Redox regulation of the immune response

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Reactive oxygen and nitrogen species (ROS–RNS) and other redox active molecules fulfill key functions in immunity. Beside the initiation of cytotoxic reactions within the pathogen defense strategy, redox reactions trigger and shape the immune response and are further involved in termination and initialization of cellular restorative processes. Regulatory mechanisms provided by redox-activated signaling events guarantee the correct spatial and temporal proceeding of immunological processes, and continued imbalances in redox homeostasis lead to crucial failures of control mechanisms, thus promoting the development of pathological conditions. Interferon-gamma is the most potent inducer of ROS–RNS formation in target cells like macrophages. Immune-regulatory pathways such as tryptophan breakdown via indoleamine 2,3-dioxygenase and neopterin production by GTP-cyclohydrolase-I are initiated during T helper cell type 1 (Th1-type) immune response concomitant to the production of ROS–RNS by immunocompetent cells. Therefore, increased neopterin production and tryptophan breakdown is representative of an activated cellular immune system and can be used for the *in vivo* and *in vitro* monitoring of oxidative stress. In parallel, the activation of the redox-sensitive transcription factor nuclear factor-kappa B is a central element in immunity leading to cell type and stimulus-specific expression of responsive genes. Furthermore, T cell activation and proliferation are strongly dependent on the redox potential of the extracellular microenvironment. T cell commitment to Th1, Th2, regulatory T cell, and other phenotypes appears to crucially depend on the activation of redox-sensitive signaling cascades, where oxidative conditions support Th1 development while ‘antioxidative’ stress leads to a shift to allergic Th2-type immune responses.

Keywords: Redox-regulated, ROS, RNS, Th1-type immune response, Th1/Th2 balance

Introduction

The maintenance of redox homeostasis is crucial for appropriate functioning of cellular processes and cell survival. Imbalances between production and removal of reactive oxygen and nitrogen species (ROS–RNS) are critically involved in pathophysiological reactions. The importance of high levels of ROS–RNS produced by the respiratory burst during pathogen defense is well established.¹ However, to avoid collateral damage, increased production of reactive molecules has to be regulated spatially as well as temporally otherwise the oxidation of biomolecules leads to toxicity towards self-cellular structures and might finally result in genotoxic damage or cell death. Altered redox balances are often associated with diseases that are characterized by chronic immune activation such as infections, allergies,

autoimmune disorders, neurodegenerative diseases, and malignancies.^{2,3}

At lower levels, ROS–RNS and other small redox molecules control many different physiological processes in a dose-dependent manner, such as induction of stress responses, detoxification, immune activation, and systemic signaling, by acting as inducers of redox-sensitive pathways.⁴ Thus, the redox state is an intrinsic indicator for cellular and systemic homeostasis.

Reactive species and redox active molecules in immune response

Small reactive molecules interfere with immunoregulatory signaling cascades by modifying redox-sensing molecules, enzyme, and transcription factor activities, and/or by depleting the endogenous antioxidant pool. The main reactive species are ROS, such as hydrogen peroxide (H₂O₂), superoxide anion (O₂^{•−}), but also RNS such as peroxynitrite (ONOO[−]), nitrogen dioxide (NO₂[•]), and nitrogen trioxide, which result

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from the reaction of nitric oxide ($\text{NO}^\bullet$) with ROS.⁵ More recently, other toxic gases, such as CO and H_2S , have been identified as important small molecule immunomodulators.^{5,6} For an immediate cellular protection against these reactive species, antioxidant molecules are provided as redox buffers in different cellular compartments, among them extensively studied are thiol/disulfide couples glutathione/glutathione disulfide (GSH/GSSG), cysteine/cystine (Cys/CySS), and thioredoxins (Trx).⁷ Besides acting as monitoring systems for the redox status, these buffer molecules regulate a variety of responsive enzymes and pathways.⁸

Cellular ROS and RNS production

Under normal conditions, mitochondria are the main site of intracellular oxygen consumption and the main source of ROS ($\text{O}_2^\bullet$) formation.⁹ Other endogenous sources of ROS include the membrane-bound NADPH-dependent oxidases (NOX), lipoxygenase, cytochrome P-450, and xanthine oxidase.^{10–12} While in general ROS production occurs as a byproduct of biological reactions, the sole function of the NOX enzyme family is the production of ROS by reducing molecular oxygen to $\text{O}_2^\bullet$, and phagocyte NADPH oxidase was the first NOX identified to be responsible for the high amounts of $\text{O}_2^\bullet$ and H_2O_2 produced during the respiratory burst reaction.^{13,14} In the resting cell, the NOX components are separated at the plasma membrane and the cytosol. Upon stimulation, membrane-bound flavocytochrome B, the catalytic core of the respiratory-burst oxidase, assembles with proteins of cytosolic origin (the small G protein Rac, p47phox, and p67phox) that activate the production of $\text{O}_2^\bullet$.¹⁵

