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REVIEWS

Review

Oxidative Stress and Redox-Modulating Therapeutics in Inflammatory Bowel Disease

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Inflammatory bowel disease (IBD) is associated with the production of reactive species that target cysteine redox switches in proteins, thereby affecting gene regulation, DNA damage, ion transport, intermediary metabolism, and mitochondrial function. Precursors of reactive species are derived from organic and inorganic compounds and their cofactors, including amino acids, vitamins, oxygen, nitrite, and sulfate. Nutrition and the gut microbiome fuel this process to a significant extent. The production of reactive species in IBD is reflected by a reduction in systemic free thiols, the major components of the antioxidant machinery. Systemic free thiols are amenable to nutritional or therapeutic intervention. This opens up future avenues for therapeutic modulation of redox status in IBD.

Inflammatory Bowel Disease and the Role of Reactive Species

IBD includes two chronic, progressive inflammatory disorders affecting the gastrointestinal (GI) tract: Crohn's disease (CD) and ulcerative colitis (UC). Both are characterized by inflammation of the gut mucosa caused by an inappropriate immune response triggered by the gut microbiome in genetically susceptible individuals [1]. The incidence of IBD is steadily increasing in westernized countries, similarly in men and women, but varies with ethnicity [2]. Causative factors remain unclear, but recent emphasis on the **exposome** (see Glossary) highlights considerations of a critical interface between chemistry and biology [3]. The exposome concept aims to capture biological responses to exposure to physical factors and synthetic chemicals in our environment, as well as to dietary constituents and psychosocial stressors from conception to death, all of which are believed to contribute to the development of chronic metabolic inflammation (metaflammation) [4–6].

The clinical signs and symptoms of IBD include abdominal pain, fatigue, diarrhea, weight loss, and several extraintestinal manifestations (e.g., arthritis, uveitis, and skin disease). Symptomatology is often non-specific, and patients frequently suffer from long-lasting subclinical disease activity that is difficult to monitor and treat [7]. Accurate disease activity monitoring and early onset of medical treatment are of crucial importance because subclinical disease activity negatively affects disease course, increasing the risk of hospitalization and the need for surgical interventions [8]. In addition, it decreases patient-reported quality of life and rates of socioeconomic participation [9].

The etiology of IBD is multifactorial and constitutes a complex interplay between host genetics, the gut microbiome, and environmental triggers that are dependent on lifestyle and diet [10]. These elements all interact with each other and contribute to IBD development and progression, resulting in disturbed gut mucosal homeostasis and distinct immunological alterations. Typically, the intestinal mucosa in IBD is characterized by infiltration with numerous inflammatory cells. A chronic uncontrolled immune response is the net result of excessive immune activity of effector lymphocytes with increased production of proinflammatory cytokines, while regulatory immune cells and mediators fail to maintain tissue homeostasis [11]. Chronically active inflammation is

Highlights

Oxidative stress is a key feature of Inflammatory Bowel Disease (IBD). However, there is still insufficient understanding of the redox architecture that defines the pathogenic mechanisms that link oxidative stress to IBD.

In addition to reactive oxygen species (ROS), there are reactive nitrogen species (RNS) and reactive sulfur species (RSS). Interactions among themselves and with downstream biological targets are defined by the recently introduced concept of the 'Reactive Species Interactome' (RSI).

The RSI is mainly fueled by nutritional components, emphasizing the importance of studying the effects of nutritional modulation on redox signaling processes in IBD.

Whereas cellular cysteine free thiols (-SH) are the major biological constituents of the RSI that fulfill important redox switch functions, free thiols in plasma/serum are a central readout of the whole-body redox status.

Redox metabolomics, in conjunction with other multi-omics technologies, may be a promising approach to improve our understanding of the pathophysiology of IBD.

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directly coupled to the generation and release by immune cells of reactive oxygen species (ROS), serving as important signaling molecules that contribute to their immunological functions [12]. However, ROS and related products can also be harmful; their continuous release in the local microenvironment of actively inflamed mucosal lesions causes collateral damage, including extensive cellular and molecular damage, perpetuating intestinal inflammation and inviting increased tissue destruction [13,14]. These processes are often associated with severe malnutrition as a result of impaired intestinal absorption [15].

In IBD, **oxidative stress**, an imbalance between the production and elimination of ROS, not only occurs in the inflamed intestinal mucosa but also extends into the deeper layers of the intestinal wall and is mirrored within the systemic circulation [16–20]. Overproduction of ROS in IBD can be quantified by measuring specific components of systemic redox status [21]. In addition to ROS, other species, in particular reactive nitrogen species (RNS) and reactive sulfur species (RSS), participate in these chemical reactions, and their mutual interaction merits further consideration.

In this review, the chemical biology behind the different **reactive species** will be described by using the recently introduced '**Reactive Species Interactome**' (RSI) framework, and its potential role in the context of IBD will be further elaborated upon. Furthermore, important biomarkers and nutritional precursors of the RSI will be highlighted, while this novel conceptual framework of redox regulation is placed within the context of IBD, followed by a description of the alterations of metabolic pathways fueling the RSI. Finally, current trends and future perspectives are described for redox-targeted therapeutics within the rapidly developing field of multi-omics technologies and **personalized medicine** in IBD.

The Reactive Species Interactome

Reactive species are highly reactive molecules that play a pivotal role in human metabolism [22]. They can be derived from oxygen (O_2), from the reduction or oxidation of nitrogenous compounds, and from the redox conversion of sulfur-containing compounds. These reactions result in the formation of ROS, RNS, and RSS, respectively. They participate in various cell signaling processes and are responsible for the modification of cysteine **thiols** (**redox switches**), leading to structural and functional modulation of proteins including enzymes (e.g., protein kinases and phosphatases), transcription factors, ion/solute transporters, and structural proteins (Box 1) [23]. To foster our understanding of the physiological functions of reactive species and their chemical interactions, the term 'Reactive Species Interactome' (RSI) has recently been

