HMGB1 moreover is highly sensitive to changes in the redox microenvironment, executing different functions (transcription factor or DAMP) dependent on its redox state.[40]

Lipid peroxidation

ROS can react with the hydrogen atoms of polyunsaturated fatty acids (PUFAs) within the cell membranes, thus creating fatty acid radicals. These newly formed unstable radicals in turn react in a chain reaction mechanism with other fatty acids, ultimately creating malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE), with the latter being mutagenic and carcinogenic as these aldehydes react with DNA[41] (Figure 1).

Peroxidation of the mitochondrial lipid cardiolipin, which is present in the inner mitochondrial membrane and is important for energy metabolism, leads to dissociation of cytochrome c and causes reduced ATP production, and even more ROS production.[42] Enzymatic and non-enzymatic lipidoxidation has been described in both development of inflammation (e.g. artherosclerosis) and anti-inflammatory effects (e.g. clearance of apoptotic cells).[43] Enzymatic membrane oxidation has been implicated to counteract tissue damage in arthritis.[44]

Nucleic acids

Elevated ROS levels can lead to nucleic acid damage, such as base and sugar modifications, covalent crosslinks, and single- and double-stranded breaks.[45] The DNA bases, especially guanine (G), are particularly susceptible to oxidation, leading to oxidized products of nucleic acids, most frequently 8-hydroxy-2′-deoxyguanosine (8-OH-dG).[46] Mitochondria under hypoxic conditions release ROS that lead to increased binding of HIF1α and subsequent expression of VEGF.[47] This finding indicates ROS as messengers between mitochondria and the nucleus (Figure 1).

Keeping these molecular targets in mind, ROS tightly interplay with other reactive molecules such as reactive nitrogen species (RNS, $NO^{\bullet}$, $NO_2^{\bullet}$), CO and H_2S. Contradictory observations regarding ROS might, therefore, not solely be based on ROS generation and function, but this intimate interplay with other redox partners.

Role of ROS in the immune continuum

Role of ROS in the innate immune system

ROS exhibit strong antimicrobial activity, therefore it is not surprising that they play a big part in the innate immune system which comprises the first line of defense in an antimicrobial attack. The ability to undergo an oxidative burst is most pronounced in granulocytes, especially neutrophils, and macrophages.

Neutrophils

Neutrophils make up the biggest part of the innate immune effector cells and are recruited first to the site of infection. They are potent phagocytes and are able to take up pathogens and eliminate them. After engulfing bacteria, the phagosomes fuse with lysosomes to form a phagolysosome in which the oxidative burst occurs. The phagolysosome is then filled with O_2^- produced by the NOX2. O_2^- can be further converted to other ROS like H_2O_2, OCl^-, and ·OH which all together act as potent antimicrobial agents.[48] Neutrophils can furthermore undergo a process called neutrophil extracellular trap (NET) formation characterized by externalization of chromatin decorated with antimicrobial peptides and proteases.[49,50] In these NETs, bacteria are trapped and degraded. The canonical process of NET formation 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.[51,52] Neutrophil-derived ROS can also influence immune responses by activating signaling pathways like the NF-κB and the ERK pathway that modulate the inflammatory answer of the cell.[53]

Macrophages

Macrophages are important players in the innate immune defense against pathogens because of their strong phagocytic activity. Distinct macrophage subsets can be found in different tissues, like alveolar macrophages in the lung or Kupffer cells in the liver. Macrophages link in the innate immune defense to the adaptive immune system by presenting processed antigens on MHC molecules to B and T cells. Apart from their secretion into phagolysosomes, macrophage-derived ROS can also have an influence on the cells' physiologic response. ROS can have activating, but also suppressing effects on intracellular signaling like the NF-κB pathway or the ERK pathway.[53–59]

Role of ROS in the adaptive immune system

In recent years, mounting evidence indicated that ROS also play a role in the adaptive immune system. Extracellular ROS, produced by macrophages or neutrophils, or intracellular ROS,

produced by lymphocytes themselves, can affect cellular pathways.