$\text{NO}^\bullet$ is synthesized during the conversion of L-arginine to L-citrulline by nitric oxide synthase (NOS) and is classically viewed as a regulator of the vasomotor tone. $\text{NO}^\bullet$ activates several signaling pathways in a dose-dependent manner as it is able to migrate through cell membranes by diffusion.^{5,16} Although of low reactivity, $\text{NO}^\bullet$ is a potent antioxidant molecule that can protect from ROS injury.^{17,18} Low amounts of $\text{NO}^\bullet$ (nM) are normally produced by the constitutively expressed endothelial and neuronal NOS and are involved in the regulation of physiological processes such as vasodilation and neurotransmission.¹⁹ Upon activation of inducible NOS (iNOS) due to inflammatory stimuli such as cytokines, e.g. interferon-gamma ($\text{IFN}\gamma$) and tumor necrosis factor-alpha, or lipopolysaccharide, $\text{NO}^\bullet$ levels in the cellular microenvironment can rise to micromolar concentrations.²⁰ These high amounts of $\text{NO}^\bullet$ may have cytostatic effects on parasitic target cells but also contribute to the pathophysiology of inflammatory diseases and septic shock.²¹ At sites of inflammation

and phagocyte activation where enhanced rates of $\text{NO}^\bullet$ and $\text{O}_2^\bullet$ production occur, $\text{NO}^\bullet$ and $\text{O}_2^\bullet$ can combine to form ONOO^- .^{22,23}

ROS–RNS production for bacterial killing during the respiratory burst is concentrated in the phagosome to minimize collateral damage. The regulation of the phagosomal pH and $\text{O}_2^\bullet$ production follows distinct schemes in neutrophils, macrophages, and dendritic cells (DC) due to differences in the localization of NOX and the phagosomal $\text{NO}^\bullet$ concentrations.²⁴ The presence of $\text{O}_2^\bullet$ and $\text{NO}^\bullet$ in the phagosome leads to the generation of nitrite and thus to the formation of $\text{NO}_2^\bullet$ and nitrogen trioxide. Also, peroxidase activity may lead to the consumption of $\text{NO}^\bullet$, leading to limited $\text{NO}^\bullet$ bioavailability and the formation of nitrite, which is further oxidized to $\text{NO}_2^\bullet$.^{5,23}

The diversity of chemical species generated from $\text{NO}^\bullet$ and ROS–RNS provides a refined pathogen defense strategy that goes far beyond the simple killing of microbes.^{24,25} The diversity of NOS activities can produce different temporal and concentration profiles of $\text{NO}^\bullet$ and, depending on the concentration of ROS, redox profiles may change. The ROS–RNS balance is crucial for the regulation of immunological mechanisms and both oxidative and nitrosative stress can interfere with intracellular redox buffer systems, resulting in a temporally decreased antioxidant capability of the affected cells.⁵

Endogenous antioxidant molecules

Antioxidant molecules provide the cell with redox buffer capacities and have been identified to play an important role in regulating immune responses. Trx, GSH/GSSG, and Cys/CySS are largely independently regulated in different subcellular compartments.⁷ Quantitatively, GSH is the major intracellular redox buffer in mammalian cells and GSH/GSSG couples can be found in cytoplasm, nucleus, mitochondria, and other organelles although with different redox potential.⁷ Upon secretion, the majority of GSH is cleaved into its components and the resulting cysteines fill the extracellular Cys/CySS pool, representing the major thiol/disulfide redox buffer outside the cell.²⁶ Mammalian Trx are selenoproteins that additionally have cytokine-like properties when secreted. In the extracellular space, Trx can act as a chemotactic factor for monocytes, polymorphonuclear leukocytes, and T cells.²⁷

Several metabolic routes link the extracellular redox potential to the intracellular buffer system thus paralleling changes that occur in the cellular environment.²⁶ Although the thiol/disulfide buffers share similar functional properties and largely compensate for each other, each system has some unique functions and may support the reduction of some different subsets of proteins.^{7,27} Beside counteracting the

influence of ROS, these redox buffers represent important elements of a variety of signal transduction networks, e.g. in the activation of T cells and their differentiation into effector T cell subsets.²⁸

