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Redox stress in COVID-19: Implications for hematologic disorders

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ABSTRACT

COVID-19 is the respiratory illness caused by the beta coronavirus SARS-CoV-2. COVID-19 is complicated by an increased risk for adverse thrombotic events that promote organ failure and death. While the mechanism of action for SARS-CoV-2 is still being understood, how SARS-CoV-2 infection impacts the redox environment in hematologic conditions is unclear. In this review, the redox mechanisms contributing to SARS-CoV-2 infection, coagulopathy and inflammation are briefly discussed. Specifically, sources of oxidant generation by hematopoietic and non-hematopoietic cells are identified with special emphasis on leukocytes, platelets, red cells, and endothelial cells. Furthermore, reactive cysteines in SARS-CoV-2 are also discussed with respect to oxidative cysteine modification and current therapeutic implications. Lastly, sickle cell disease will be discussed as a hematologic disorder with a pre-existing prothrombotic redox condition that complicates treatment strategies for COVID-19. An understanding of the redox mechanism may identify potential targets for COVID-19-mediated thrombosis in hematologic disorders.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative viral agent for the respiratory illness Coronavirus Disease 2019 (COVID-19) and has millions of cases, deaths, and hospitalizations worldwide [1]. Individuals with underlying hematologic disorders are at an increased risk for developing severe respiratory conditions and adverse clotting events [2,3]. Several mechanisms promote the thrombogenic and hypercoagulable state in COVID19. These mechanisms include dysregulated platelet activation [4–6], endothelial dysfunction and vasculitis [7], the activation of immune cells [8], and the activation of clotting factors and subsequent deficiency in fibrinolytic mechanisms [9]. Although the coagulopathy and vasculitis phenotype in COVID-19 are likely driven by a combination of factors, recent evidence suggests that the redox environment also contribute to SARS-CoV-2 infection and disease severity.

The redox environment is sensitive to the flux of oxidants and the associated antioxidative buffering capacity. The general mechanisms of oxidant and antioxidant generation have been discussed elsewhere [10] and will only be briefly discussed in this review as they relate to the activation of hematopoietic (*e.g.* neutrophils, T lymphocytes, platelets, and red blood cells) and non-hematopoietic

Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, Coronavirus Disease 2019; ACE2, angiotensin-converting enzyme 2; DIC, disseminated intravascular coagulation; NET, neutrophil extracellular trap; ROS, reactive oxygen species; NADPH, reduced nicotinamide adenine dinucleotide phosphate; MPO, myeloperoxidase; DNA, deoxyribonucleic acid; PMA, phorbol 12-myristate 13-acetate; PKC, protein kinase C; ATP, adenosine triphosphate; eNOS, endothelial nitric oxide synthase; RBD, receptor binding domain; MPro, 3CL main protease; RNS, reactive nitrogen species; GSH, reduced glutathione; GSSG, oxidized glutathione; AE1, Band 3.

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vascular endothelial cells. Importantly, during viral infection the activation of these cells contributes to the generation of potent oxidative species (Fig. 1). These oxidants modify a myriad of cellular constituents, including redox-sensitive cysteines of proteins, that may contribute to the coagulopathy associated with SARS-CoV-2. Indeed the thiol-disulfide balance during SARS-CoV-2 infection has been proposed as an important mechanism for viral entry relating to angiotensin-converting enzyme 2 (ACE2), the receptor for SARS-CoV-2 [11]. Disruption in cellular metabolism and a dysregulated glutathione balance also contributes to oxidative stress during infection [12]. However, the role of virally induced oxidant generation and the redox imbalances during SARS-CoV-2 infection are still poorly understood.

The mode of SARS-CoV-2 viral infection have been published elsewhere [13,14]. In this review, the sources of oxidant generation during infection from non-hematopoietic and hematopoietic cells are discussed. As oxidative stress promotes alteration in protein function, the role of redox-sensitive cysteines during SARS-CoV-2 infection will also be discussed. Lastly, as COVID-19 complicates treatment strategies for hematologic disorders, sickle cell disease will be discussed as an example where the pre-existing redox state impacts COVID-19 severity.