Box 1. Redox Switches as Biological Targets of Reactive Species

Redox switches consist of protein targets that carry a functional cysteine group (i.e., protein thiols) and can be chemically and reversibly modified by reaction with reactive species such that their biological function is altered. Reactive cysteine residues form key parts of many biological proteins and are characterized by unique biochemical properties, including high redox sensitivity, reactivity, and the capacity to bind metal ions. As such, they constitute the central building blocks of redox switches and are highly redox-sensitive to (reversible) thiol modifications in response to ROS, RNS, and RSS. Depending on the type of reactive species that induces the modification, redox switches can undergo a variety of different oxidative modifications. These often lead to specific alterations in the conformational or functional state of the protein, in some cases triggering a change in subcellular localization. Relevant examples of redox switches include metabolic enzymes, protein kinases and phosphatases, ion channels, transporters, structural proteins, and transcription factors. Modulation of redox switches may involve short-term alterations such as modified protein structure and/or activity, as well as longer-term adaptations operating via redox switches that regulate gene expression by epigenetic control mechanisms or transcription factors, for example NF-κB, HIF-1α, and Nrf2/Keap1. The net result of interactions between reactive species and cysteine-based redox switches depends on the type of reactive species involved in the oxidative modification, the electrochemical environment (e.g., pH, cofactor availability), and the redox state, pK_a value, and structural environment of the targeted thiol. Redox switches are considered to be the transducing components of the RSI because they allow rapid sensing of reactive species production and the translation of redox signals into both short-term adjustments of metabolic pathways and longer-term adaptations by activating downstream effector pathways.

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introduced [24]. The RSI constitutes an integrative, conceptual framework that aims to describe (i) chemical interactions between reactive species, (ii) the pathways involved in their generation through cellular intermediary metabolism, (iii) transducing elements of redox regulation involved in modulating downstream intracellular targets to adapt in relation to a change in metabolic demand, and (iv) their role in sensing changes in the extracellular environment.

Of the constituents of the RSI (Figure 1), O_2-derived species (i.e., ROS) are the best-established. In aerobic energy metabolism, O_2 serves as ultimate electron acceptor leading to the formation of superoxide anions ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH), collectively termed ROS, although they have very distinct chemical reactivities and biological effects [23]. Similarly, NO-derived species are referred to as reactive nitrogen species (RNS) [25]. RNS can modify protein tyrosine residues, affect mitochondrial energy production, and alter the structure of lipids and DNA strands [13]. A prominent role in the RSI has been attributed to hydrogen sulfide (H_2S)-derived reactive sulfur species (RSS). Thiols (R-SH) have a central place in redox biology, not only because they form a crucial biological target for other reactive species (including higher oxidized sulfur compounds known as polysulfides) but also because they are the transducing elements that link the sensing and adaptation machinery [24,26].

Quantification of Oxidative Stress in Inflammatory Bowel Disease

Continuous exposure of the inflamed intestinal mucosa to the damaging effects of ROS overproduction compromises gastrointestinal function, including nutritional **malabsorption**, increased intestinal permeability, and disturbed gut motility [27–29]. Therefore, oxidative stress is an important effector mechanism in the pathogenesis of IBD that is closely associated with its development and clinical manifestations [30,31].

Numerous attempts have been made to quantify oxidative stress in patients with IBD (reviewed in [12,15,32]). Most evidence for oxidative stress in IBD is derived from studies that measured oxidized biomarkers in tissue or blood. Oxidative stress markers are generally related to the oxidation of proteins, peroxidation of lipids, oxidative DNA damage, or reduced antioxidant capacity, for example through measurements of the levels and activities of antioxidant enzymes or thiol/disulfide redox couples of glutathione and cysteine. However, it is not clear what particular processes these different biomarkers actually mark within the redox signaling network; thus, a quantitative assessment may not be representative of the overall redox state or representative of a crucial redox hub, and may instead reflect spill-over products originating from local inflammatory processes [24]. In addition, interpretation of reported concentration differences remains challenging because many different methodologies have been adopted, the biomarkers themselves are prone to **diurnal variation**, their analytical determination is laborious, and the chosen methodology is often unsuitable to discriminate between distinct types of reactive species [32–34]. Similarly, direct quantification of ROS is difficult because ROS are characterized by very short biological half-lives, and the available detection techniques are characterized by a lack of sufficient sensitivity and/or specificity despite requiring highly sophisticated laboratory facilities [32]. Many different systemic biomarkers of the human redox system are currently in use, but existing knowledge is insufficient to accurately define the relative contributions of individual compounds and their specific roles in specific redox signaling pathways. A comprehensive understanding of their *in vivo* interactions across different levels of biological organization to correctly interpret redox measurements is lacking [35].

Serum Thiol Status as Integrative Biomarker of the Systemic Redox Status

Free sulfhydryl (R-SH) groups of cysteine in proteins (e.g., albumin), as well as low-molecular-weight (LMW) **free thiols** [e.g., cysteine, glutathione (GSH), homocysteine, and related species], are common targets of the RSI constituents [24,36,37]. Thiols are the main biological targets of

Glossary

Adjunctive therapy: a treatment that is given in addition to an existing primary treatment. It usually serves to support the primary treatment. It is also known as adjuvant therapy or add-on therapy.
Calciphylaxis: a clinicopathological syndrome resulting from calcification of small blood vessels in the deeper layers of the skin and fatty tissues. It is commonly, but not exclusively, observed in patients with end-stage chronic kidney disease. It is associated with poor disease prognosis.
Diurnal variation: variation of a specific variable over the course of a day (a period of 24 h).
Exposome: the diversity and range of all exposures individuals experience during their lifetime, from conception onwards, and how these exposures are related to health.
Free thiols: sulfhydryl-containing compounds that are in a reduced state (R-SH), in contrast to an oxidized or 'bound' state in which the same compounds are bound to other thiols via a disulfide bridge (R-SS-R'). Free thiols can be quantified by derivatization with a thiol-reactive agent, and may consist of protein-embedded free thiols and low molecular weight (LMW) free thiols, the sum of which is referred to as total free thiols.
Malabsorption: inadequate absorption of nutrients across the gastrointestinal tract because of defective digestion, absorption, or transport. It can affect all types of nutrients and occurs in several types of disease including inflammatory bowel disease (IBD).
Oxidative stress: an imbalance between oxidant and antioxidant substances that favors; oxidative stress is associated with disturbed redox regulation and may result in cellular/molecular and tissue damage.
Personalized medicine: medicine tailored to an individual's specific needs. This is the opposite of the 'one size fits all' approach in which all patients are treated in a similar manner irrespective of their specific individual characteristics, for instance genetic differences and/or environmental exposures.
Reactive species: highly reactive small molecules that fulfill important physiological functions, although overproduction may result in oxidative stress.
Reactive Species Interactome (RSI): a recently introduced biological concept describing the chemical interactions of

