

REVIEW ARTICLE

ROS in gastrointestinal inflammation: Rescue Or Sabotage?

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Received 23 September 2015; **Revised** 09 December 2015; **Accepted** 7 January 2016

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The intestine is composed of many distinct cell types that respond to commensal microbiota or pathogens with immune tolerance and proinflammatory signals respectively. ROS produced by mucosa-resident cells or by newly recruited innate immune cells are essential for antimicrobial responses and regulation of signalling pathways including processes involved in wound healing. Impaired ROS production due to inactivating patient variants in genes encoding NADPH oxidases as ROS source has been associated with Crohn's disease and pancolitis, whereas overproduction of ROS due to up-regulation of oxidases or altered mitochondrial function was linked to ileitis and ulcerative colitis. Here, we discuss recent advances in our understanding of how maintaining a redox balance is crucial to preserve gut homeostasis.

LINKED ARTICLES

This article is part of a themed section on Redox Biology and Oxidative Stress in Health and Disease. To view the other articles in this section visit <http://onlinelibrary.wiley.com/doi/10.1111/bph.v174.12/issuetoc>

Abbreviations

5-ASA, 5-aminosalicylic acid; CD, Crohn's disease; CGD, chronic granulomatous disease; DC, dendritic cells; DSS, dextran sodium sulphate; FAK, focal adhesion kinase; GI, gastrointestinal; IBD, inflammatory bowel disease; IEC, intestinal epithelial cells; LP, lamina propria; mETC, mitochondrial electron transport chain; mtROS, mitochondrial ROS; NSAIDs, nonsteroidal anti-inflammatory drugs; PHB, prohibitin; PMN, polymorphonuclear leukocyte; RNS, reactive nitrogen species; SI, small intestine; SOD, superoxide dismutase; TNBS, 2,4,6-trinitrobenzenesulfonic acid; UC, ulcerative colitis; VEOIBD, very early onset IBD

Tables of Links

TARGETS		LIGANDS	
Enzymes^a	p38	5-ASA, 5-aminosalicylic acid (mesalazine)	IL-17
eNOS, NOS3	RIPK3	Allopurinol	Infliximab
iNOS, NOS2	Catalytic receptors^b	Epiregulin	LPS
nNOS, NOS1	NLRP3	H ₂ O ₂	NO
ERK	PRR, pattern recognition receptors	IFN- γ	PGI ₂
Focal adhesion kinase	TLR2	IL-1 β	TGF- β
Mitochondrial F ₁ F ₀ -ATPase	GPCRs^c	IL-10	TNF- α
Myeloperoxidase	FPR1, formyl peptide receptor 1	IL-13	

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 (^{a,b,c}Alexander *et al.*, 2015a,b,c).

Introduction

The production of ROS is a key event in the progression of many inflammatory disorders, including those involving the gastrointestinal (GI) tract. In the gut, disruption of the mucosal barrier will rapidly activate the innate immune system and set off an acute inflammatory response that begins in the lamina propria (LP). Polymorphonuclear leukocytes (PMNs) migrate to the site of infection or injury, engulf invading pathogens and secrete ROS, granular enzymes, and vasoactive and pro-inflammatory mediators (Mittal *et al.*, 2014). Production of ROS by PMNs can create a hypoxic niche due to oxygen consumption, which may aid in the resolution of inflammation (Campbell *et al.*, 2014). However, uncontrolled and persistent oxidative stress with overproduction of ROS and/or inadequate removal of ROS by antioxidant systems will cause apoptosis and tissue injury (Rezaie *et al.*, 2007). In particular, deregulation of the mitochondrial electron transport chain (mETC), with increased mitochondrial ROS (mtROS) levels, was observed in inflammatory bowel disease (IBD) patients and decreasing mtROS ameliorated colitis (Dashdorj *et al.*, 2013). Tightly controlled ROS generation by enzymes expressed in non-phagocytic cells, for example enterocytes or fibroblasts, paved the way towards the concept of ROS as regulators of intracellular processes. Indeed, low physiological levels of ROS are indispensable for a plethora of signalling pathways (Holmstrom and Finkel, 2014), including gene transcription, protein kinase activation and phosphatase inhibition, thereby regulating cytokine production and coordinated cell motility (Peterson and Artis, 2014). The importance of maintaining physiological levels of ROS in the gut is highlighted by the chronic intestinal inflammation present in patients with inactivating gene defects in NADPH oxidase. The consequences of altered ROS levels on multifactorial GI inflammation are still not well understood, but the importance of maintaining the redox balance is emerging. This review aims to highlight the most relevant findings in the field of redox regulation of GI inflammation by discussing the intriguing and debated role of ROS at the crossroad between antimicrobial and pro-inflammatory agents, and our view that a tight regulation of ROS is a prerequisite for a healthy gut.

Mucosal immunity in the GI tract

From the oral cavity to the anus, the GI epithelium functions as a physical barrier confining an extraordinary number of bacteria, collectively known as microbiota, and potentially harmful pathogens or substances, to the lumen of the GI tract. The GI epithelium is composed of a single cell layer that undergoes turnover approximately every 4–5 days and has the task of sensing the *phyla* and quantity of bacteria and their distance from the cellular surface. Total bacterial numbers increase along the GI tract, ranging from 10^2 – 10^3 per mL in the highly acidic environment of the stomach to 10^5 per mL in the upper small intestine (SI) and up to 10^{12} per mL in the colon (Sekirov *et al.*, 2010). The intestinal mucosa responds to infectious pathogens with inflammatory signals

and to harmless commensals with immune tolerance signals. General defence mechanisms include the secretion of polysaccharides by goblet cells that form a mucus bilayer towards the lumen, release of antimicrobial peptides by Paneth cells and secretory IgA by plasma cells (McGuckin *et al.*, 2011; Peterson and Artis, 2014). Maintaining the integrity of the barrier is an essential prerequisite for intestinal homeostasis. In the case of barrier damage, the capacity of rapidly resealing the epithelial layer is critical to avoid or minimize the exposure of immune cells to microbiota, which would lead to the initiation of an inflammatory response (Figure 1).

During homeostasis, when the epithelial barrier is intact, the sampling of luminal gut antigens that determines the mucosal 'tolerance' is mediated by microfold cells in the dome epithelium overlying Peyer's patches, intestinal epithelial cells (IECs), subsets of innate lymphoid cells (Hepworth *et al.*, 2015), $\gamma\delta$ T cells (Nanno *et al.*, 2008) and dendritic cells (DCs), the latter directly extending dendrites into the gut lumen (Chieppa *et al.*, 2006). Antigenic signals are transduced into the LP, a loose connective tissue containing lymphocytes, plasma cells, eosinophils, mast cells, neutrophils and a large number of macrophages (Figure 1). Macrophages and DCs are maintained in an anergic, non-inflammatory state by the predominance of T regulatory cells, which secrete the immunosuppressive cytokines IL-10, an inhibitor of effector T cell responses, and TGF- β , an inducer for differentiation of conventional CD4⁺ T cells into T regulatory cells. The epithelial cell, once thought to be a purely absorptive cell type, is now considered similarly to LP cells a crucial effector of mucosal immunity. IECs express a collection of membrane and cytosolic pattern recognition receptors (PRRs) that recognize distinct microbial ligands. These receptors include toll-like receptors (TLRs), NOD-like receptors and RIG-I-like receptors (Peterson and Artis, 2014). Seminal studies have defined beneficial roles of PRR signalling by IECs. These include the expression of the EGF receptor ligands, amphiregulin and epi-regulin, the production of the cytoprotective trefoil factor-3, the activation of the NLRP3 inflammasome and NEMO (I κ B kinase- γ)-dependent NF- κ B signalling (Wullaert *et al.*, 2011; Fukata and Arditi, 2013).