2. Oxidative stress from hematopoietic and non-hematopoietic cells during COVID-19

The initial findings in patients with severe COVID-19 suggest that SARS-CoV-2 infection promotes microvascular clots and greatly elevates fibrinogen and D-Dimer levels [15,16]. The hematologic parameters during COVID-19 are similar to but different from disseminated intravascular coagulation (DIC). The microvascular clots during COVID-19 are caused by the activation of many different cell types including those from hematopoietic and non-hematopoietic origin. Several reviews already detail the mechanisms by which vasculopathy contributes to a DIC-like phenotype in COVID-19 [7,9]. However, oxidative stress is a component to COVID-19 severity. Hematopoietic and non-hematopoietic cells are sources of oxidants during SARS-CoV-2 infection and are detailed in Fig. 2A–D. Additional disparity between mortality rates of males compared to females with COVID-19 suggests that males are at an increased risk for severe infection, intensive care treatment, and death [17]. This disparity could be linked to differences in gender-mediated responses to stressors, including anti-oxidative signaling during oxidative stress [18]. Specifically, women have lower levels of oxidative stress signals compared to men from estrogen-mediated anti-oxidative signaling [19]. Oxidant generation is thus a contributing factor to the coagulopathy observed with COVID-19.

2.1. Oxidant generation by immune cells during SARS-CoV-2 infection

There is undoubtedly a direct role for the innate immune system during SARS-CoV-2 infection in COVID-19 pathophysiology. In response to SARS-CoV-2 infection, the levels of neutrophils [20–23], macrophages [23,24], and dendritic cells [25,26] are elevated. Earlier evidence suggests that T lymphocyte levels were also increased and contributes to disease severity [23,27,28]. Lymphocyte activation during the initial phases of the infection promotes the generation of cytokines and chemokines against the viral particles resulting in a cytokine storm—an uncontrolled pro-inflammatory response that further triggers more inflammation [29,30]. Two mechanisms which enhances inflammation are the formation of neutrophil extracellular traps (NETs) and reactive oxygen species (ROS) generation (reviewed in [30]). In addition to the activation of immune cells, lymphocyte numbers are increased in COVID-19 patients compared to healthy controls [31]. The functional impact of oxidant generation from immune cells during SARS-CoV-2 infection is not well-understood but is attributed to inflammation and cell death during the disease.

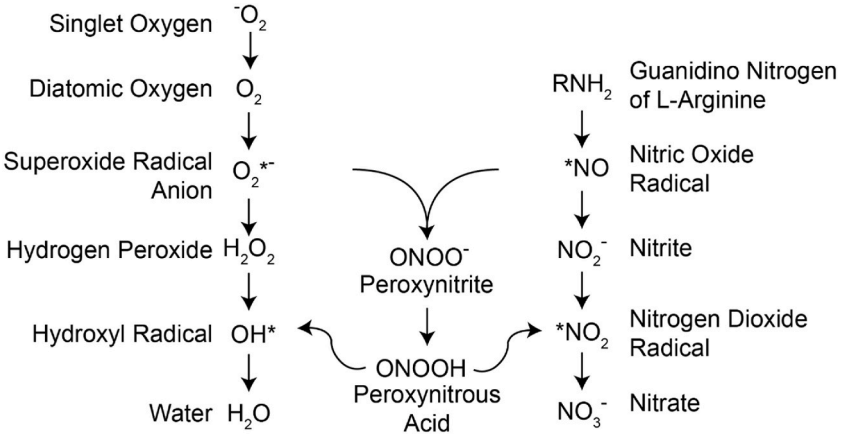

Fig. 1. Oxidant species of oxygen and nitrogen in hematologic disorders. On the left side, reactive oxygen species (ROS) are generated from the chemical reduction and oxidation of oxygen. On the right side, reactive nitrogen species (RNS) are generated from the chemical reduction of the guanidino nitrogen of L-arginine. In some instances, highly reactive oxygen-nitrogen species are generated from the combination of both ROS and RNS. This figure was modified from Yang et al. [71].