the RSI and have a myriad of functions, enabling short-term and longer-term adaptations. Modification of thiol groups can be induced by a variety of reactive species, is intimately linked to the global extracellular redox state, and is likely modifiable by nutritional means and changes in gut microbiota composition (Figure 2). Because thiols play a pivotal role in redox regulation, their extracellular fraction may be viewed as a systemic redox buffer. In addition, the extracellular compartment serves as a communication conduit that connects the intestinal supply of RSI precursors with the full complement of intracellular thiol targets.

Extracellular free thiols are a reliable reflection of the overall *in vivo* reduction–oxidation (redox) status because they capture the balance between total oxidant burden and antioxidant capacity [24]. Free thiols are representative of an intricate and dynamic redox regulation system that has significant antioxidant buffering reserve because they act as major scavengers of reactive species [21]. In contrast to the intracellular environment, extracellular thiols are relatively more oxidized (with the exception of the free cysteine group of human albumin, which is ~75% reduced) and are present at much lower concentrations. Blood proteins harbor the largest amount of thiol groups, representing ~60–75% of the total circulating thiol pool [36]. Albumin is the most abundant serum protein in humans, and is therefore quantitatively the most important molecule within this pool, primarily based on its single free cysteine residue (Cys34), but also on its ability to transport LMW thiols [24,38]. Assessment of systemic free thiol concentrations is an easy, minimally invasive, and reproducible method to evaluate the global redox state in normal physiology, aging, and in a variety of disease conditions.

Extracellular free thiol status has been related to (cardiovascular) risk factors, including aging, obesity, smoking, and alcohol consumption [39,40]. Altered levels of free thiols have been observed in a multitude of disease conditions, including cardiovascular diseases, type 2 diabetes mellitus, and renal failure [41–45]. Similarly, reduced levels of systemic free thiols have been observed in patients with CD compared with healthy individuals, and the reduction correlated significantly with systemic markers of inflammation [16]. In another study, free thiols were observed to be a strongly discriminating biomarker for endoscopic disease activity in IBD because they were significantly reduced in patients with severe disease activity compared with patients having only mild disease activity [46]. Although these studies were of a retrospective and cross-sectional nature, they fueled the hypothesis that quantification of serum free thiols may be a novel and minimally invasive strategy to monitor disease activity in IBD. Larger prospective cohort studies will fully determine the clinical utility of thiol redox status in the context of IBD.

Metabolic Precursors of the Reactive Species Interactome and Nutritional Modulation

The significance of nutrition for human health and its importance in IBD is well recognized [47]. Nevertheless, to the best of our knowledge nutrition has not been discussed in the context of the RSI. The RSI is fueled by a variety of precursor compounds in tandem with the activity of specific enzymes necessary for the production of ROS, RNS, and RSS. The composition of the human diet is therefore crucial to achieve an appropriately balanced RSI output, suggesting a potentially important relationship between the local production of reactive species (ROS, RNS, and RSS) and dietary intake, intestinal absorptive function, and adequate metabolic processing to secure adequate precursor availability.

Metabolic Precursors and Modulation of Reactive Oxygen Species

Molecular oxygen (O_2) is the main precursor for the enzymatic and non-enzymatic formation of individual ROS. Two main sources of ROS include mitochondrial respiratory chain activity and the activity of various oxidative enzymes residing in the intestinal mucosa, including NADPH oxidases (NOX enzymes) and dual oxidases (DUOX), which produce excessive amounts of superoxide ($O_2^{\cdot-}$)

reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive sulfur species (RSS) among themselves and with downstream biological targets.
Redox switches: protein thiol groups that can be chemically and reversibly modified by oxidation reactions, resulting in alterations to the structural and/or functional state of the protein that contains the targeted thiol group(s).
Thiols: inorganic (e.g., hydrogen sulfide) or organic (often cysteine-derived) compounds containing a sulfhydryl (-SH) functional group. These are the 'sulfur analog' of alcohols (compounds containing an -OH group). Thiols are good metal ligands and, when oxidized, can give rise to several different RSS.