Key signaling cascades during the Th1 immune response

In a cell-mediated immune response, various T cell subsets are involved, e.g. antigen-specific activated Th1-type cells secrete cytokines and activate T effector cells such as cytotoxic T cells and nonspecific effector cells such as natural killer cells and macrophages. The pro-inflammatory cytokine IFN γ plays a central role in the Th1-type immune response, as it induces a variety of cellular responses.²⁹ IFN γ signaling initiates pathogen and tumor defense mechanisms in target cells and triggers NOX-mediated ROS formation during the respiratory burst reaction.³⁰ Further, several immunoregulatory enzymes such as guanosine triphosphate-cyclohydrolase-I (GTP-CH-I), indoleamine 2,3-dioxygenase (IDO), and iNOS are activated during the immune response (Fig. 1). Pro-inflammatory cytokines, most importantly again IFN γ , as well

as lipopolysaccharide-induced Toll-like receptor 4 signaling activate GTP-CH-I and IDO.^{31–33}

The activation of GTP-CH-I leads to the production of neopterin and 5,6,7,8-tetrahydrobiopterin (BH₄), an essential cofactor for several monooxygenases, including iNOS.³³ While human fibroblasts or endothelial cells preferentially produce BH₄, human and primate monocytes/macrophages are the most relevant source of neopterin and its reduced form 7,8-dihydroneopterin, which are both present at a relatively constant ratio in human serum.³ Both neopterin and 7,8-dihydroneopterin interfere with oxidative signaling pathways, e.g. they are involved in the activation of the redox-sensitive transcription factor nuclear factor kappa B (NF- κ B).³⁴ Reduced pteridine derivatives like 7,8-dihydroneopterin are potent antioxidants, while neopterin was found to support the generation of chloride metabolites and to enhance their toxic effects during the respiratory burst reaction in human neutrophils.^{35,36} Thus, neopterin and 7,8-dihydroneopterin may influence cellular and systemic redox balances.³⁷ High levels of neopterin were found to be associated with increased production of ROS and with low serum concentrations of antioxidants.^{3,38} Aside from its possible pathophysiologic relevance, neopterin has gained importance in clinical diagnostics as a way to monitor Th1-type responses, which are associated with a variety of disorders.^{39,40}

IDO catalyzes the rate-limiting step in the oxidative degradation of the essential amino acid tryptophan (Trp). The depletion of the essential amino acid Trp produces an antiproliferative environment that counteracts the growth of pathogens or malignant cells and thus represents a primary mechanism of cytotoxicity.⁴¹ The ratio of kynurenine to Trp (Kyn/Trp) can be used as an estimate of IDO activity.⁴² Kyn/Trp has been determined in a variety of patient cohorts and, like increased neopterin, elevated Kyn/Trp has been revealed to be a sensitive indicator for an activated immune system.⁴³

Beside monocytes/macrophages, a variety of other cell types such as endothelial cells, epithelial cells, and fibroblasts exhibit IDO activity. IDO is a haem-containing enzyme that exerts antioxidant activity by scavenging of O₂^{•-}, which can be utilized as a substrate for the oxidative metabolism of Trp but also as a cofactor. Reductants such as O₂^{•-} bring (Fe₃⁺)-IDO to its active ferrous form.⁴⁴ NO[•] and H₂O₂ inhibit IDO enzyme function and interfere with various steps of Trp catabolism.⁴⁴

In addition to its importance in protein biosynthesis, Trp is a precursor for the generation of the neurotransmitter 5-hydroxytryptamine (serotonin) by the BH₄-dependent Trp 5-hydroxylase, thus providing a rational link to inflammation-induced depression. This could be due to the serotonin deficiency caused

Figure 1 During an immune response, the neopterin-producing enzyme GTP-cyclohydrolase-I (GTP-CH-I), the nitric oxide (NO[•])-forming nitric oxide synthase (iNOS), and tryptophan-converting enzyme indoleamine 2,3-dioxygenase (IDO) are stimulated. Macrophages (M ϕ) and dendritic cells (DC) produce neopterin. Other cells like endothelial cells (EC) and fibroblasts (FB) form tetrahydrobiopterin (BH₄), the cofactor of iNOS, thus contributing to the release of NO[•]. As NO[•] inhibits the expression and function of IDO, tryptophan levels accumulate.

by the limited supply of Trp and the oxidation of BH₄ in a pro-oxidative environment, and due to the accumulation of neurotoxic Kyn metabolites.^{45,46}

iNOS expression is upregulated in macrophages upon stimulation, but can also be found in a number of cell types including epithelial cells, astrocytes, and endothelial cells.^{5,21} iNOS activity does not depend on Ca²⁺/calmodulin and produces high rates of NO• in the presence of L-arginine and NADPH.