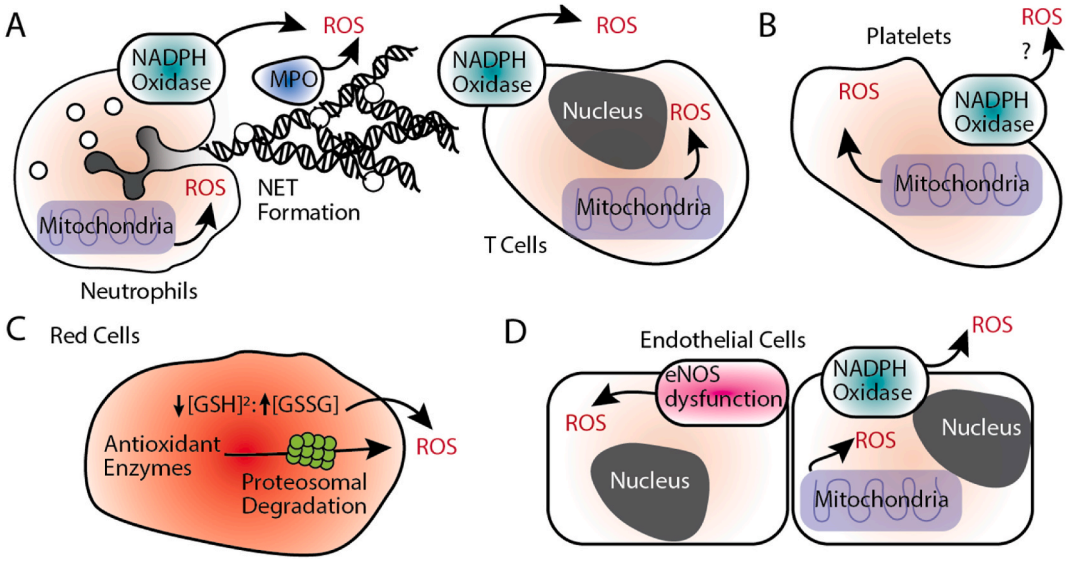

Fig. 2. Oxidant generation from neutrophils, T cells, platelets, red cells, and endothelial cells during SARS-CoV-2 infection. (A) Left: in Neutrophils, NADPH Oxidase activity and metabolic dysregulation generates reactive oxygen species (ROS). Furthermore, neutrophil extracellular trap (NET) formation and the release of myeloperoxidase (MPO) promotes ROS formation. Right: in T cells, ROS is generated through the mitochondria and NADPH oxidase. (B) Platelets generate ROS through the mitochondria although it is likely that NADPH oxidase is also a major contributor. (C) Red cells have a decrease in the antioxidative buffering capacity (decrease glutathione (GSH) and increase oxidized glutathione (GSSG)) and decrease in the levels of antioxidant enzymes that is likely through ubiquitin-mediated degradation pathways to promote ROS generation. (D) In endothelial cells, eNOS dysfunction, NADPH oxidase activity, and electron leakage from the mitochondria generates ROS.