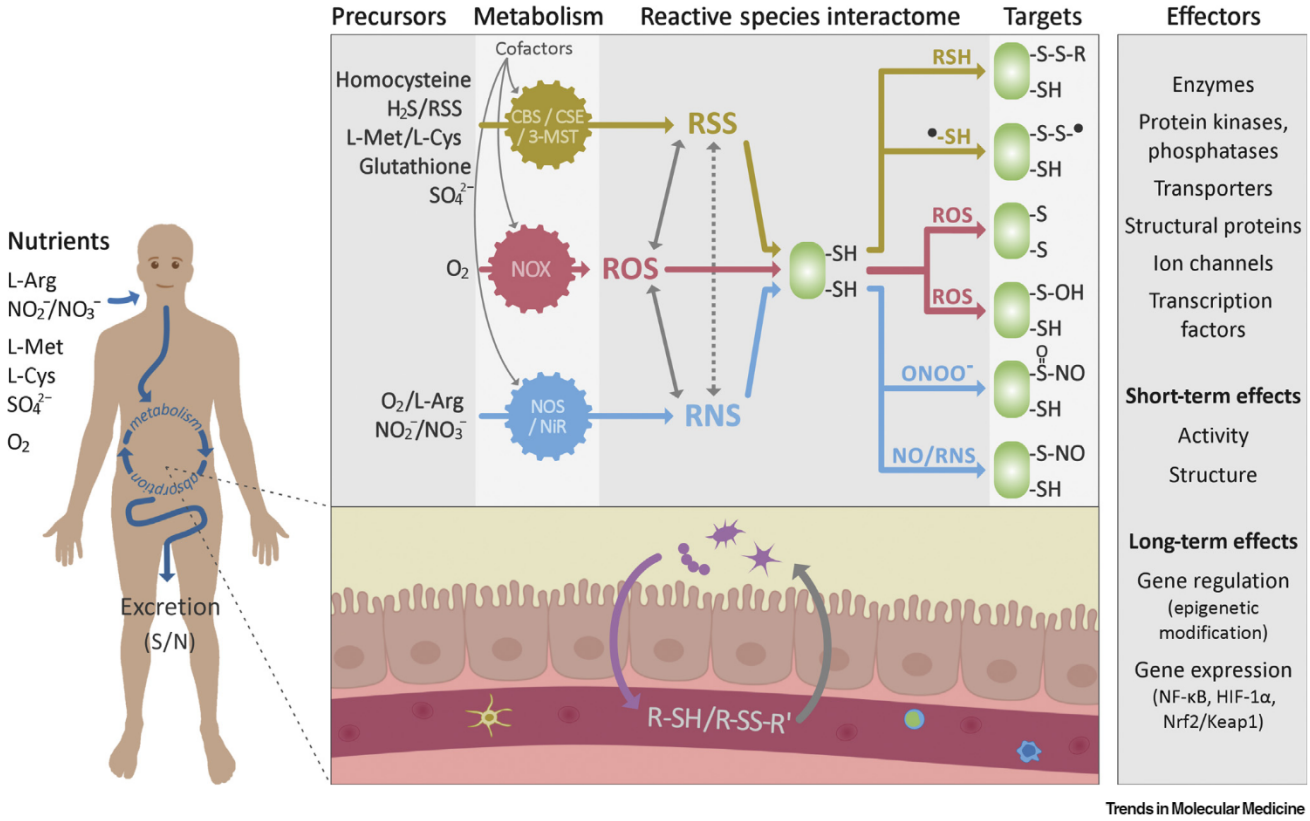

Figure 1. Main Constituents of the Reactive Species Interactome (RSI) and Its Intra- and Extracellular Role in Human Physiology. Precursors of the RSI include both organic (e.g., L-arginine, L-methionine, homocysteine) and inorganic (e.g., oxygen, nitrite, H_2S) compounds as well as cofactors (e.g., vitamins B_6, B_{12}, and C). Both chemical interactions among RSS (reactive sulfur species), ROS (reactive oxygen species) and RNS (reactive nitrogen species) and their interactions with downstream biological targets (i.e., protein cysteine-based thiols and metal centers) constitute the RSI. By interacting with these biological targets, reactive species can lead to short-term adaptive responses, resulting in altered protein structure or activity, as well as longer-term adaptations such as modification of redox switches that are involved in gene regulation. The net physiological state of the RSI is dependent on precursor availability (nutritional intake or protein turnover), absorptive and metabolic capacity (intestinal mucosa and gut microbiota), and excretion (skin, lung, urine, and feces). The intestinal barrier plays a crucial role in fueling the RSI by governing the passage of dietary precursors, but also interfaces microbial redox signaling with that of the human host via the systemic circulation where the RSI may be viewed as a transducing and communicating modality, mediated by cysteine-based redox relays and the interplay between circulating reduced, oxidized, and protein-embedded thiols. Abbreviations: CBS, cystathionine-β-synthase; CSE, cystathionine-γ-lyase; L-Arg, L-arginine; L-Cys, L-cysteine; L-Met, L-methionine; 3-MST, 3-mercaptopyruvate sulfurtransferase; NIR, nitrite reductase; NOS, nitric oxide synthase; NOX, NAPDH oxidase; R-SH, thiol; R-SS-R′, disulfide.

and, in the presence of superoxide dismutase (SOD), hydrogen peroxide (H_2O_2) [13]. Other ROS-producing enzymes include xanthine oxidoreductase, cyclo-oxygenase, myeloperoxidase, 5-lipoxygenase, and cytochrome P450 enzymes. In physiology, ROS are crucially important for cellular signaling and tissue homeostasis because they modulate different transcription factors, enzymes, and other proteins, leading to activation of signal transduction pathways that are responsible for proper cell functioning [48,49]. By contrast, in the context of inflammation or hypoxia, as occurs in IBD, ROS are produced in excessive amounts, leading to cellular and molecular damage and collateral tissue damage (e.g., oxidation of DNA, lipid peroxidation, protein oxidation). ROS levels can be nutritionally modulated by the supply of antioxidant substances that fuel the cellular antioxidant machinery, which comprises both enzymatic (e.g., SOD, catalase, or glutathione peroxidase) and non-enzymatic (e.g., vitamins C and E, polyphenols, or uric acid) antioxidants. The human diet contains several antioxidant micronutrients that could contribute to beneficial modulation of ROS levels. In particular, plant-derived foods are notable sources of antioxidants, encompassing thousands of bioactive compounds with potential antioxidant activity [50]. Fruit-