Altered barrier function (or 'leaky gut') permits the translocation of bacteria and microbial products into the LP, inducing a series of events ranging from activation of professional phagocytes (PMNs and macrophages) and ROS production for antimicrobial defence to pro-inflammatory cytokine generation by effector Th1, Th17 and NK cells. At the same time, epithelial cells produce ROS, which participate in regulating intracellular signals for barrier repair and healing (Figure 1).

Dysfunction of biological systems regulating the balance between 'low, tolerable' and 'high, toxic' levels of ROS can lead to persistent and unresolved mucosal inflammation. Incorrect levels of oxidants, too low or too high, alter the redox balance, thereby triggering pathological processes. Deregulation of the redox balance compromises antimicrobial defence, prolongs immune activation and alters innate and adaptive immune responses such as activation of transcription factors, inflammasome function or autophagy. The ensuing incomplete pathogen clearance and/or oxidative stress not only perpetuates inflammation but may also eventually lead to other chronic complications, such as fibrosis, neoplasia and extra-intestinal symptoms (Neurath, 2014).

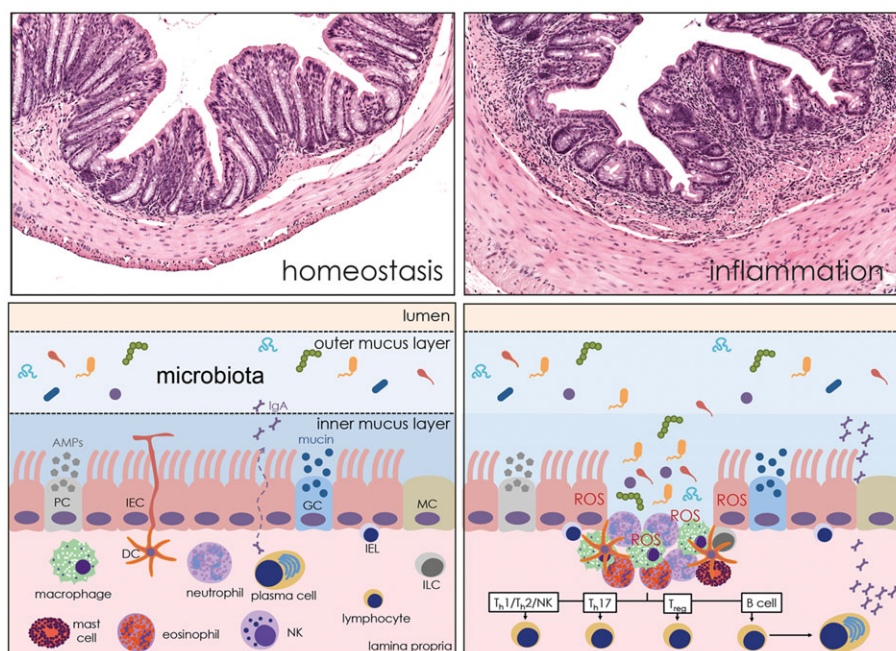


Figure 1

Schematic representation of the GI mucosal immunity. Gut tolerance results from the coordinated action of epithelial, innate and adaptive immune cells. In the event of epithelial barrier failure, the microbiota is detected by innate immune cells of the lamina propria, which then orchestrate appropriate inflammatory and healing responses, in part by releasing ROS. AMP, antimicrobial peptides; GC, goblet cell; IEL, intraepithelial lymphocyte; ILC, innate lymphoid cell; MC, microfold cell; PC, Paneth cell.

Sources of ROS in the GI tract

ROS are small molecules, including the oxygen radicals [superoxide ($O_2^{\bullet-}$) and hydroxyl ($\bullet OH$)] and non-radicals such as hypochlorous acid (HOCl), singlet oxygen (1O_2) and hydrogen peroxide (H_2O_2). Unstable oxygen-centred radicals are highly reactive and can lead to deleterious effects in cells and tissues. $O_2^{\bullet-}$ can be converted to more stable and diffusible H_2O_2 by superoxide dismutase (SOD)-mediated catalysis (Figure 2). H_2O_2 is decomposed to water and oxygen or can be converted to HOCl (100 times more toxic than H_2O_2) by peroxidases, such as phagocytic myeloperoxidase, in the presence of chloride ions (Cl^-). H_2O_2 can also react non-enzymically with $O_2^{\bullet-}$ to form $\bullet OH$ in the presence of ferrous (Fe^{2+}) iron in the so-called Fenton reaction (Winterbourn, 1995). In the presence of NO, a crucial mediator of GI physiology, $O_2^{\bullet-}$ rapidly reacts with NO, thereby forming the highly reactive peroxynitrite ($ONOO^-$) (Figure 2). Additional reactions with peroxynitrite give rise to many other NO-derived compounds, collectively known as reactive nitrogen species (RNS) (Mittal *et al.*, 2014). Prolonged production of high concentrations of ROS can irreversibly destroy or alter the function of target molecules, causing DNA damage, lipid peroxidation and protein oxidation. Although historically viewed as harmful, low and moderate concentrations of ROS are vital for homeostasis and beneficial in physiological processes, including the regulation of intracellular signalling pathways such as reversible thiol oxidation of reactive cysteine residues within regulatory proteins (Holmstrom and Finkel, 2014). In the GI tract, the main sources of ROS and RNS are NADPH oxidase enzymes [NOX/dual oxidase (DUOX)], the mETC and NOSs.

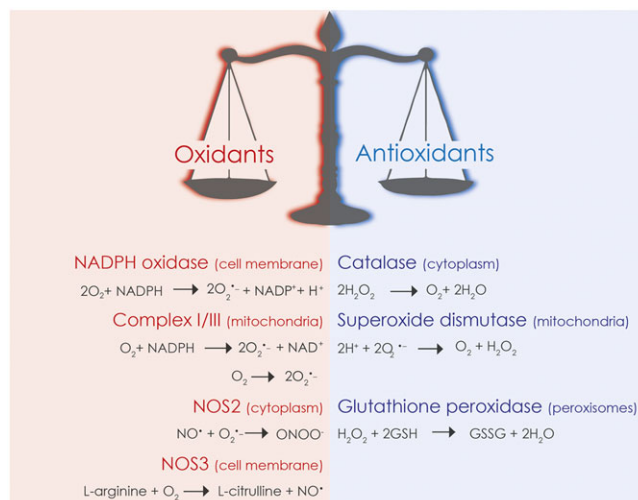


Figure 2

Main sources of ROS/RNS and antioxidant defence systems in the GI tract. Gut homeostasis will be compromised when the capacity of antioxidant enzymes is overcome by enhanced oxidant production.

NADPH oxidases

The membrane-bound multimeric NOX and DUOX complexes are the only known enzymes generating ROS not as byproduct but rather as their primary function (Lambeth and Neish, 2014). The mammalian oxidase family comprises five NOX members (NOX1–5) and two dual oxidases (DUOX1–2). With the exception of NOX5 that is not expressed in rodents, both

humans and mice express all oxidase isoforms. NOX and DUOX homologs differ in their structures, mechanisms of activation and tissue expression patterns. The main oxidases expressed along the GI tract are NOX1 and DUOX2 found in the epithelium (NOX1 in the ileum, cecum and colon; DUOX2 in all segments of the gut), NOX2 expressed by professional phagocytes and DCs, and NOX4 present in the epithelium, fibroblasts and smooth muscle cells (Bedard and Krause, 2007; Mandal *et al.*, 2010) (Figure 3).