2.1.1. Oxidant generation from neutrophils

Neutrophil infiltration into the pulmonary capillaries was documented in autopsy samples of COVID-19 patients [32] indicating their direct involvement in COVID-19 pathogenesis. Neutrophilia, a condition of elevated neutrophil count in the circulation, is also associated with COVID-19 severity [33]. NETs are formed when neutrophils go through programmed cell death and release their nuclear contents to the extracellular milieu. NETs were found in elevated levels in SARS-CoV-2 infection [34–37]. In the context of thromboinflammation, NETs activate complement factors, promote platelet activation, and enhance coagulation factor activities [34, 38]. In the process of NET formation, neutrophils are one of the most potent generators of ROS where micromolar levels of oxidants are produced. This process termed oxidative burst kills pathogens and is a protective mechanism for the host [10]. In neutrophils, reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and dual oxidases are the enzymes generating ROS for oxidative burst. As the enzymes require electron carriers, oxidative burst is delicately linked to the metabolic function of the cell. Indeed, neutrophils are highly glycolytic and mitochondrial respiration uncoupling levels are elevated in severe COVID-19 [39]. Circulating myeloperoxidase (MPO) and MPO-deoxyribonucleic acid (DNA) complexes are also elevated, suggesting degranulation of granulocytes [39,40]. The altered metabolism promotes the generation of ROS by providing electron carriers for NADPH oxidase or having a backup of electrons in the electron transport chain of the mitochondria. ROS from isolated neutrophils were increased in COVID-19 patients that was further augmented by phorbol 12-myristate 13-acetate (PMA), a compound that activates protein kinase C (PKC) [41]. PKC is an intracellular serine/threonine kinase that links signaling from the receptor level to the generation of ROS by phosphorylating and activating the p47 phox subunit of NADPH oxidase [42]. Thus, PMA was used in this study as a tool to test NADPH oxidase-mediated oxidative burst from neutrophils [41], suggesting that the potential for ROS generation is increased during COVID-19. The findings also suggest that neutrophils are a contributor to oxidative signaling during SARS-CoV-2 infection.

2.1.2. Oxidant generation from T lymphocytes

T cells are essential to immunity during SARS-CoV-2 infection. T-cell responses are mediated through $CD4^+$ and $CD8^+$ cells and the assessment of T cell responses and humoral immunity in COVID-19 patients have been extensively characterized [43–45]. In the context of SARS-CoV-2 induced T cell oxidative stress, the mitochondria and the metabolic network participate in ROS generation [46, 47]. Specifically, T cell intracellular ROS were accumulated in COVID-19 patients and was associated with mitochondrial mass and architecture [47]. Further analyses identified increased fatty acid uptake, mitochondrial ROS generation, and altered mitochondrial respiration [47]. Additionally, NADPH oxidase activation was also implicated. ROS generation from COVID-19 patient T-cells was prevented by the treatment with dexamethasone, an anti-inflammatory agent, and an inhibitor to NADPH oxidase [47]. These findings suggest that in COVID-19, T-cell oxidative stress could be linked to an increase immunogenic demand and a hypoxic condition.

2.1.3. Oxidant generation from monocytes and macrophages

Macrophages are part of the immune system's arsenal against SARS-CoV-2 infection. Specific measurements of ROS and their

potential sources have not been investigated in macrophages during infection. Like other immune cells discussed above, macrophage ROS generation during SARS-CoV-2 infection is likely driven through specific oxidases. Given their role in immunometabolism, macrophage ROS is also likely to be generated through metabolic dysfunction.

2.2. Oxidant generation from platelets

Platelets are anucleated cell "fragments" that are derived from megakaryocytes. During viral infection, platelets are prothrombotic [48]. Not surprisingly, SARS-CoV-2 infection is associated with increased platelet reactivity [4–6] and the microvascular thrombis in COVID-19 are also platelet-rich [49,50]. SARS-CoV-2 was hypothesized to be recognized by platelets through toll-like receptors, which are a class of receptors that recognizes specific motifs of pathogens and contributes to ROS generation. A potential role for ACE2, the natural receptor for SARS-CoV-2, was also hypothesized to be present on platelets and megakaryocytes [29,51]. ROS generation in platelets by SARS-CoV-2 infection further augments pro-aggregatory and procoagulant responses through a mechanism that is yet to be defined.