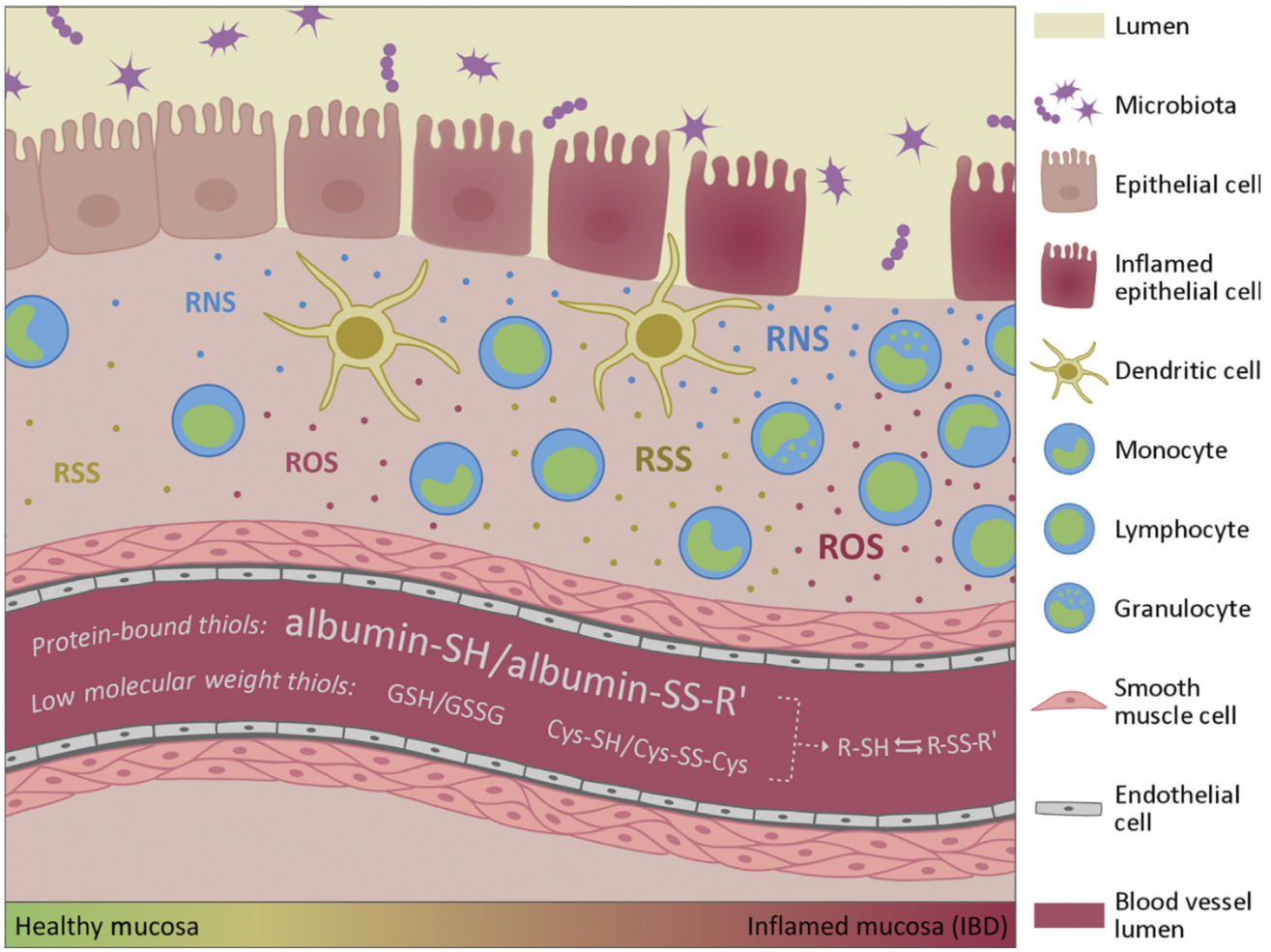

Figure 2. Interface between the Intestinal Reactive Species Interactome (RSI) and Systemic Redox Status in the Healthy and Inflamed Intestinal Mucosa. Extracellular free thiols are represented by high-molecular-weight thiols (i.e., protein-bound thiols, e.g., albumin) and a fraction of low-molecular-weight thiols (e.g., glutathione or free cysteine), the sum of which is collectively referred to as total free thiols. Systemic free thiols (R-SH) are in equilibrium with their oxidized counterparts, disulfides (R-SS-R'). In the context of inflammatory bowel disease (IBD), systemic oxidative stress is reflected by reduced total free thiol levels. The inflamed intestinal mucosa in IBD is typically characterized by the infiltration of many different immune cells, which generate and release large amounts of reactive species that serve as important signaling molecules and contribute to their immunological functions. However, continuous exposure of the intestinal mucosa to considerable amounts of reactive species leads to a pathological shift of the thiol/disulfide equilibrium (R-SH vs. R-SS-R') as antioxidant defense mechanisms become overwhelmed. This is accompanied by collateral tissue damage (e.g., epithelial cell injury and/or increased intestinal permeability) and perturbations in gastrointestinal physiology. These sequential processes are probably influenced by absorbed nutrients and gut microbes that reside above the intestinal epithelium. Abbreviations: RNS, reactive nitrogen species; ROS, reactive oxygen species; RSS, reactive sulfur species.

and vegetable-rich diets contain the largest amount of antioxidant substances; examples include cruciferous vegetables, tomatoes, and legumes, as well as apples, grapes, berries, and citrus fruits [51]. However, in the context of IBD, further research is warranted to investigate effects of ROS-scavenging compounds under various conditions and in different preclinical models. Few compounds have been tested more than once in IBD, limiting the progress of human studies [17]. Nonetheless, some human intervention trials that tested a small number of different antioxidants in the context of IBD failed to demonstrate any beneficial effect on disease activity or disease course (section on Redox Medicine in Inflammatory Bowel Disease: Promises and Pitfalls). One of the main challenges will lie in providing additional antioxidant protection in such a manner that ROS levels stay within physiological limits to support cellular physiology.

Metabolic Precursors and Modulation of Reactive Nitrogen Species

L-arginine (L-Arg) is the main precursor for NO. In intestinal physiology, NO is important for the induction of gut peristalsis and regulates mucosal blood flow while also preventing leukocyte aggregation [52]. L-Arg is a semi-essential amino acid that is crucial for protein biosynthesis. It is endogenously produced by the intestinal epithelium, the liver, and the kidney. In the early years of life, intestinal epithelial cells can synthesize L-Arg themselves, mainly by using dietary glutamine and proline to synthesize L-citrulline in mitochondria, which is secreted into the systemic circulation and eventually converted by the kidney to L-Arg (this is known as the intestinal–renal axis, that is responsible for ~60% of total L-Arg synthesis) [24]. Another source includes the hepatic urea cycle in which L-citrulline is produced from bicarbonate (HCO_3^-) and ammonia (NH_3), a process which takes place in the mitochondria [53].

Inadequate nutritional supply of L-Arg is associated with immune deficits and correlates with intestinal inflammation or infections, situations that are characterized by increased protein breakdown [54,55]. Thus, in addition to endogenous production of L-Arg, there is an increased need for its dietary supply in pathological conditions to maintain adequate NO production and protein biosynthesis. There are evident correlations between dietary L-Arg intake and serum NO metabolites, which is not surprising given the fact that systemic L-Arg levels are the main contributor to NO production (~54%) [56,57]. Inflammatory conditions are associated with enhanced expression of arginase, which leads to a shunting of L-Arg away from NOS to produce ornithine and urea [58]. Dietary sources rich in L-Arg include, among others, soy, nuts, and meats.