Originally discovered in neutrophils in the late 1980s, the O_2^- generating NOX2 oxidase constitutes an essential part of the antimicrobial repertoire of innate immune cells. The NOX2 enzyme is a multi-subunit complex with a catalytic core termed flavocytochrome b_{558} , composed of the two transmembrane proteins $p22^{phox}$ and $gp91^{phox}$ (renamed NOX2). NOX2 is typically inactive in resting cells, but in the presence of pathogen-derived stimuli, multiple activation steps take place, initiating post-translational modifications and translocation of cytosolic subunits ($p40^{phox}$, $p47^{phox}$ and $p67^{phox}$) and activated Rac GTPase to the membrane. Association of the NOX2- $p22^{phox}$ heterodimer, and assembly of a functional complex, permits electron flow from NADPH as

electron donor to FAD, both located in the cytosolic region of NOX2, to two low potential haems to convert molecular oxygen (O_2) to $O_2^{\bullet-}$. This so-called oxidative burst into the phagosome or to the outside environment supports elimination of pathogens. Activated neutrophils can capture and kill pathogens by receptor-mediated phagocytosis and by releasing complex three-dimensional structures called neutrophil extracellular traps containing nuclear chromatin, associated mainly with nuclear histones, and several antimicrobial granule proteins, a process that requires NOX2-derived superoxide (Kirchner *et al.*, 2013). If the ability of phagocytes to generate $O_2^{\bullet-}$ is compromised, the host's antimicrobial defence is weakened leading to an increased risk of developing infections with significant morbidity and/or mortality. This condition is known as chronic granulomatous disease (CGD) (van den Berg *et al.*, 2009; O'Neill *et al.*, 2015) and is an inherited immunodeficiency disorder caused by inactivating mutations in the genes encoding the NOX2 complex (*CYBB*, *CYBA*, *NCF1*, *NCF2* and *NCF4*) and is characterized by recurring and life-threatening bacterial and fungal infections due to diminished or abolished pathogen killing. CGD consists of a heterogeneous group of disorders ranging from pulmonary to dermatological and GI complications, often accompanied by the formation of granulomas in several organs. Loss-of-function variants of X-linked *CYBB* occur in about 70% of the reported CGD cases, while variants in *CYBA*, *NCF1* and *NCF2*, encoding $p22^{phox}$, $p47^{phox}$ and $p67^{phox}$, respectively, are less frequent, with *NCF1* occurring in 25% of CGD patients. So far, only one *NCF4* variant (encoding $p40^{phox}$) has been described (Matute *et al.*, 2009; O'Neill *et al.*, 2015).

Following the discovery of NOX2, homology screening revealed six additional NADPH oxidase family members in mammals. Here, we will focus primarily on oxidases expressed in the gut. X-linked NOX1, evolutionarily close to NOX2 (sharing ~60% homology with NOX2), requires heterodimerization with $p22^{phox}$ and assembly with cytosolic subunits and activated Rac GTPase to produce $O_2^{\bullet-}$. In the case of NOX1, the cytosolic subunits are termed NOXO1 and NOXA1 (for NOX1 organizer and activator respectively) and are homologues of $p47^{phox}$ and $p67^{phox}$. Although found in LP lymphocytes and other cell types (Szanto *et al.*, 2005), NOX1 is often called 'colon NADPH oxidase' due to the high expression in colon epithelium. The enzyme remains inactive until pro-inflammatory cytokines, such as IFN- γ (Geiszt *et al.*, 2003a), or bacterial products, such as LPS induce up-regulation, translocation and activation (Corcionivoschi *et al.*, 2012; O'Leary *et al.*, 2012).

Commonly found in kidney cortex, but also in fibroblasts, smooth muscle cells and the intestinal epithelium, NOX4 shares only 39% identity with NOX2. NOX4 regulation is distinct as it is considered constitutively active and preferentially releases H_2O_2 . NOX4 was shown to be involved in oxygen sensing and cellular senescence (Geiszt *et al.*, 2000; Shiose *et al.*, 2001). Unlike other NOXs, NOX4 does not require regulatory binding partners after the NOX4- $p22^{phox}$ complex is formed (von Lohneysen *et al.*, 2008). Certain stimuli including TGF- β or hypoxia induce transcriptional up-regulation of NOX4 accompanied by continuous H_2O_2 generation (Hecker *et al.*, 2009; Diebold *et al.*, 2010).

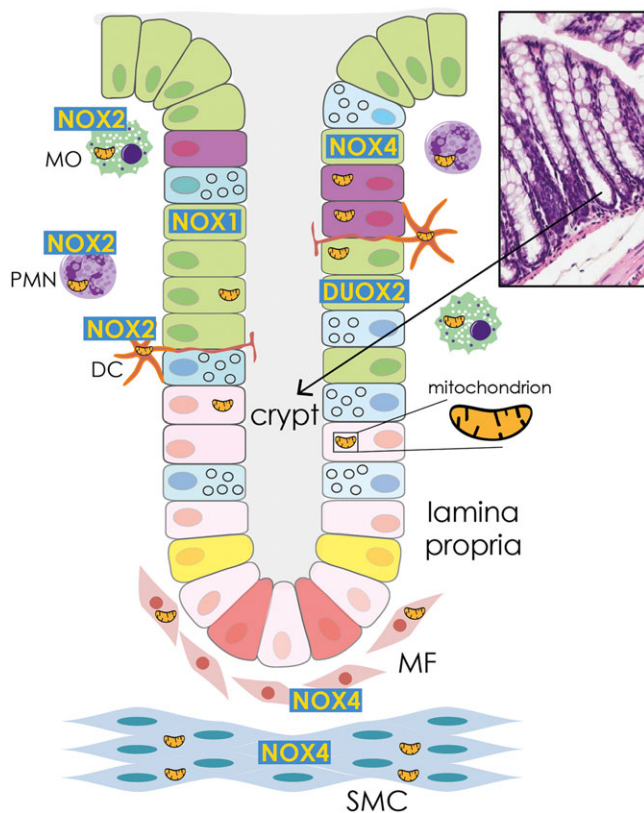


Figure 3

Localization of NADPH oxidase isoforms in the GI mucosa. NOX1 and DUOX2 are present in the epithelium, NOX2 is mainly expressed in professional phagocytes and DCs, while NOX4 is found in fibroblasts, smooth muscle cells and epithelium. MO, macrophage; PMN, polymorphonuclear cell; MF, myofibroblast; SMC, smooth muscle cell. Colour code for crypt cells: green, enterocyte absorptive cell; purple, enteroendocrine cell; pink, progenitor cell; blue, goblet cell; yellow, +4 cell; red, crypt base columnar cells.