A major source of ROS in platelets is the mitochondria. Platelets contain roughly 5–8 mitochondria and are metabolically active [52]. The mitochondria orchestrate the metabolism of the cell to generate energy in the form of adenosine triphosphate (ATP). During this process, electron leakage generates ROS that are physiologically scavenged by catalase, a mitochondrial antioxidant enzyme. Platelets produce mitochondrial ROS when stimulated [53]. Oxidant generation in this context increases phosphatidylserine externalization to promote the tenase and prothrombinase complexes for procoagulant activity. In a small cohort of patients with COVID-19, Sumbalova et al. found that platelet mitochondria are dysfunctional and the electron carrier co-enzyme Q10 levels are decreased [54]. They also showed elevated levels of lipid peroxidation in the plasma in COVID-19 patients, suggestive of oxidative stress. The role of platelet mitochondria dysfunction during SARS-CoV-2 infection and how this contributes to a prothrombotic response requires further investigation as was suggested by others [55].

It will not be surprising if viral infection is linked to the activity of platelet NADPH oxidase as NADPH oxidase is also a large source of oxidants. In HIV patients, markers of NADPH oxidase activation were increased compared to healthy human subjects [56]. It is likely that NADPH oxidase is activated in the earlier phases of viral infection as a potential defense mechanism against pathogens [48]. Platelet NADPH oxidase activity during COVID-19 requires further investigation since HIV and SARS-CoV-2 have differing pathophysiology. The difference could be multifactorial; however, in the context of ROS generation during infection, these differences could be due to the fate of the oxidants (*e.g.* in the oxidation of proteins, lipids, or nucleotides).

2.3. Oxidant generation from red blood cells

Inflammation changes the redox statuses and metabolism within red blood cells. In an elegant proteomic and metabolomic study, Thomas et al. showed that red blood cells from COVID-19 patients have significantly altered glycolysis and have elevated levels of ribose phosphate—the end product of the pentose phosphate pathway [57]. Although indicative of ROS production, oxidant generation are supported by elevated levels of oxidized glutathione in red blood cells. Red blood cells also have lower levels of antioxidant enzymes potentially due to degradation by the ubiquitin-proteasomal pathway and contributing to an overt oxidative stress. Functionally, the damage induced by ROS affects red blood cell structural and membrane integrity as evidenced by increased oxidation of the N-terminus of band 3 (AE1), a red blood cell membrane protein that senses the cell's redox status and metabolism [58]. Based on these findings, it is not inconceivable that red blood cells could be important contributors of redox signaling during COVID-19 pathogenesis in addition to their potential role as a reservoir for the virus.

2.4. Oxidant generation from endothelial cells

Endothelial cells line the lumen of the vessel and are essential for vascular quiescence. Endothelial dysfunction is a contributor to the pathogenesis of COVID-19 [7]. During SARS-CoV-2 infection, the endothelium undergoes a proinflammatory and prothrombotic state that includes decrease nitric oxide production, increase vascular permeability, decrease glycocalyx function and cytoprotective signaling, and increase exocytosis of thrombogenic substances (reviewed in [7]). There are three major sources of ROS in endothelial cells: nitric oxide synthase, NADPH oxidase, and the mitochondria.

Endothelial nitric oxide synthase (eNOS) promotes the generation of nitric oxide by utilizing oxygen and L-arginine. Nitric oxide production is needed to maintain vascular quiescence and is a potent anti-thrombotic factor. In the context of oxidant generation during SARS-CoV-2 infection, eNOS activity is decreased, leading to a loss of net nitric oxide production. In addition, L-arginine levels were found to be decreased in acute respiratory conditions [59], which potentially describes a net decrease in nitric oxide production. During SARS-CoV-2 infection, the endothelium generates ROS and down-regulates eNOS [60]. It is likely that eNOS activity is uncoupled and switches over from a nitric oxide producing enzyme to a ROS-generating enzyme, as is the case in certain pathological conditions [61]. eNOS uncoupling could also be caused by low levels of L-arginine and promotes increased vasoconstriction as well as the thrombogenic potential of endothelial cells [62].