A large body of evidence supports **adjunctive therapy** with L-Arg for patients with IBD. Dietary supplementation with L-arginine has protective effects in animal models of colitis and improves gut microbial diversity [59,60]. In intestinal tissue of UC patients, diminished L-arginine availability has been demonstrated, which strongly correlates with inflammation [55]. L-Arg is proposed to alleviate intestinal inflammation most probably by promoting the activation of NOS enzymes, thus increasing endogenous NO production [61]. Dietary supplementation with L-Arg could be a potential therapy for patients with IBD, especially in combination with dietary glutamine, which has been suggested to exert strong anti-inflammatory effects on the inflamed intestine [62].

Metabolic Precursors and Modulation of Reactive Sulfur Species

RSS are beneficially involved in antioxidant mechanisms, blood clotting, and vasodilation [63]. In *in vitro* and animal studies, hydrogen sulfide (H_2S) can be delivered by parenteral administration of soluble sulfide salts such as sodium hydrosulfide (NaHS) and sodium sulfide (Na_2S) or by inhalation [64,65]. RSS can also be administered using sodium thiosulfate (STS, $Na_2S_2O_3$), as an intermediate precursor and oxidation product of RSS [66]. Thiosulfate can generate RSS through both a non-enzymatic and an enzymatic glutathione-dependent pathway. In humans, thiosulfate is used for short-term treatment of **calciphylaxis** and is also applied as treatment in cyanide poisoning and cisplatin toxicity [67]. Vasodilating and antioxidative properties of thiosulfate have also been described. These beneficial effects of thiosulfate have already made it an attractive therapeutic dietary supplement for reducing the damaging effects of hypertension and renal damage [68]. Importantly, the administration of H_2S donors has been demonstrated to alleviate intestinal inflammation in several animal studies, providing a rationale for testing the therapeutic potential of these compounds in patients with IBD [69–71].

The precursors for RSS are derived from dietary sulfur compounds. Sources of sulfur include inorganic sulfur compounds (e.g., sulfate, SO_4^{2-}) and sulfur-containing amino acids (SAAs) such as L-methionine, homocysteine, and cysteine. Protein-rich and alliaceous or cruciferous vegetable-rich diets contain high amounts of sulfur owing to their high content of SAAs and

inorganic sulfur compounds, respectively [72]. SAAs are important substrates for the methionine recycling (one-carbon metabolism) and transsulfuration pathways in which sulfate, taurine, and hydrogen sulfide (H_2S) can be generated through the consecutive actions of the enzymes cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE), 3-mercaptopyruvate sulfurtransferase (3-MST), and cofactors including serine and vitamin B_6 [24,73].

It is unclear whether methionine availability in IBD has protective or harmful effects. In animal models of IBD, methionine-restricted diets have been demonstrated to enhance epithelial barrier function, reduce proinflammatory cytokine levels, enhance antioxidant enzyme activity, and suppress colonic carcinogenesis [74,75]. By contrast, methionine-rich animal diets improved antioxidant machinery, villus morphology, and intestinal absorptive capacity [76,77]. Hyperhomocysteinemia is reflective of poor nutritional status or metabolic perturbation, and is a risk factor for cardiovascular disease. Likewise, hyperhomocysteinemia has consistently been reported in IBD patients [78,79]. This is believed to be the consequence of nutritional deficiencies in essential cofactors such as vitamins B_6 and B_{12}, as well as folate, which are necessary for adequate functioning of the methionine recycling and transsulfuration pathways [80].

Nutritional Modulation of Reactive Species: Future Challenges

Nutritional modulation of reactive species production and the assessment of downstream effects on physiology and IBD remain challenging because the modulated redox processes cannot be reliably quantified with a single biomarker. To better control the dynamics of these processes, combined readouts in healthy individuals under various physiological conditions and in patients with IBD are needed. Studying the effects of lifestyle and nutritional modulation on readouts of the RSI may eventually lead to targeted modulation of redox signaling in IBD.

Redox Medicine in Inflammatory Bowel Disease: Promises and Pitfalls

With the invention of multi-omics technologies, a systems-biology approach is increasingly advocated to identify disease biomarkers and therapeutic targets for IBD. Multi-omics datasets are being constructed to gain comprehensive insights into the genetic, microbiome-related, metabolic, and biochemical factors underlying the pathogenesis of IBD. Recent breakthroughs have been made by combining metagenomics, metatranscriptomics, metaproteomics, and metabolomics [81]. The main challenge with the results obtained thus far is that between-subject differences or cohort heterogeneity continue to explain the majority of data variation. To resolve this issue, it will be crucial to carefully characterize individual patient phenotypes while taking into account demographic, clinical, dietary, environmental, and disease-specific information, enabling detailed phenotypic stratification of individual patients during their disease course [82].

In line with these principles, ongoing exploration of the RSI should ideally follow an omic approach to identify central regulators and key determinants of the redox system that could be integrated with other single-omics datasets, ultimately leading to the identification of functionally relevant disease markers and treatment targets in IBD (Figure 3). By comparison with other layers of biological regulation, such redox-related omic approaches are at their infancy [83]. To some extent, this is due to several methodological constraints, such as the lack of sensitivity and specificity of available detection techniques, the fact that direct measurement of reactive species in intestinal cells or tissues is technically challenging because of their short biological half-lives, and that most techniques require highly sophisticated and expensive laboratory facilities [32,84]. Most importantly, insufficient understanding of the entire redox regulation system constrains the identification of outcome measures that are of biological relevance in that they represent a substantial portion of the human redox architecture [35]. Similarly, there is no clear definition of what criteria