B cells

Upon B cell receptor (BCR) activation or CD40 receptor ligation ROS can be detected in B cells.[60–62] Signal transduction and intracellular signaling in B cells can be influenced by ROS, for example by promoting tyrosine phosphorylation, by amplifying a BCR signal, or by functioning as downstream second messengers.[61,63–66] Moreover, ROS can modulate the interaction of B cells with $CD4^+$ T cells by promoting MHC II antigen presentation and influence the number of circulating memory B cells.[67–69]

T cells

T cells are often found in the vicinity of potent oxidative burst-mounting phagocytes. ROS influence T cell activation, modulate T cell receptor signaling pathways, suppress T cell proliferation, and downregulate their responsiveness.[5,70–73] A recent study reported that suppression of CD4+ effector T cells by regulatory T cells (T_{reg}) is dependent on a functional ROS production.[74] ROS are also proposed to have an influence on T cell differentiation and lineage commitment and promote the development to a T_H17 or T_H1 phenotype.[13,75] Dependent on the T cell subset ROS can act as inducers of apoptosis. Memory T cells and CD4+ T cells are stimulated to undergo apoptosis, whereas ROS have no or only limited effect on naïve and regulatory T cell death.[76–78]

Effects of ROS on autoimmune and inflammatory diseases

Chronic Granulomatous Disease

Chronic granulomatous disease (CGD) is an inherited immunodeficiency characterized by recurring bacterial and fungal infections and the formation of granulomas that obstruct vital organs.[79] Typical clinical manifestations of CGD are pneumonia, suppurative adenitis, subcutaneous and/or hepatic abscesses, osteomyelitis, and sepsis.[80] 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.[81] The most common form of CGD is X-linked and comprises around 70% of CGD patients, mostly males. It results from a mutation in $gp91^{phox}$, encoding for the catalytic subunit of NOX2.[80] Regarding the remaining patients, who inherited the mutations in a recessive autosomal manner, mutations in $p47^{phox}$ (*Ncf1*) are most common (~49%), followed by mutations in $p22^{phox}$ (~16%), and $p67^{phox}$ (*Ncf2*) (~8%).[82] The susceptibility of CGD patients to microbial infections is due to an impairment in phagolysosomal killing of pathogens by ROS and to the inability to form NETs.[4,83] It is also demonstrated that CGD patients have a reduced memory B cell compartment which has a decreasing effect on the maintenance of long term memory defense against pathogens. The same observation was made in *Ncf1* mutated mice, which exhibit a strongly reduced ROS production because of a single nucleotide polymorphism in the *Ncf1* gene.[9] There is evidence for ROS having an influence on the process of memory B cell formation, as there is a correlation between the amount of neutrophils with normal NOX2 activity and the percentage of memory B cells in CGD patients.[68,84] In contrast, circulating $CD19^+$ B cells and naïve IgD^+CD27^- B cells are elevated in CGD patients.[9] It was also shown in *in vitro* studies that ROS can have an influence on activation and proliferation of B cells. Neutralization of ROS in B cells after stimulation attenuated B cell receptor signaling.[85] Type I IFNs are known to contribute to the promotion of autoimmune diseases by inducing dendritic cell differentiation, which in turn are able to present self-material.[86] When genome-wide gene expression analysis of blood of CGD patients and *Ncf1 mutated* mice was performed, a pronounced type I IFN signature in ROS deficient individuals was found, comparable to the one in SLE patients.[9] CGD patients have an increased risk of developing SLE and have an elevated frequency of other autoimmune diseases. Case studies reported further manifestations such as antiphospholipid syndrome, juvenile idiopathic arthritis, IgG nephropathy, and Crohn-like inflammatory bowel disease.[87–92]

Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is a complex systemic autoimmune disease which affects 40 to 200 people out of 100,000, with the highest prevalence in women of child-bearing age.[93] SLE is characterized by the occurrence of autoantibodies directed against nuclear components and inflammation in multiple organs like kidneys, lung, and joints. The cause for the loss of tolerance in SLE is not yet know, but genetic predispositions associated with a higher risk of developing lupus have

been documented.[94] Jacob *et al*. identified a SNP in the *Ncf2* gene that causes a twofold decreased ROS production and showed a significant association with the occurrence of SLE.[8]