Another important gut NADPH oxidase is DUOX2 (thyroid oxidase), an EF-hand motif containing protein that forms a membrane-bound heterodimer with its maturation factor DUOXA2 and generates H_2O_2 in a Ca^{2+} -dependent manner. In *Drosophila*, the DUOX orthologue dDuox was required for the clearance of bacterial intestinal infections (Ha *et al.*, 2005; Lee *et al.*, 2015). In mammals, the expression of DUOX2/DUOXA2 was detected throughout all of the digestive tract, particularly in caecum and colon epithelium (El Hassani *et al.*, 2005). Microbial stimuli, and in particular microbiota, pathogenic bacteria and viruses induce marked up-regulation of the DUOX2 enzyme, and thus, DUOX2 is thought to participate in antimicrobial defence in the host mucosa (Geiszt *et al.*, 2003b; Harper *et al.*, 2005; Lipinski *et al.*, 2009; Grasberger *et al.*, 2015; Sommer and Backhed, 2015). Microbial induction of murine Duox2 expression in the ileum required TIR-domain containing adapter inducing interferon-beta (TRIF) signalling to NF- κ B p50/p65, whereas in the colon, MyD88 and p38 MAPK pathways were necessary (Sommer and Backhed, 2015). In IECs, Duox2, similarly to Nox1, is upregulated following *in vitro* stimulation with colitogenic IL-13 (Mandal *et al.*, 2010). DUOX2 up-regulation was also observed upon IFN- γ stimulation (Harper *et al.*, 2005). Albeit mostly expressed in thyroid and lung epithelium, the closely related DUOX1/DUOXA1 enzyme complex has been detected in the stomach lining and selected gastric and colorectal cancer cell lines (El Hassani *et al.*, 2005; Juhasz *et al.*, 2009; Strickertsson *et al.*, 2013).

The mitochondrial electron transport chain (mETC)

Mitochondria are another source of ROS generation in physiological and pathophysiological conditions (Balaban *et al.*, 2005). mtROS are produced by the oxidative phosphorylation pathways involved in energy production. Mitochondria generate high-energy ATP molecules by an electrochemical proton gradient maintained by the transfer of electrons to protein carriers in the mitochondrial membrane, namely, Complex I–IV. Mitochondrial oxidative phosphorylation is a tightly controlled process; however, 1–2% of oxygen consumed during this process is converted to $O_2^{\bullet-}$ when electrons leak out from the mETC and are aberrantly transferred to molecular O_2 (West *et al.*, 2011). Superoxide generated by mETC reacts with NO radicals formed by the interaction of NO with mitochondrial redox-metal centres. The reactive ONOO-product can cause mitochondrial integrity disruption due to irreversible nitration of proteins, inactivation of enzymes and DNA damage. In the mitochondrial matrix, $O_2^{\bullet-}$ can react with SOD2, leading to the formation of diffusible H_2O_2 that regulates cellular functions by modulating the activity of redox-sensitive transcription factors (Nrf2, HIF-1 and NF- κ B), altering kinase activity (MAPK and I κ B kinase- β) and inhibiting phosphatases (PTEN and PTP1B) (Tanner *et al.*, 2011; Marinho *et al.*, 2014). In health and disease, crosstalk between similar or distinct ROS sources can occur. This includes NOX to NOX/DUOX signalling but may also involve mitochondria-NOX crosstalk in various scenarios, including serum withdrawal, increased glycolysis or hypoxia, leading to mtROS-mediated up-regulation and activation of NOX isoforms (Lee *et al.*, 2006; Daiber, 2010; Lu *et al.*, 2012).

Mitochondrial dysfunction and the overproduction of mtROS play an important role in inflammation, degenerative diseases and ageing (Liochev, 2013). However, similarly to NADPH oxidase-derived ROS, mtROS may predominantly regulate redox signalling, and inducing senescence could be the result of generally compromised signals, rather than being caused by direct damage to sensitive targets (Liochev, 2013). In a high turnover tissue like the digestive tract, factors that regulate enterocyte lifespan are of special interest when considering inflammatory disease and colon cancer (Kajla *et al.*, 2012; Pelicci *et al.*, 2013).

NO synthases (NOS)

Three isoforms of NOSs have been identified: two are constitutively present in either neuronal (NOS1; nNOS) or endothelial (NOS3; eNOS) tissues and are termed constitutive NOS, while a third isoform is expressed by immune, vasculature and neuronal cells after induction by certain cytokines or microbial products and is thus termed inducible NOS (NOS2; iNOS) (Kolios *et al.*, 2004). All these isoforms of NOS catalyse the oxidation of the terminal nitrogen of the amino acid L-arginine to produce NO and L-citrulline (Figure 2). The production of NO by constitutive NOS is low (nanomolar concentrations), short-lasting and highly controlled by Ca^{2+} -mobilizing agents, while NOS2 generates high levels of NO (micromolar concentrations) that can persist for days. Transient, low levels of NO are beneficial to control gastric mucosal blood flow, gut motility and barrier integrity, whereas prolonged NO generation is often accompanied with ROS production, leading to the formation of RNS with potentially deleterious effects.

The interaction of NO with O_2 or $O_2^{\bullet-}$ gives rise to two types of chemical stress: nitrosative or oxidative stress (Figure 2). Both types of stress are associated with inflammation and *de novo* expression of iNOS (Kolios *et al.*, 2004; Lanzetti *et al.*, 2012). The interaction of NO with O_2 triggers the auto-oxidation of NO and the formation of nitrogen dioxide and dinitrogen trioxide, potent nitrosating agents with carcinogenic effects, while the interaction of NO with $O_2^{\bullet-}$ produces cytotoxic peroxynitrite (ONOO $^-$) (Kolios *et al.*, 2004). ONOO $^-$ can cause lipid peroxidation, or changes in protein structure and folding by oxidation of sulphhydryl groups in proteins, and nitration of tyrosine, yielding nitrotyrosine, and eventually leading to loss of protein function and tissue damage (Pereira *et al.*, 2013).

Inflammation and repair in the GI tract

Here, we discuss the role of ROS or RNS during the inflammatory response in three different regions of the digestive tract: stomach, ileum and colon. As prompt resealing of the epithelial surface barrier following injuries is essential to preserve gut homeostasis, the role of ROS and oxidative stress during wound healing will be also discussed.

Gastritis

Acute inflammation of the stomach mucosa is the consequence of a number of triggers including bacterial infections, uremia, ischaemia, nonsteroidal anti-inflammatory drugs (NSAIDs) and corrosive agents. Infectious gastritis develops

when the gastric pH rises above pH 4 and stomach acid is incapable of neutralizing food-borne pathogens. The most common bacterium leading to severe stomach inflammation is *Helicobacter pylori* (*H. pylori*).

H. pylori is a Gram-negative, helical, microaerophilic bacterium that selectively colonizes the antrum of the human stomach inducing chronic inflammation that eventually evolves into peptic ulcer (10–15% of cases), gastric adenocarcinoma (1–3% of cases) or mucosa-associated lymphoid tissue lymphomas (<0.1% of cases) (Wroblewski *et al.*, 2010). *H. pylori* is highly adapted to occupy the special ecological niche of the stomach lining by producing large amounts of urease, thereby creating an alkaline environment. Although the majority of *H. pylori* reside within the mucus layer, ~20% of the bacteria will bind to gastric epithelial cells (Peek *et al.*, 2010). *H. pylori*-induced gastritis is predominantly caused by a Th1/Th17 cell response (Shi *et al.*, 2010) occurring after recruitment of a large number of ROS-producing neutrophils into the mucosa. Indeed, an excessive production of ROS and RNS was observed in *H. pylori*-infected human gastric mucosa, which correlated with mucosal tissue damage and bacterial colonization (Davies *et al.*, 1994). In *H. pylori*-infected stomach tissue, possible sources of ROS/RNS include gastric mucosal cells and *H. pylori* itself, apart from neutrophils (Handa *et al.*, 2010; Naito and Yoshikawa, 2002). While *H. pylori* infection promoted the recruitment of neutrophils into the mucosa, the bacterium can survive in this hostile environment by manipulating neutrophil defence mechanisms such as phagocytosis and the oxidative burst (Peek *et al.*, 2010). Secretion of the *H. pylori* virulence factor neutrophil-activating protein engaged TLR2 resulting in NF- κ B activation and Th1 cytokine up-regulation. This pro-inflammatory response was accompanied by recruitment of PMNs and generation of O₂^{•-} via NOX2 activation. Simultaneously, *H. pylori* can evade neutrophil-dependent killing by inhibiting opsonisation (Peek *et al.*, 2010). In epithelial cells, *H. pylori* infection stimulated ROS production via NOX1 activation (Kawahara *et al.*, 2005). *H. pylori* can also increase the production of mtROS via type IV secretion system-mediated injection of the virulence factors CagA and VacA, which alter mETC function and elevate oxidative stress (Galmiche and Rassow, 2010; Handa *et al.*, 2010). These studies suggest that the production of pro-inflammatory ROS as the host's primordial defence mechanism against bacterial invasion can eventually turn deleterious for the host through ROS-mediated tissue damage and inflammation.