Endothelial cells possess NADPH oxidase for the generation of ROS. During SARS-CoV-2 infection, endothelial cells may have increased NADPH oxidase expression and activity as was suggested by Youn et al. [63]. In this study, the authors have added the viral spike (S) protein to bovine aortic endothelial cells and showed by electron paramagnetic resonance spectroscopy, a biophysical method for ROS, that oxidants were increased. In addition, the increase in ROS production and NADPH oxidase expression could be prevented with 17β-estradiol, suggesting that sex differences or estrogen levels could account for COVID-19 severity during disease progression.

Just like with platelets, electron leakage from the mitochondria contributes to endothelial cell ROS generation. Electron leakage from Complex I and Complex III causes chemical reduction of oxygen generating ROS [64]. In a study by Costa et al., SARS-CoV-2 infection causes redox imbalances within the endothelium and this promotes mitochondrial ROS. The mechanism of mitochondrial ROS generation is not very clear but could be due to a decrease in the protein levels of Complex I and an increase in cytosolic calcium that causes cellular dysfunction. The active S protein of SARS-CoV-2 also causes a decrease in mitochondrial respiration compared to control conditions in brain endothelial [65] and pulmonary endothelial cells [66]. A decrease in mitochondrial respiration suggests that the proton motive force in the mitochondria is impaired, which leads to ROS generation from the electron transport chain or by shuffling electrons to other means (e.g. oxidases).

3. Cysteine reactivity during SARS-CoV-2 infection

During oxidative stress, excess levels of ROS target many components of the cell including lipids, nucleic acids, carbohydrates, and proteins. With relevance to protein oxidation, cysteines are one of the most sensitive amino acids to electron withdrawal. Cysteines have nucleophilic properties (*e.g.* their ability to give electrons) and electrophilic properties (*e.g.* their ability to take electrons) [67,68]. In this regard, the cysteine is a versatile amino acid relevant to redox chemistry. The versatility of the cysteine to be oxidized is linked to the intrinsic redox potential of the amino acid. If a cysteine has a lower redox potential (more electronegative) relative to the oxidizing agent (more electropositive), electrons will thus move from the more electronegative to electropositive. This electron flow will cause the cysteine to be oxidized while the oxidizing agent gains electrons and is therefore reduced. Oxidative stress in the setting of viral infection promotes cysteine oxidation as stoichiometrically more levels of oxidants are generated to overcome the thiol buffering capacity. This would influence the thiol-disulfide balance by shifting the levels of free thiols to oxidative cysteine modification, including disulfides [11,69]. Importantly, the prothrombotic nature of oxidizing cysteines during inflammation is not limited to viral infection. Cysteine oxidation in metabolic disorders, such as in dyslipidemia, where excessive levels of oxidants are generated promotes thrombosis [70,71]. Targeting cysteines could be an approach to limit SARS-CoV-2 infection and its associated prothrombotic coagulopathy.

3.1. Reactive cysteines of SARS-CoV-2 spike protein

The mature virion comprises a nucleocapsid (N) protein, an envelope protein (E), a membrane protein (M), and the spike (S) glycoprotein [13,72]. The S protein has the receptor binding domain (RBD) essential for recognition by its cognate ACE2 receptor and the crystal structure of the protein with the receptor has been solved [73,74]. Allosteric disulfides in the S protein have been suggested to be redox active and a lack of a reducing environment during infection may support cysteine oxidation [69,75]. Structural and computational analysis by Singh et al. of the disulfide pairs in the RBD domain suggest that there are 8 cysteines that play structural roles (Cys336-Cys361, Cys379-Cys432, Cys391-Cys525, and Cys480-Cys488) as shown in Fig. 3A [75]. The Cys480-Cys488 pair is located in a loop region of the RBD that interacts with ACE2 and was suggested to influence the formation of a receptor-ligand complex [75]. In molecular dynamic simulation studies, Hati and Bhattacharyya further showed that reduction of all of the disulfided cysteines to free thiols decreases binding to ACE2 (*e.g.* decreases thermodynamic favorability) [69], which further supports the notion that disulfides are essential for SARS-CoV-2 infection and that the use of selective reducing agents as potential therapeutics (see section*3.3* below) could limit infection potential.