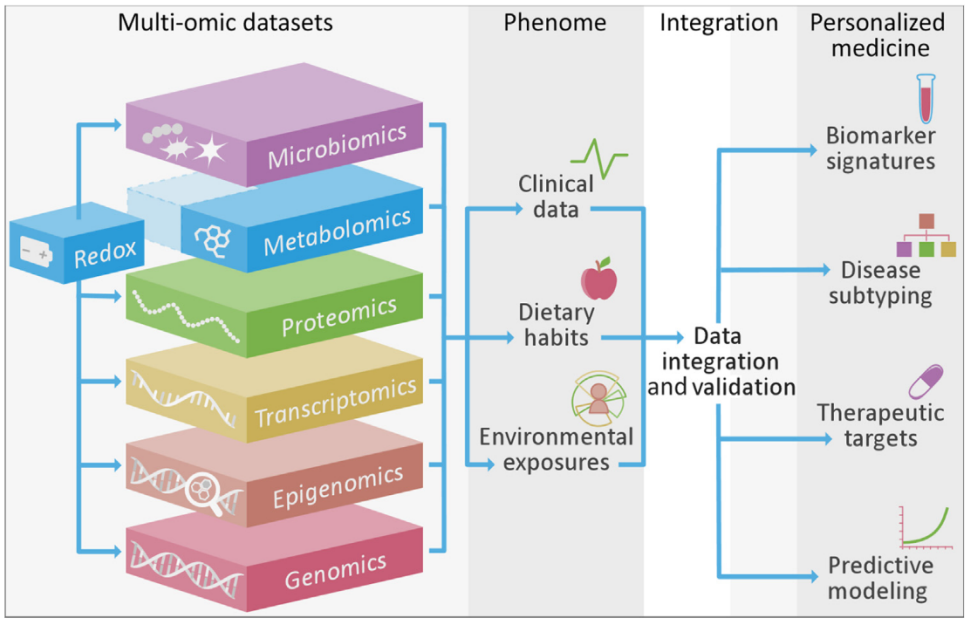

Figure 3. Integration of Multi-Omics Biological Datasets and Phenotypic Data Is Key to the Development of Personalized Medicine in Inflammatory Bowel Disease (IBD). A redox metabolomics approach should be followed to identify key regulators of the Reactive Species Interactome (RSI) and integrative biomarkers of the redox system that could be integrated with other omics datasets. However, when combining different single-omics datasets, one should carefully stratify patients by taking into account detailed phenome data, including demographic, clinical, and disease-specific characteristics, dietary habits, and environmental exposures. After integrating these different data sources with each other, the next step includes both internal and external validation of molecular profiling data in large, preferably prospective, longitudinal patient cohorts. Ultimately, this will lead to the identification of unique biomarker signatures, accurate disease subtyping, potential novel therapeutic targets, and relevant prediction models that could be used in the context of personalized medicine in patients with IBD.

potential redox readouts should fulfill to be able to reliably assess the redox system on a large-scale basis [24].

Considering the role of oxidative stress in IBD, implementation of antioxidant therapy would seem to be a rational therapeutic choice. In fact, commonly prescribed 5-aminosalicylic acid (5-ASA) compounds exert their therapeutic effects largely through antioxidant mechanisms because they are important scavengers of reactive species [25,85]. 5-ASA derivatives are known to inhibit peroxynitrite ($ONOO^-$)-mediated reactive species formation [20]. Nonetheless, many antioxidant therapies have so far been disappointing [18]. These failures could be attributed to the fact that reactive species are part of an elaborate and highly complex redox signaling network that primarily serves a physiological purpose, and patients display considerable heterogeneity in their responsiveness to treatment. This makes it unreasonable to assume that a single antioxidant supplementation would confer marked health benefits in all patients. Antioxidant therapies might better be targeted to patients preselected for pathological overproduction of select reactive species, with careful monitoring to avoid interference with physiological redox signaling. Recently, a prospective clinical study involving patients with CD demonstrated that, in addition to standard medical treatment, supplementation with the antioxidant riboflavin (vitamin B_2) resulted in improved systemic redox status [86].

Given the complex pathophysiology of IBD and the indeterminate role of individual RSI constituents, many potential factors need to be controlled for to obtain unequivocal evidence for the effects of

Clinician's Corner

Inflammatory bowel diseases (IBD), namely Crohn's disease and ulcerative colitis, are characterized by aberrant redox signaling mechanisms that affect the structure and function of the gastrointestinal tract.

A novel biological concept was recently introduced to capture the entire redox regulation system. This so-called Reactive Species Interactome (RSI) covers precursor substances, intermediary metabolism, the generation of reactive species, and modifications of biological targets.

The metabolic state of the RSI is significantly dependent on the availability of dietary precursors, including organic and inorganic compounds and their cofactors (e.g., amino acids, vitamins, oxygen, nitrite, and sulfate). Nutrition may therefore be considered as a major contributor to adequate functioning of the RSI. In addition, dietary modulation of gut microbiota composition fuels the RSI because the gut microbiota also provides a large amount of precursor substances for reactive species production.

Predictive biomarkers from the RSI could improve monitoring of disease activity, risk stratification, and prediction of therapeutic success and disease prognosis. As a first convincing step in this direction, free thiols have been demonstrated to be closely associated with disease activity in IBD.

A redox metabolomics approach, including therapeutic interventions aimed at modifying the thiol redox metabolome, may open new avenues for successful redox-targeted therapeutic interventions in IBD.

antioxidant therapy in IBD. Only a few randomized controlled trials (RCTs) have rigorously investigated the therapeutic efficacy of antioxidants in IBD [87–90]. One RCT investigated the combined effect of vitamin C and E supplementation versus placebo in CD patients, resulting in significant reduction in oxidative stress, but unaltered disease activity [87]. Another RCT in patients with CD evaluated the combination of fish oil and antioxidants (vitamins A, C, E, and selenium) and observed decreased inflammatory cytokine production [88]. In UC patients, RCTs have evaluated the effect of *N*-acetylcysteine (NAC) and curcumin supplementation in addition to 5-ASA therapy, and both trials observed an improvement in disease activity [89,90]. Most of these RCTS were performed using relatively low antioxidant dosages, low-potency antioxidants, short follow-up periods, and patient groups with only quiescent or mild disease activity. The remaining body of clinical trials investigating antioxidant therapies consists of largely uncontrolled studies in patient populations of considerable phenotypic heterogeneity.