A role for Duox2 in the pathogenesis of *H. pylori*-associated gastritis was first suggested in Rhesus macaques (Hornsby *et al.*, 2008). *H. pylori* infection in mice is usually characterized by limited colonization and variable pathology, while infection with *H. felis* is more reliable to reproduce human disease. Grasberger *et al.* (2013) used L-thyroxine supplemented mice with combined deficiency in *Duoxa1* and *Duoxa2* to determine the role of Duox in *H. felis* infection (Grasberger *et al.*, 2013). Mucosal colonization with *H. felis* was increased in *Duoxa*^{-/-} mice. The elevated bacterial load caused enhanced shedding of bacterial antigens and virulence factors, leading to severe gastritis in *Duoxa*^{-/-} mice. These results suggest that the release of H₂O₂ by Duox at

the apical surface of the gastric epithelium controls growth of *H. felis* in the mucus layer and provides antimicrobial protection (Grasberger *et al.*, 2013).

Ileitis

The intraluminal pH shifts from pH 1.5–3.5 in the stomach to pH 6 in the duodenum and pH 7.4 in the terminal ileum. This alkalization alters the environment and promotes the development of specific microbiota (Hayashi *et al.*, 2005). For example, the SI hosts a considerable number of commensal bacteria (up to 10⁸ per mL in the ileum) and is the site of colonization for a number of enteritis-causing pathogens, such as *Vibrio cholerae*, pathogenic forms of *Escherichia coli* and *Salmonella* (Garner *et al.*, 2009). Ileitis, an inflammation of the SI, develops often as a consequence of Crohn's Disease (CD); however, a number of other conditions including tuberculosis, spondyloarthropathies, ischaemia or sarcoidosis can be associated with ileitis (Dilauro and Crum-Cianflone, 2010). Our knowledge regarding redox regulation in ileitis is limited by the paucity of suitable animal models. Transgenic mice with conditional deletion of caspase-8 (*Casp8*^{ΔIEC}) (Gunther *et al.*, 2011) or FADD (*FADD*^{IEC-KO}) (Welz *et al.*, 2011) in the intestinal epithelium spontaneously developed inflammatory lesions in the terminal ileum, lacked Paneth cells and exhibited severe colitis in a dextran sodium sulphate (DSS) colitis model. These changes correlated with RIPK3-dependent IEC necrosis and altered TNF- α levels. Although RIPK3-mediated cell death has been associated with oxidative stress, this link was not investigated. Recently, Esworthy *et al.* (2014) reported that transgenic mice with double knockout (dKO) for the antioxidant enzymes GSH peroxidase (GPx)-1 and GPx-2 developed spontaneous mild-to-moderate ileocolitis. This pathology disappeared in triple knockout (GPx-dKO/*Nox1*^{-/-}) mice, suggesting that Nox1-generated ROS promotes intestinal inflammation when the antioxidant systems fail. Interestingly, GPx-dKO/*Nox1*^{-/-} mice showed reduced TNF- α levels in the ileum, indicating crosstalk between TNF- α and redox signalling in the development of ileitis (Esworthy *et al.*, 2014). Nox1-generated ROS was also connected to ileal mucositis induced by administration of 5-fluorouracil. In this mouse model, characterized by an abnormal inflammatory response, intestinal malabsorption and dysfunction, Nox1 deficiency correlated with reduced TNF- α -mediated and IL-1 β -mediated crypt apoptosis (Yasuda *et al.*, 2012).

Similarly to NADPH oxidase-produced ROS, mtROS were linked to SI inflammation. In a model of NSAID-induced mucosal injury, increased barrier permeability and inflammation were associated with enhanced mtROS production and changes in mitochondrial morphology towards vacuolation, swelling and loss of cristae (Handa *et al.*, 2014). In enteritis induced by *Clostridium difficile* infection, the increased fluid and electrolyte secretion, intestinal permeability and loss of electrical resistance were accompanied by increased ROS levels, although the source and mechanism of action were not determined (Qiu *et al.*, 1999). Other studies associated Rac1-dependent intracellular oxidative stress and necrosis in colonic tissue with the action of *C. difficile* toxin B (Farrow *et al.*, 2013). Taken together, this evidence suggests a pro-inflammatory role of Nox1-derived ROS and mtROS in the development of ileitis.

IBD

Oxidative stress is considered a potential etiological and/or triggering factor for the development of colon inflammation (Biasi *et al.*, 2013). IBD, consisting of CD and ulcerative colitis (UC), are defined as idiopathic, chronic and relapsing intestinal disorders occurring in genetically predisposed individuals exposed to environmental risk factors, such as diet, hygiene, stress and microbiome changes (Kaplan, 2015). Both forms of IBD are associated with significant morbidity and characterized by gut inflammation, diarrhoea, abdominal pain, weight loss and a number of extra-intestinal manifestations including arthritis, psoriasis, erythema, cholangitis and eye inflammation (Levine and Burakoff, 2011).

CD can affect any part of the GI tract including the terminal ileum with deep and extended ulcers involving all layers of the bowel wall, while UC is limited to the colon and rectum with inflammation occurring in the innermost layer of the intestinal lining. The maladaptive chronic inflammatory responses observed in IBD patients are characterized by alterations of the physiological mechanisms regulating the host's immune tolerance, leading to an abnormal sensing of commensal microorganisms by the LP immune system.

NADPH oxidases and IBD

A chronic inflammatory process affecting both small and large bowels and resembling CD occurs in up to 40% of CGD patients (Marciano *et al.*, 2004; Marks *et al.*, 2009). The GI involvement seems to correlate rather with *CYBB* deficiency than with autosomal-recessive forms (*CYBA*, *NCF1*, *NCF2* and *NCF4*) or with the remaining ROS levels (Agarwal and Mayer, 2013; Holland, 2013). CGD patients may present with perirectal abscesses, GI tract obstruction and recurrent diarrhoea. Colon endoscopy often reveals colonic narrowing, a thickened bowel wall, pancolitis and pseudopolyps, whereas the histology shows submucosal oedema, crypt abscesses, inflammatory cell infiltration, cryptitis, epithelioid granulomas (usually in the *muscularis*) and large pigment-laden histiocytes in the LP (Agarwal and Mayer, 2013; Barbato *et al.*, 2014). Decreased NOX2 activity, above the threshold leading to CGD, has also recently been linked to very early onset (VEO)IBD (defined as IBD in children <6 years of age according to the modified Paris classification) (Eszter Muller *et al.*, 2014). VEOIBD patients present predominantly with pancolitis and occasionally perianal disease and are hyporesponsive to conventional therapy leading to high morbidity and mortality (Uhlir *et al.*, 2014). In contrast to the multifactorial nature of adult IBD, VEOIBD is considered a consequence of monogenic defects (Uhlir *et al.*, 2014). Several functionally altered variants in NOX2 complex components have been identified in VEOIBD (Dhillon *et al.*, 2014a). Certain SNPs in *NCF1* and *NCF2* are described in up to 10% of VEOIBD patients. Up to now, only one VEOIBD patient was identified with a *NCF4* variant, which negatively influenced the binding of this protein to p67^{phox} (O'Neill *et al.*, 2015). This variant has been previously associated with ileal adult CD in genome-wide association studies (Rioux *et al.*, 2007; Roberts *et al.*, 2008). Recently, we identified two functionally altered *NOX1* and *DUOX2* variants in VEOIBD patients, both associated with severe pancolitis and reduced ROS production *in vitro* (Hayes *et al.*, 2015), suggesting an important role of these epithelial oxidases

in the pathogenesis of IBD. Interestingly, patients carrying these *DUOX2* variants did not present with hypothyroidism at newborn screening.