These findings are supported by analysis of lupus-prone ROS-deficient mice. Crossing of MRL.*Fas*^{lpr} with NOX2-deficient mice results in elevated features of lupus pathogenesis, which was determined by an increased production of anti-RNA and anti-Sm antibodies, exacerbated renal disease, and enhanced spleen weight. An increased number of antibody-forming cells and an expanded myeloid compartment were also found in the spleen of NOX2-deficient MRL.*Fas*^{lpr} mice.[3] Furthermore, *Ncf1* mutated mice show elevated basal levels of anti-dsDNA and anti Sm/RNP antibodies, and enhanced deposition of IgG and complement C3 in glomeruli, which suggests a promoting role of ROS-deficiency to a lupus-like phenotype.[9] Our own unpublished results show a strongly exacerbated disease course in a *Ncf1* mutated lupus mouse model induced by intraperitoneal injection of the hydrocarbon oil pristane.[95] These mice develop higher levels of anti-dsDNA, anti-histone, anti-Sm/RNP and other antibodies, suffer from lung hemorrhages, and show an enhanced glomerulonephritis as well as increased clinical signs of arthritis. In this model, worsening of the disease might be driven by aberrant phagocytosis or the inability to form NETs. Another feature of SLE is an increased risk of atherosclerosis which leads to a high mortality in affected people.[96] An early marker for the development of atherosclerosis is chronic endothelial dysfunction associated with decreased $NO^{\bullet}$ bioavailability and increased generation of ROS.[97] Lupus-prone NZBWF1 mice show an overexpression of NADPH oxidase subunits in aortic tissues, an elevated NADPH oxidase activity in aortic rings, and enhanced systolic blood pressure, next to typical lupus symptoms like renal disease and anti-dsDNA production. Administration of hydroxychloroquine, a drug used in SLE treatment, downregulated NADPH oxidase expression and increased NO production, thus improving endothelial function.[6,98] Taken together, these observations suggest that ROS might be necessary to prevent autoimmune events leading to lupus, but also might cause collateral damage during the later stages of the disease.[99–101]

Rheumatoid arthritis

Rheumatoid arthritis (RA) is an inflammatory disease characterized by persistent joint inflammation and accompanying bone and cartilage destruction, and the development of autoantibodies. Detrimental mutations in genes which encode for components involved in the oxidative burst are suggested to cause a disposition to the development of RA. Olofsson *et al*. identified gene regions in a rat model of arthritis (pristane-induced arthritis, PIA) which were involved in controlling different phases of the disease and regulating PIA severity.[102] A polymorphism in the *Ncf1* gene was associated with the arthritis phenotype. Recovery of ROS production by activation of NOX2 by phytol was shown to have a protective effect on arthritis development.[10,103] A more severe arthritis with earlier onset accompanied by enhanced cartilage destruction and higher levels of anti-collagen antibodies can also be observed in a collagen-induced arthritis (CIA) mouse model in *Ncf1*-mutated mice. Interestingly, female *Ncf1*-mutated mice also develop a spontaneous arthritis postpartum.[2] Autoimmune responses directed against cartilage-derived molecules play an important role in the development of arthritis. Therefore, recognition of type II collagen (CII) bound to MHCII on antigen-presenting cells by T cells is important for the initiation of the disease, but even so, the expression of a CII-binding MHCII is not enough to break the tolerance and promote spontaneous development of arthritis. Mice on a B10.Q background with a transgenic expression of rat CII were resistant to immunization with rat CII in a CIA-model, which showed a persisting tolerance to self-CII.[104] However, introduction of an *Ncf1* mutation in those mice led to chronic CIA, suggesting that the lack of ROS induced break of tolerance against collagen by modulation of T cell activation.[7] In line with that, a study in RA patients which analyzed various SNPs in genes encoding for the NADPH oxidase complex identified associations of changes in *Ncf4*, *Ncf2*, and *Rac2* with arthritis incidence.[105] Also polymorphisms in genes encoding for antioxidant enzymes like SODs or catalase were correlated with disease activity in RA patients.[106] Furthermore, an increased copy number of *Ncf1* can be a protecting factor, as RA patients are prone to have lower copy number of *Ncf1* compared to healthy controls.[107] Reduction of ROS by dietary intake of antioxidants seems to have an influence on arthritis incidence, too. A study showed a correlation between a high consumption of vitamin C or vitamin E by women and the occurrence of RA.[108]

These dampening effects of ROS on the development of arthritis are opposed by its deleterious actions during established joint inflammation.