The amount of NO• production differs in distinct mammalian species, e.g. NO• is more prominently produced in rodents than in humans and primates. One explanation might be that, in human macrophages, activation of GTP-CH-I during a Th1-type immune response leads to the production of neopterin at the expense of BH₄, a cofactor required for normal NOS function.³³ This peculiarity of BH₄ and neopterin biochemistry might serve as an explanation for why in humans the inflammation is often associated with high blood pressure due to vasoconstriction, rather than vasodilation as is for example the case in coronary artery disease. Interspecies variability in NO• production represents one of the limitations that can be faced when experimental data are extrapolated from murine data to the human situation.

NOS enzymes can produce O₂^{•−} in addition to or instead of NO• and this balance is regulated by ROS–RNS in a dose-dependent manner. Oxidant stress decreases NO• generation, this effect being largely mediated via BH₄ depletion.²² While ROS induce a reversible inhibition of iNOS, ONOO[−] promotes an irreversible enzyme inhibition. In the absence of L-arginine or because of BH₄ depletion, production of NO• from iNOS becomes uncoupled from the oxidation of NADPH, thus resulting in prominent O₂^{•−} generation.⁴⁷ iNOS uncoupling is further associated with NF-κB activation.

Both oxidative and nitrosative stress are associated with the expression of pro-inflammatory genes and cytokines such as tumor necrosis factor-α, interleukin-6 (IL-6), and IL-1β.^{48,49} In this context, the most prominent regulatory molecule is the redox-sensitive transcription factor NF-κB, which regulates a variety of genes that control the immune response.⁵⁰ There are many inducers of NF-κB, including cytokines or reactive small molecules, and, depending on the cell type, the route of induction, the presence of synergistically acting signals, and the cross-talk with other pathways, the expression of selected gene sets is induced.⁵¹ Gene induction follows a temporal order, early genes are involved in neutrophil recruitment to the site of inflammation, then genes that are responsible for macrophage and lymphocyte recruitment are expressed to provide a second wave of defenders, and late gene products are involved in restorative processes such as wound healing, thus

resolution of inflammation is also a critical aspect of NF-κB control.^{51,52} NF-κB activity is regulated by ROS–RNS dose-dependently, thereby reactive species might not only interfere directly with the transcription factor or its inhibitor complex, but may react also with the redox-sensitive upstream phosphatases and kinases that are involved in NF-κB regulation.⁴⁹ Also, the modulation of the cytosolic and nuclear redox status might indirectly interfere with redox-sensitive transcription events.

Redox regulation of T cell activation and differentiation

The generation of both humoral and cell-mediated responses depends on T helper activation and this process is strongly dependent on the redox potential of the microenvironment.²⁶ Lymphocytes require a reducing milieu for optimal activation, e.g. immunization is associated with a striking increase in free thiols in lymphoid tissue.^{53,54}

The priming of naive T cells involves the specific engagement of the T cell receptor by antigen–MHC class II complex, the interaction between co-stimulatory molecules (CD28 on T cells and CD80/86 on the antigen presenting cells (APCs)), as well as secretion of cytokines.²⁶ Further, a reducing extracellular microenvironment in the immune synapse is needed to facilitate an immune response. Naive T cells are metabolically dependent on APCs as they do not express the cystine transporter x_c[−], and thus require exogenous thiols for activation and function.⁵³ Upon cross-talk with T cells, APCs stimulate cysteine production and secretion via different metabolic routes and also extracellular Trx1 is augmented.^{26,53,55}

The reducing microenvironment might provide local protection from oxidative stress during T cell activation, but low levels of ROS are essential for the onset of the immune response.^{26,56} T cell receptor engagement results in enhanced intracellular H₂O₂ production that is needed for IL-2 and IL-2 receptor α chain gene transcription and NF-κB activation, thus expression of inflammatory genes and cytokines.⁵⁷ Furthermore, when a response to antigen is induced GSH synthesis is stimulated, which serves an important proliferative signal for T cells and further redox-sensitive signaling cascades that promote activation and proliferation, e.g. AP1, cell cycle proteins are activated.^{58,59}