Allosteric disulfides are not the only modification present on the S protein. Cysteine acylation is the addition of an acyl chain onto a cysteine to help anchor the protein to membranes. Evidences indicating the role of palmitoylation on the S protein for viral entry into the cell have been reported [76–80]. Specifically, the S protein is cysteine palmitoylated by the zDHHC family of palmitoyltransferases. These studies suggest that selectively targeting palmitoylation of the S protein could prevent viral infection.

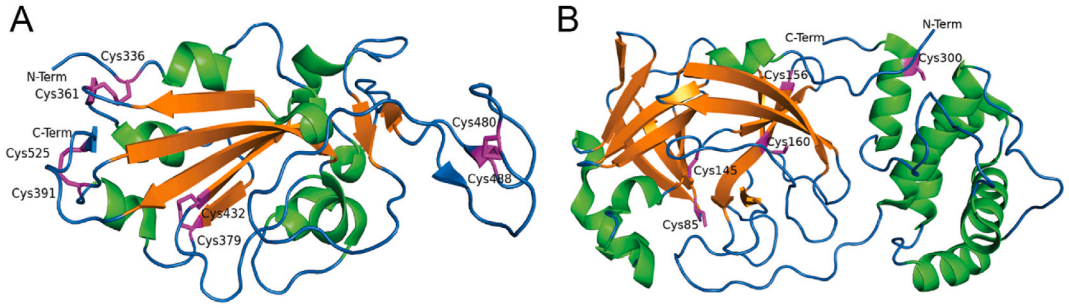

Fig. 3. Reactive cysteines in the SARS-CoV-2 Receptor Binding Domain of the Spike protein and Mpro. (A) Disulfides in the Receptor Binding Domain (RBD) of the Spike protein are indicated in *magenta* color. The disulfide Cys480-Cys488 is located in a flexible loop that is recognized by the ACE2 receptor (PDB: 6m0j; ACE2 receptor not shown from the original file). (B) Exposed cysteines in MPro are indicated in *magenta*. Glutathionylation of Cys300 impacts dimerization of the protein and are also present on other cysteines. Cys145 is the catalytic site of the protein. (PDB: 7vk1). In both panels, α helices are colored *green* and β sheets are colored *orange*.

3.2. Reactive cysteines of the SARS-CoV-2 MPro cysteine protease

Unlike structural disulfides of SARS-CoV-2, other forms of oxidative cysteine modification are also present to regulate the virus. The 3CL main protease (MPro) is a cysteine protease that catalyzes peptide hydrolysis to form the mature virion. MPro contains 11 cysteine residues of which Cys145 is at the catalytic site and Cys85, Cys156, Cys160 and Cys300 are exposed as shown in Fig. 3B. The other cysteines are located within the enzyme for structural support. In x-ray crystallographic studies, Cys145 was observed to be persulfenated, which is a modification with the adduction of an OOH group onto the sulfur atom of the thiol [81]. This modification was likely caused by reaction with oxygen during the crystallization process. Interestingly, Cys156 was reactive to the thiol labeling agent N-ethylmaleimide when Cys145 was oxidized [81], suggesting structural movement by the protein that allows it to become accessible to the probe. Data from this study suggest that other modification could also be present depending on the type of oxidant and that the protease is sensitive to redox control of its conformation. Further experimental evidence would be required to see if these modifications are present in solution.