In ongoing arthritic inflammation, ROS act as inflammatory mediators and contribute to tissue destruction by direct effects like the degradation of collagen by O_2^- and indirect effects.[109,110] In osteoarthritis an overproduction of ROS seems to be associated with cartilage degradation. Chondrocytes, which are essential for cartilage formation and functionality, are stimulated to undergo apoptosis by O_2^- and $NO^\bullet$. A decreased numbers of chondrocytes impairs self-repair of the cartilage and therefore promotes the breakdown of the extracellular matrix in joints.[111,112] Furthermore, chondrocyte-produced ROS like H_2O_2 lead to chondrocyte lipid peroxidation, which is linked to cartilage matrix protein oxidation and degradation.[113,114]

Gout

Arthritis in gout is caused by elevated levels of uric acid which precipitate as monosodium urate (MSU) crystals in tissues. The immense inflammatory potential of MSU crystals during acute gout is explained by induction of inflammatory cytokine release from monocytes and neutrophils.[115–117] Remarkably, gouty attacks usually fade within 3–10 days, though MSU crystals are still present in the tissue.[118] Recently, evidence for an indirect role of ROS in the resolution of these attacks was found. Schauer *et al.* showed that MSU crystals induce formation of larger aggregates of NETs that trap and degrade pro-inflammatory mediators by inherent serine proteases and therefore limit and resolve inflammation. MSU-induced formation of aggregated NETs depends on ROS production in mice and men. Therefore, induction of gouty arthritis by injection of MSU in foot pads of ROS deficient mice lead to a chronic disease course with persistent paw swelling and higher concentrations of inflammatory cytokines and chemokines.[1] These observations suggest that a functional oxidative burst is crucial for maintenance of immune tolerance and resolution of inflammation in gout.

Psoriasis

Psoriasis is a chronic, immune-mediated, skin inflammatory disease comprising a number of comorbidities and a complex, multifactorial etiology. Oxidative stress is believed to be a key factor in the pathogenesis of psoriasis.[3] Yet again, there is evidence for an alleviating effect of ROS: T_{reg} hyperfunctionality and enhanced expression of indoleamine 2,3-dioxygenase was triggered by elevated levels of ROS in an imiquimod-induced psoriasis model. Similarly, the increase of cellular ROS by hyperbaric oxygen therapy in patients suffering of psoriasis vulgaris or in imiquimod-treated mice showed a beneficial outcome. Recent studies also demonstrated the effect of photo(chemo)therapy, another ROS-inducing regime, by increasing T_{reg} cell function and reducing circulating T_h17 cells.[6] Finally, a recent paper described exacerbated mannan-induced psoriasis and development of mannan-induced arthritis in *Ncf1*-mutated mice.[7] Hence, the normal production of ROS by a functional NOX2 seems to be critical for attenuating psoriasis and arthritis phenotypes in mice.

Sepsis

In septic humans and rodents, the development of severe inflammatory response syndrome (SIRS) is connected to the loss of redox balance. In the inflammatory phase, neutrophils and macrophages produce prodigious amounts of ROS and reactive nitrogen species (RNS). This leads to continuous consumption of antioxidants together with decreased mitochondrial ATP generation and increased lipid peroxidation as well as a multitude of biochemical modifications (reversible and irreversible) on DNA, proteins and lipids (see above). Peroxidation of the mitochondrial cardiolipin leads to further mitochondrial dysfunction and further ROS production.[42] Persistently high ROS levels lead to a decreased translocation of Nrf2 further reducing the antioxidative response.[119,120]

Diabetes mellitus (type I)

Diabetes mellitus is a metabolic disease in which high blood sugar levels are caused by a dysregulation of insulin production. Type I diabetes occurs when the pancreas does not produce insulin because of autoimmune destruction of β-cells and emerges at early age. In the last years more and more evidence emerged that increased oxidative stress might play a role in the hyperglycemia-induced onset of diabetes.[121–123] An experimental mouse model demonstrated that the production of ROS plays an important role in the development of type I diabetes, as ROS deficient nonobese diabetic mice were protected in contrast to their ROS-producing counterparts.[12] Reduced ROS levels help shift macrophages to an M2 phenotype, instead to the proinflammatory M1 phenotype which induces type I diabetes.[11] It was shown that in cells of affected people, an elevated production of O_2^- occurs but, furthermore, the neutralization of ROS was impaired via depletion of antioxidants or inhibition of antioxidant enzymes.[124–130] In consequence of the hyperglycemic conditions the

increased oxidative stress can lead to damage of proteins, DNA, lipids, and also to a displaced activation of signaling pathways, for example islet β-cells are susceptible to damage through ROS.[131]