Human IBD can be mimicked in mice by altering the epithelial barrier integrity with chemicals such as DSS or 2,4,6-trinitrobenzenesulfonic acid (TNBS). Additionally, transgenic mice with IL-10 (Berg *et al.*, 1996), *Muc2* (Van der Sluis *et al.*, 2006) or *NLRP6* (Elinav *et al.*, 2011) deficiency will permit studying the development of spontaneous chronic enterocolitis. However, with respect to ROS generation, current animal models seem often not to reflect human disease. For example, *Cybb* knockout mice do not develop the spontaneous CD-like phenotype observed in many CGD patients. *Nox2* deficient mice were protected against the mucosal damage induced by DSS (Bao *et al.*, 2011), but succumbed to colitis in the TNBS model (Campbell *et al.*, 2014). In mice harbouring a point mutation in *Ncf1*, more severe colitis was observed (Rodrigues-Sousa *et al.*, 2014), while *Ncf1* knockout mice showed no colitogenic phenotype (Kriegelstein *et al.*, 2001). Mice lacking *Ncf4* exhibited exacerbated colitis (Conway *et al.*, 2012) that was absent in *Cybb* (*Nox2*)-deficient mice. As IBD development is not only associated with genetic susceptibility but also with environmental factors, some of the apparent inconsistencies in animal studies are likely due to different housing conditions, nutrition or commensal communities present.

Experimentally, ROS produced by *Nox1* during colitis has been studied using *Il10*^{-/-} mice as background, as *Nox1* deficiency alone did not produce a colitis phenotype in DSS or TNBS models (Leoni *et al.*, 2013; Treton *et al.*, 2014). IL-10 negatively regulates *Nox1*-derived ROS production after barrier insult (Li *et al.*, 2014) as well as following TNF- α (Kuwano *et al.*, 2008; Kamizato *et al.*, 2009), IFN- γ (Kuwano *et al.*, 2006; Kamizato *et al.*, 2009) and LPS (O'Leary *et al.*, 2012) stimulation. When *Nox1*^{-/-} mice were crossed with *Il10*^{-/-} mice, spontaneous colitis developed mimicking clinical and histological features of UC patients (Treton *et al.*, 2014). Consistent with findings that *Nox1* regulates the balance between goblet and absorptive cell types in the murine colon (Coant *et al.*, 2010), *Nox1*^{-/-}/*Il10*^{-/-} mice displayed reduced and aberrant goblet cells and decreased *Muc2* and *Muc4* mucin expression in ulcerated sites of the intestine (Treton *et al.*, 2014). Others reported that ROS generated by an unspecified *Nox* isoform was critical for mucin granule accumulation in murine colonic spheroids (Patel *et al.*, 2013).

Interaction between the CD susceptibility genes *NOD2*, *ATG16L1* and ROS sources has been proposed, thus connecting microbial signalling, autophagic clearance and ROS production. Stimulation of *NOD2* by its ligand muramyl dipeptide increased ROS generation in Caco-2 cells and in murine colon biopsies derived from wild type but not *Nod2*-deficient mice (Lipinski *et al.*, 2009). A physical interaction between these two antimicrobial effectors was found in TNF- α /muramyl dipeptide stimulated Caco-2 cells (Lipinski *et al.*, 2009) but not in *DUOX2*/*DUOX2* expressing HT29 cells challenged with enteropathogenic *E. coli* (Hayes *et al.*, 2015). In autophagy, contrasting views exist in regard to ROS as essential physiological regulator or to ROS/oxidative stress being the result of disturbed autophagic processes. Even the source of ROS is debated with some studies favouring mtROS, while others show the importance of *Nox*-ROS

signalling. A direct effect of ROS on microtubule-associated protein 1A/1B-light chain 3 conjugate recruitment to autophagosomes and co-localization of Nox with light chain 3 was reported (Huang *et al.*, 2009; Patel *et al.*, 2013), while others implicated mtROS in redox regulation of autophagy and particularly in mitophagy (Frank *et al.*, 2012; Kurihara *et al.*, 2012). Crosstalk between inflammasomes and autophagy is also connected to ROS (Saitoh *et al.*, 2008; Scherz-Shouval and Elazar, 2011) as NLRP3 and AIM2 inflammasomes required mtROS, but not Nox-derived ROS, for transcriptional up-regulation and activation respectively (Zhou *et al.*, 2011; Crane *et al.*, 2014). Taken together, both human and animal data suggest that ROS play an important role in preserving processes essential for intestinal homeostasis and that the impairment or deficiency of phagocytic/epithelial oxidases (ROS low) or mitochondrial dysfunction (ROS high) predispose or exacerbate intestinal pathology. In particular, with respect to patients with defective NADPH oxidase function, these data indicate that reduced innate immune cell or intestinal epithelial ROS generation will increase susceptibility to IBD and colon inflammation.

Conversely, up-regulation of *DUOX2* has been detected in paediatric (Haberman *et al.*, 2014) and adult (Csillag *et al.*, 2007) CD and UC patients. Prominent expression of *DUOX2* was also found in all segments of UC patients' large bowel (MacFie *et al.*, 2014), accompanied by an increase of *Proteobacteria phylum* in the microbiota (Haberman *et al.*, 2014). Interestingly, *in vitro* stimulation of inflamed UC biopsies with 5-aminosalicylic acid (5-ASA) used as first-choice intervention in the treatment of UC and mild to moderate CD (Dignass *et al.*, 2012), induced up-regulation of *DUOX2* expression (MacFie *et al.*, 2014). The mechanism underlying these *ex vivo* observations remains unknown, and so far, the use of *Duox2* knockout mice is hindered by the severe congenital hypothyroidism present in animals with loss of *Duox2* function (Grasberger *et al.*, 2012) and the required continuous L-thyroxine treatment, both likely altering the microbiome and metabolome.

Little is known regarding the role of NOX4-derived ROS in the GI tract. Nox4 has been detected in murine IECs upon stimulation with colitogenic IL-13 (Mandal *et al.*, 2010) and following *Listeria* infection (Dolowschiak *et al.*, 2010), but its role in IEC physiology or pathophysiology has not been studied. In contrast, an intimate connection exists between TGF- β signalling, NOX4-mediated H₂O₂ production and myofibroblast transformation in lung, kidney and liver fibrosis (Jiang *et al.*, 2014; Paik *et al.*, 2014). TGF- β 1 is a central mediator of fibrosis in CD (Jiang *et al.*, 2014) with up to 30–40% of CD patients developing intestinal fibrosis and ileal strictures within 10 years of disease onset (Lawrance *et al.*, 2015). Recurrent or persistent epithelial injuries, accompanied by local chronic inflammation, are responsible for initiating intestinal fibrogenesis in CD. At which point of this disease process, ROS will trigger development or progression of intestinal fibrosis has not yet been assessed, but NOX4 is a prime candidate for one of the key steps involved.