In addition to anti-inflammatory and antioxidant therapy in IBD, dietary provision of nutritional precursors is a key determinant of an individual's redox status by driving intermediary metabolism in the RSI. To improve the effectiveness of these nutritional precursors, a promising future therapeutic strategy consists of the application of colon-targeted delivery systems (CTDSs) to modulate the RSI of the local intestinal microenvironment [91,92]. This avoids absorption of RSI precursors in the small intestine and may permit direct modulation of the mucosal RSI. In circumstances of uncontrolled oxidative stress, this pharmacological approach could become an intestinal RSI-modulating route for restoring gut redox balance by enabling effective and gut-selective delivery of precursors exclusively to the site of inflammation and/or oxidative stress [93]. Furthermore, as our understanding of the molecular signatures characterizing the RSI grows, this will lead to a better understanding of how to modulate this system for improved outcome using rational nutritional interventions tailored to an individual's nutritional status in a personalized medicine approach. Extracellular thiol status could operate as a monitoring tool to identify patients who may benefit most from redox-targeted therapy. One should aim to optimize current therapeutic strategies in IBD by combining standard anti-inflammatory treatments with individually tailored nutritional therapy. Following this combined approach, suppression of intestinal inflammation, reduction of oxidative mucosal damage, and restoration of intestinal epithelial integrity will contribute to improve intestinal absorptive function which is crucial to maximize nutritional precursor availability.

Further research should be directed to understanding the role of the RSI in IBD and to establishing targeted therapy using 'redox metabolomics', which can be defined as the analysis of stable products of metabolic pathways within the redox system (Figure 3). It is essential to focus on the components of the RSI that constitute central regulatory hubs of redox metabolism or represent 'integrative biomarkers' that combine readouts of multiple redox-regulated metabolic pathways. Several suggestions for potential (key) targets of the RSI have been put forward in the context of redox metabolomics [23] (some of which are listed in Table 1). To fully capture the metabolic status of the RSI, integrative measurements of (i) precursor availability, (ii) the complement of transducing components, and (iii) the amounts of stable end-products of S-, N-, and O-related metabolites could potentially provide us with a targeted omic approach to redox biology and disease.

Concluding Remarks

Oxidative stress is a key factor in the pathophysiology and progression of IBD, and is intimately associated with the development and course of intestinal inflammation. In addition to ROS, there are several regulatory elements within the IBD redox network. This review has introduced the concept of the RSI that describes the interactions between ROS, RNS, and RSS among themselves and with downstream biological effector proteins. It is essential to comprehensively disentangle the role of the RSI in IBD to provide a distinct rationale for novel therapeutic

Outstanding Questions

What alterations in RSI status can be observed in IBD patients?

What is the potential role of RSS with regard to the onset and progression of IBD?

What is the impact of the gut microbiome on the RSI of the host in healthy conditions and in IBD?

What methodological platforms should be used or need to be developed to comprehensively assess the entire redox metabolome?

What is the clinical utility of predictive redox biomarkers in the context of IBD?

What are potential components of the RSI that could be integrated into a redox metabolomics approach, and what criteria should they ideally fulfill?

What metabolic pathways should be considered as promising avenues for targeted therapeutic modulation of aberrant redox homeostasis in IBD?

What potential options are available for targeted personalized nutritional modulation of redox signaling in IBD?

Table 1. Potential Integrative Biomarkers of the Reactive Species Interactome That Could Be Incorporated into a 'Redox Metabolomics' Approach Together with Their Corresponding Metabolic Representations

Components	Potential targets	Metabolic representations
Precursors and substrates of the RSI	Arginine	The main nutritional precursor for the formation of RNS and the main substrate for NO-related chemistry
	Homocysteine	The main nutritional precursor for the formation of RSS and substrate for H_2S production. Readout of methionine availability and cofactor availability (betaine, folate, vitamin B_6, vitamin B_{12}), the methionine recycling pathway, tetrahydrofolate pathways (one-carbon metabolism), and the transsulfuration pathway
	Glycine	The main nutritional precursor for glutathione synthesis (in addition to glutamine and cysteine) and porphyrin synthesis
Transducing components of the RSI	Cysteine-based redox switches, systemic thiol pool (reduced vs. oxidized, free, and protein-bound thiols)	The central biological targets of the RSI that function as redox relays leading to multiple short-term and longer-term biological adaptations
Stable end-products of the RSI	Sulfur (S)	Relatively stable metabolites include hydrosulfides (R-SH), persulfides (R-SSH), polysulfides (R-SS_nH), thiosulfate ($S_2O_3^-$), and sulfate (SO_4^{2-})
	Nitrogen (N)	Relatively stable metabolites include ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), and nitroso- or nitrosyl (R_xNO) species
	Oxygen (O)	Relatively stable metabolites include peroxides (H_2O_2), lipid peroxidation products (F_2-isoprostanes, malondialdehyde), protein carbonyl (CO) groups, total antioxidant capacity (TAC), individual redox couples (e.g., GSH/GSSG and cysteine/cystine)

approaches, ranging from dietary modulation to biological therapies, targeted at integrative components of the redox network (see Clinician's Corner).

The extracellular thiol pool lies at the center of the RSI, and represents the main downstream biological targets of reactive species as well as acting as multimodal redox relays that govern protein functions including structural, catalytic, and regulatory alterations. Extracellular thiols act as major scavengers of reactive species and provide a robust monitoring tool for translational studies. Based on current insights, extracellular thiols merit further investigation as integrative biomarkers of systemic oxidative stress in prospective, longitudinal cohort studies. It may be more informative to focus on a broad panel of redox-related substances instead of one or two biomarker(s) of oxidative stress to accurately determine the dynamics of reactive species in IBD. Redox system components could be integrated into machine-learning algorithms for predictive modeling in large population cohorts.

Redox metabolomics, in conjunction with other multi-omics technologies, may be a promising approach to improve our understanding of the pathophysiology of IBD. To make further progress, studies are needed that integrate redox signaling readouts with other multi-omics datasets, including genomics, transcriptomics, metagenomics, metabolomics, and proteomics, accompanied by detailed phenotypic patient stratification. This will allow the interplay between the host redox system and its determinants to be unraveled from different biological angles, and will finally provide a basis for the development of targeted therapeutic approaches to tackle IBD (see Outstanding Questions).

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