A pro-oxidant environment in the initial phase of an immune response might facilitate priming of T cells, but sustained pro-oxidant conditions would lead to the inhibition of T cell proliferation and finally induce apoptosis.⁶⁰ Furthermore, redox signaling appears to influence T cell commitment and various T cells have different redox statuses and thus different ROS susceptibility.²⁸

As T cell proliferation also depends on Trp availability, the metabolic control via IDO activation represents a negative feedback loop for immune activation.⁶¹ IFN γ -induced IDO signaling is crucial for the generation of regulatory T cells that are involved in the suppression of autoimmune responses and promote tolerance.^{62,63} The control of Trp metabolism by IDO in mouse DC mediates protection against exaggerated responses through the combined effects of Trp starvation and Kyn metabolites acting via the aryl hydrocarbon receptor of T cells.⁶⁴ Also

arginase secreted by neutrophils during inflammation plays a role in T cell regulation by competing for arginine and thus suppressing T cell proliferation.^{5,65}

Furthermore, T cell commitment to Th1 or Th2 appears to crucially depend on the activation of redox-sensitive signaling cascades, where oxidative conditions support Th1 development while 'antioxidative' stress leads to a shift to allergic Th2 responses (Fig. 2).^{66–68}

It has been shown in several *in vitro* studies with PBMC from healthy donors that a variety of antioxidant compounds including vitamins, phytochemicals,

Figure 2 Redox balance is critically involved in the regulation of T cell activation, proliferation, and in shifting of the T cell phenotype. Stimulation of naive T cells (Th0) by dendritic cells (DC) is followed by thiol induction that is critical for the survival of T cells in an oxidative milieu. A Th1-type immune response is characterized by the secretion of pro-inflammatory cytokines, mainly interferon gamma (IFN γ) and interleukin-2 (IL-2), and leads to the activation of macrophages (M ϕ) and cytotoxic T cells (CTL). During a Th2-type immune response, IL-4, -5, -6, and -13 are secreted and B cells are stimulated to produce antibodies. Th1 and Th2-type immune responses are cross-regulated, suppression of Th1 reactions favors Th2 responses and *vice versa*. Whereas oxidative conditions are involved in Th1 reactions, antioxidative stress preferentially activates Th2-type responses, which may result in ineffective pathogen defense and development of allergies. On the other hand, overwhelming production of ROS–RNS due to a chronic activation of the immune system might lead to severe damage due to the oxidation of biomolecules.

preservatives, and colorants were able to suppress mitogen-induced Trp degradation and neopterin production in a dose-dependent manner.⁶⁹ Also, DCs treated with vitamin C and E become resistant to phenotypic and functional changes following stimulation with pro-inflammatory cytokines, and allogeneic T cells were anergized following exposure to vitamin-treated DCs and secreted higher levels of Th2 cytokines and IL-10 than cells incubated with control DCs.⁷⁰ Due to the balance of Th1 and Th2 immunity, antioxidants may facilitate a shift towards Th2-type responses by suppression of Th1-type mechanisms.⁷¹

NO• represents an additional signal for the induction of the T cell subset response.⁵ NO• generally decreases T cell responses but low doses of NO• selectively enhance the differentiation of murine and human Th1, but not Th2, cells *in vitro*.⁷² This enhancement is achieved by the direct action of NO• on T cells in synergism with IL-12 produced by APCs.^{72,73}

Conclusion

ROS–RNS intermediates serve as an arsenal for pathogen defense but in lower amounts they act as signaling molecules. Small redox molecules are effective immunomodulators that regulate cellular metabolism as well as multiple inflammatory pathways and are crucially involved in restorative and immunosuppressive processes. Effects depend on the cellular context, the location, and duration of exposure. Several immune-associated redox pathways are further involved in the regulation of metabolic functions and thus control the proliferative capacities of immunocompetent cells. Redox imbalances are implicated in the development and progression of a variety of pathological conditions and this is mostly suggested to be mediated through a sustaining pro-oxidant environment. However, oxidative stress is not necessarily an unwanted situation and may also result in beneficial reactions. In this context the consumption of large amounts of ‘health-promoting’ exogenous antioxidants, which will reach relatively high concentrations in the gastrointestinal tract, should be considered with caution, since an exaggerated increase of the antioxidant potential might cause adverse effects due to ‘antioxidative stress’.

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