Mitochondria and IBD

Functionally compromised mitochondria are an important source of ROS in chronic inflammation. mtROS are involved

in overproduction of the cytokines IL-1 β and IL-18 by up-regulating the nucleotide-binding domain, leucine rich family, pyrin containing 3 inflammasome (Mittal *et al.*, 2014) and in intestinal tumorigenesis (Bellafante *et al.*, 2014). Marked mitochondrial damage in the colonic mucosa was found in IBD patients, correlating with increased oxidative stress (Biasi *et al.*, 2013). Furthermore, the expression of prohibitin (PHB), an important membrane regulator of mitochondrial respiratory function, was decreased during oxidative stress (Theiss *et al.*, 2007). PHB levels were reduced in mucosal biopsies of IBD patients and in animal models of colitis (Hsieh *et al.*, 2006; Theiss *et al.*, 2007). PHB overexpression in intestinal cells protected against oxidant-induced permeability changes, suggesting a beneficial role of this protein in preventing mucosal barrier disruption by excessive production of ROS (Theiss *et al.*, 2007). A pro-inflammatory role of mtROS in intestinal inflammation is supported by analysis of transgenic mice with compromised mitochondrial function due to genetic deficiencies. *Ucp2*^{-/-} mice lacking the negative regulation of mtROS production exhibited severe colitis (Zhang *et al.*, 2012). This phenotype correlates with findings in patients, where *UCP2* polymorphisms were associated with UC and CD (Yu *et al.*, 2009). mtROS homeostasis in the epithelium is also regulated by the stress-inducible immediate early response gene *IEX-1* through a mechanism that targets the mitochondrial F₁F₀-ATPase inhibitor IF1 for degradation, resulting in elevated F₁F₀-ATPase activity, acceleration of ATP hydrolysis and reduced mtROS (Shen *et al.*, 2009). *Iex1*^{-/-} mice showed diminished pathology in DSS colitis, possibly by up-regulating IL-17 (Ustyugova *et al.*, 2012).

NOS enzymes and IBD

NO directly or indirectly regulates several manifestations of IBD, including mucosal vasodilatation, increased epithelial permeability and altered motility. Cumulative evidence indicates that NO production is enhanced during IBD (Kolios *et al.*, 2004; Winter *et al.*, 2013), and recently two hyperactive NOS2 variants were linked to increased susceptibility to VEOIBD (Dhillon *et al.*, 2014b). The main cellular sources of NO in the inflamed gut tissue are monocytes/macrophages (Bain and Mowat, 2014), IECs (Kolios *et al.*, 2004) and myofibroblasts (Wu *et al.*, 2013). In both animals and humans with colitis, the generation of ONOO⁻ is favoured by the concomitant presence of NO and O₂^{•-} produced *via* NOS2 and a superoxide source such as NOX1 or NOX2 respectively. This peroxynitrite stress can cause nitrosative DNA damage by forming 8-nitroguanine (Kawanishi *et al.*, 2006). Several studies have been carried out to uncover the importance of enhanced production of O₂^{•-} and NO and/or ONOO⁻ in the aetiology of IBD; however, so far, this issue remains controversial. For example, one hypothesis is that ONOO⁻ by inducing DNA damage can activate poly(ADP-ribose) synthetase, which in turn induces increased epithelial permeability as a result of IEC apoptosis (McCafferty, 2000; Pacher *et al.*, 2007). In general, it seems that low and transient levels of NO are an important regulator of intestinal homeostasis as mice deficient in NOS3 (*eNos*^{-/-}) are highly susceptible to colitis (Sasaki *et al.*, 2003). On the other hand, higher concentrations and prolonged production of NO generated by NOS2 seem to augment the inflammatory response in

the colon because the lack of inducible NOS (*iNOS*^{-/-}) conferred protection in the DSS model (Kriegelstein *et al.*, 2001). These data suggest that the protective role of endothelial NO in disease may disappear at some point, possibly overwhelmed by an excess of OONO⁻ supplied by activated immune cells, which ultimately contributes to chronic intestinal inflammation.

Wound healing

Intestinal inflammation severely compromises the epithelial barrier function, resulting in the exposure of underlying tissues to luminal bacteria and their antigenic products. Hence, wound closure of the epithelium must occur efficiently to rapidly restore barrier function and homeostasis. Certain drugs such as NSAIDs can exacerbate pre-existing IBD, increase flare-ups and may lead to the development of gastric and intestinal ulcers. Recently, the pivotal role of PGI₂ in preventing penetrating ulcers after colonic mucosal injury was reported (Manieri *et al.*, 2015). In general, a deregulated wound healing response often results in fibrotic disease. H₂O₂ is the most relevant ROS produced during wound healing, and it plays a role in every phase of tissue repair, including haemostasis, inflammation, vascularization and re-epithelialization (Cordeiro and Jacinto, 2013). Following tissue injury, ROS participate in wound healing and tissue regeneration by conferring resistance to wound infection, altering signalling pathways, modulating leukocyte adhesion molecule expression and inducing fibroblast, smooth muscle and epithelial cell proliferation and migration (Chan *et al.*, 2009). Cooperation between phagocytic and epithelial NADPH oxidases in this process has been visualized in a *Xenopus* wounding model (Love *et al.*, 2013), while others reported mtROS-induced redox modifications of the GTPase Rho1 as crucial for promoting skin wound healing responses in *Caenorhabditis elegans* (Xu and Chisholm, 2014). Although perhaps counterintuitive, the oxidative burst of PMNs accumulating in crypt abscesses during mucosal inflammation is crucial for the resolution of gut inflammation (Campbell *et al.*, 2014). ROS generation by transmigrating PMNs during acute colitis causes an oxygen depleted microenvironment, which transcriptionally up-regulates hypoxia genes in IECs, resulting in protection of the mucosa (Robinson *et al.*, 2008; Campbell *et al.*, 2014). In general, acute mild to moderate hypoxia supports adaptation and survival, while chronic extreme hypoxia leads to cell death (Sen, 2009).

A growing body of evidence indicates that Nox1-generated ROS co-ordinately drive colon epithelial proliferation. Although absent (Kawahara *et al.*, 2004) or barely detectable (Laurent *et al.*, 2008) in the SI mucosa by real-time PCR, Nox1 is highly expressed in the large bowel (Kikuchi *et al.*, 2000), where it may participate in regulating IEC proliferation and differentiation. Nox1 deficiency in mice promoted the rapid differentiation of intestinal progenitor cells into post-mitotic goblet cells, suggesting that Nox1 signalling is required within the crypt compartment to maintain the undifferentiated state of crypt progenitors. These data are important in the context of epithelial barrier

repair, but they also indicate that Nox1 may have oncogenic potential (Coant *et al.*, 2010). Indeed, Nox1 is overexpressed in human colon cancers harbouring activating mutations of K-Ras (Laurent *et al.*, 2008). The K-Ras oncogene-mediated up-regulation of Nox1 was required for transformation and resulted in premature senescence (Mitsushita *et al.*, 2004; Kodama *et al.*, 2013). In contrast, oxidative damage of mtDNA by mtROS with an increased rate of mutations was observed in UC patients developing colorectal cancers (Nishikawa *et al.*, 2005).