Conclusion

The myriads of data from different pathological conditions, cells types, and models show a very heterogeneous picture of how ROS influence the immune system and orchestrate autoimmune and inflammatory diseases. We conclude that ROS have a beneficial impact on the prevention of autoimmune diseases, because ROS deficiency contributes to their initiation and facilitates a disease progression. This effect is clearly demonstrated for CGD, which is the prototypic disease resulting from ROS deficiency. Furthermore, the same trend can be seen in other autoimmune manifestations such as SLE and diabetes. However, the underlying mechanisms on how ROS exert impact on disease initiation, progression and resolution still remain elusive. Defective ROS production leads to recurring microbial infections and eventually to chronic inflammation which might result in the establishment of autoimmune diseases. Additionally, decreased ROS levels may contribute to the break of tolerance by diminishing T cell anergy, and therefore boosting their reaction to self-antigens. The influence of ROS deficiency on the distribution of different cell subsets, like memory B cells, and M1 and M2 macrophages, might furthermore alter immune reactions and hence influence the development of autoimmune diseases. However, an increased ROS production can influence several signaling pathways, and therefore could drive a regular immune response toward autoimmunity. In chronic stages of autoimmune diseases, high ROS levels are often observed, causing cell and tissue damage, for example destruction of β-cells in the pancreas, or degradation of cartilage in the joints (Figure 2). Furthermore, the levels of ROS in sepsis

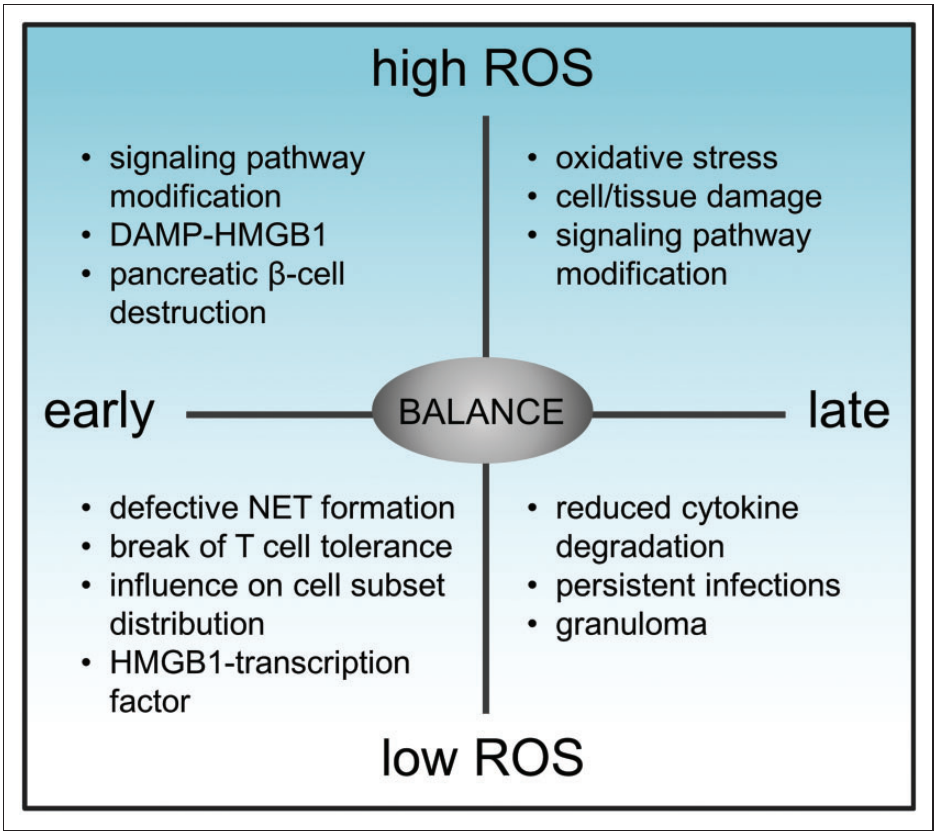

Figure 2 Influence of skewed ROS homeostasis on early and late stages of autoimmune diseases.
Low ROS levels can influence onset of autoimmune diseases by altering cellular composition, contributing to breakdown of T cell tolerance, and defective NET formation. In late stages the impaired NET formation results in reduced cytokine/chemokine degradation and hence promotes inflammation. High ROS production leads to protein modification, like the oxidation of HMGB1, resulting in its subsequent function as transcription factor or DAMP. Elevated ROS levels can modulate cell signaling to increase inflammation at onset and advanced stages of disease. During chronic phases oxidative stress can cause tissue damage.

and ischemia reperfusion injury are dramatically increased, resulting in excessive tissue damage and ultimately multi organ failure.

We conclude that balanced levels of ROS are essential to sustain health and avoid the development of autoimmune diseases. While ROS is necessary to prevent or forestall autoimmunity, it might perpetuate its progression and cause severe damage at later stages of the disease.

Declaration of Conflicting Interests

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