The microbiota is an important player in gut barrier repair. Signals delivered by pathogens and commensals up-regulate Nox1 and/or Duox2 and stimulate ROS generation (Corcionivoschi *et al.*, 2012; Sommer and Backhed, 2015). *Lactobacilli* spp., particularly *Lactobacillus rhamnosus*, stimulated Nox1-dependent ROS generation in murine enterocytes, driving cell proliferation (Jones *et al.*, 2013). The importance of Nox1-derived ROS and mucosal wound repair was also evident in ligand-mediated stimulation of the formyl peptide receptor FPR1 present on IECs (Leoni *et al.*, 2013). This GPCR, mainly characterized on neutrophils, responds to microbial-derived formylated peptides and gives rise to Nox-dependent ROS production. Specific members of the commensal flora can stimulate FPR1 signalling leading to the generation of ROS via enterocyte Nox1 and to phosphorylation of focal adhesion kinase and Erk1,2 MAPK, which ultimately enhances proliferation and migration of enterocytes towards the site of injury (Alam *et al.*, 2014).

Current IBD treatments

The eradication of inflammation results in decreased surgery and hospitalization rates in both UC and CD patients (Costa *et al.*, 2013); therefore, one of the goals of IBD therapy is to reduce intestinal inflammation and immune system hyper-responsiveness. Conventional therapy involves aminosalicylates, corticosteroids, thiopurines, antimetabolites, calcineurin inhibitors and biological agents. Although debated, the use of 5-ASA as the first-choice drug in the treatment of UC is positively influenced by tolerability, dose schedule and cost (Mowat *et al.*, 2011). Different mechanisms of action have been attributed to 5-ASA (Desreumaux and Ghosh, 2006) including the potential to scavenge ROS/RNS (Couto *et al.*, 2010; Managlia *et al.*, 2013) and to potentially inhibit ROS-dependent neutrophil extracellular trap formation in activated neutrophils (Kirchner *et al.*, 2013). Although the notion that balanced ROS levels are essential for gut health is gaining traction, in general oxidative stress with increased ROS levels has been considered a culprit in the GI inflammatory response. Whether antioxidant therapy will be a valid means in arresting inflammation in patients with gastric or intestinal inflammation remains largely unresolved. For example, the beneficial effect of SOD-based treatments in experimental models of colitis (Keshavarzian *et al.*, 1990) was not replicated by SOD therapy in human IBD (Emerit *et al.*, 1991). Although 5-ASA is considered an antioxidant (Managlia *et al.*, 2013), two clinical studies indicated that 5-ASA is more effective in the treatment of UC when combined with other antioxidants, such as *N*-acetyl cysteine (Guijarro *et al.*, 2008), or allopurinol, a xanthine oxidase inhibitor (Jarnerot *et al.*, 2000), suggesting that prevention of oxidative damage may be beneficial when treating IBD. The therapeutic

value of 5-ASA most likely relies, not on its putative antioxidant activity, but on a broad anti-inflammatory mechanism as patients with reduced ROS generation due to NADPH oxidase loss-of-function variants respond well to 5-ASA treatments. Antioxidant and vitamin dietary supplements are still considered a therapeutic option due to their low risk of side effects. Polyphenols were found clinically beneficial in both UC (Dryden *et al.*, 2013) and CD patients (Kolacek *et al.*, 2013), whereas vitamin supplementation particularly vitamin D, whose deficiency is associated with IBD incidence/activity (Torki *et al.*, 2015), reduced the risk of relapse in a cohort of patients with CD (Jorgensen *et al.*, 2010).

Managing IBD in CGD patients

Special therapeutic considerations are vital for managing IBD in CGD patients. These patients are severely immunocompromised due to impaired host defence by ROS-deficient innate immune cells and tend to present with hyperinflammation as regulation of cytokine signalling is dependent on NOX2-derived ROS. Hyperinflammation is frequently observed in CGD patients, predominantly due to deregulation of cytokine signalling pathways and the inability of macrophages to efficiently perform efferocytosis (uptake of apoptotic cells) (O'Neill *et al.*, 2015). The average age of onset of GI symptoms in susceptible CGD patients is approximately 5 years, but some intestinal manifestations may even precede the CGD diagnosis in 5% of cases (Huang *et al.*, 2006; Towbin and Chaves, 2010). When GI symptoms occur, a misdiagnosis of IBD remains a possibility, thereby compromising the therapeutic management of CGD. Although the optimal treatment of CGD colitis is not established yet, the first recommended approach is similar to IBD, often involving the administration of 5-ASA.

Corticosteroids and other immunomodulatory agents such as methotrexate, azathioprine and its metabolite 6-mercaptopurine are indicated when patients show a diminished response (or are completely refractory) to 5-ASA. On the other hand, the use of the monoclonal antibody Infliximab, a selective TNF- α blocker, is considered a major advance in IBD therapy, although its use is strongly discouraged in CGD patients, even when combined with antibiotic prophylaxis, due to the high risk of developing severe infections (Uzel *et al.*, 2010). Therefore, more effective management of intestinal inflammation in CGD patients is needed. Recently, a pro-oxidant therapy has been suggested. Pro-oxidants are molecules capable of inducing mild oxidative stress and therefore could partially restore ROS levels in CGD patients. For instance, some vitamins such as vitamin C (ascorbate) can induce the generation of free radicals (\bullet OH) by reducing Fe^{2+} to Fe^{3+} via the Fenton reaction, although the efficacy of this treatment for colitis remains to be established (Aghdassi *et al.*, 2003). NOX2 agonists as ROS-inducing drugs have been identified by large-scale screening, but their pharmacological use, therapeutic index and side effects have not yet been determined (Hultqvist *et al.*, 2015).

To date, the only curative treatment for CGD and accompanying GI symptoms is haematopoietic stem cell transplantation from human leukocyte antigen-matched siblings or unrelated donors, correcting the immune deficiency with engrafted donor cells. In a recent multi-centre study, haematopoietic stem cell transplantation cured colitis in 22 out of 24 patients with CGD (Gungor *et al.*, 2014). Current clinical trials, using gene therapy with improved vectors or

gene editing approaches in CGD iPS cells, may point to a valid alternative for effective treatment of CGD patients when a match is not available or the patient is too ill for a transplant (Flynn *et al.*, 2015; Grez *et al.*, 2011; Kang and Malech, 2012).

Conclusions and future remarks

Over the last few years, the pivotal contributions of the redox balance to GI physiology and gut health have become apparent, significantly changing our understanding of how oxidants maintain the intestinal barrier and control complex host-pathogen interactions in the stomach and intestine. It is now clear that ROS are not only harmful byproducts of cell metabolism but also crucial homeostatic regulators in the gut. Physiological levels of ROS are likely to act as signals governing the interaction of the mucosa with the microbiome, potentially even shaping commensal communities. Likewise, mtROS exert essential immunoregulatory functions, but their link to endoplasmic reticulum stress, the unfolded protein response and apoptosis/necrosis may be essential in triggering intestinal inflammation and tissue injury *via* ROS overproduction. Until now, antioxidants have failed in the treatment of IBD in clinical trials, possibly by not reaching therapeutic concentrations or due to their broad inhibitory spectrum. One may argue that more specificity in terms of targeting the deregulated ROS source is required or that even ROS-enhancing options will be beneficial for particular patient cohorts. We believe that future therapeutic approaches will need to consider the dual role of ROS in health and disease.

Acknowledgements

The authors apologize to those researchers whose work could not be included in this review due to space limitation. We thank S. O'Neill for critical reading of the manuscript. Funding was provided by the National Children's Research Centre (UGK, GA), Science Foundation Ireland (UGK), and the European Crohn's and Colitis Organization (GA). The present work was supported by the European Cooperation in Science and Technology (COST Action BM1203/EU-ROS).

Conflict of interest

The authors declare no conflicts of interest.

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