In support of the evidence that the enzyme is sensitive to the redox environment, glutathionylation of many proteins in Vero E6 kidney epithelial cells were observed in response to SARS-CoV-2 infection [12]. This could be the result of the generation of oxidized glutathione from ROS or for metabolic support. Cysteine glutathionylation is a reversible modification where the sulfur group of a glutathione is adducted onto a cysteine [82]. The MPro protein was shown to be glutathionylated at Cys85, Cys156, and Cys300 [83]. Glutathionylation of Cys300 is a regulatory mechanism to prevent dimerization and thus prevent enzymatic activity since dimerization is needed to be active.

Cysteine nitrosation is the addition of the nitroso group of nitric oxide onto the thiolate of a cysteine [84]. Nitric oxide is a potent vaso-dilating, anti-inflammatory, and anti-microbial molecule. In an *in vitro* study using Vero-E6 cells, SARS-CoV-2 viral replication was concentration-dependently inhibited by the presence of a nitric oxide donating agent, s-nitroso-N-acetylpenicillamine [85]. The enzymatic activity of MPro was also concentration-dependently inhibited. These effects were not observed with a control compound, N-acetylpenicillamine, that does not liberate a nitroso group; N-acetylpenicillamine instead acted as a reducing agent and thus increased MPro activity. Although direct nitrosation of the enzyme was not investigated in this study, given the reactivity of cysteines on MPro, nitrosation is likely a modification to be present and could be exploited therapeutically.

3.3. Targeting reactive cysteines of SARS-CoV-2

The nucleophilic sulfur of the catalytic Cys145 in MPro has been a therapeutic target of much interest. Covalent modification of cysteines using electrophilic warheads have been utilized to develop potent inhibitors in many context (reviewed in [86]). Indeed, targeting Cys145 with the electrophiles chlorofluoroacetamide [87], α-ketoamides [88], ketones [89–92], vinyl sulfones [93], nitriles [94,95], and aldehydes [96–98] showed strong potency against the enzymatic activity of MPro. In addition to covalently labeling the catalytic cysteine of MPro, the oxidation statuses of cysteines in the mature virion have been targeted with specific probes. Chemical probes to selectively reduce the allosteric disulfides of the S protein have been published by the Carroll laboratory [99]. Specifically, they found that the thiol-based reducing agents P2119 and P2165 target Cys379-Cys432 and Cys391-Cys525 in the RBD domain and prevents the S protein from recognition by ACE2. These compounds are much more potent than N-acetylcysteine, which is an anti-oxidative and anti-inflammatory agent proposed to decrease COVID-19 severity. This study demonstrates the utility of targeting redox-sensitive disulfides as a potential anti-viral approach if potency and selectivity could be achieved. Furthermore, it would be interesting to see if extracellular oxidoreductases (*e.g.* thiol isomerases) that catalytically break allosteric disulfides target the S protein as anti-viral defense mechanism. Lastly, anti-oxidative treatments (e.g. N-acetylcysteine [100–102], ebselen [103,104], flavonoids [105,106]) to limit oxidative stress and inflammation have been suggested and could potentially control the redox sensitivity of cysteines during COVID-19.

4. Oxidative stress in hemoglobinopathies may contribute to COVID-19 disease severity

Sickle cell disease is an inherited hemoglobinopathy complicated by recurrent vaso-occlusive events. The Center for Disease Control and Prevention listed sickle cell disease and thalassemia as medical conditions that could pre-dispose individuals to a higher risk for severe COVID-19. A compiled list of observational and case studies relating to sickle cell patients acquiring COVID-19 have been published (reviewed in [107]). A summary of the current evidence suggests that sickle cell disease patients have a higher rate of hospitalization, pneumonia, and pain with COVID-19 compared to individuals without the hemoglobinopathy [108]. Clinical predictors of severe COVID-19 in patients with sickle cell disease (*e.g.* presence of end-organ disease of the brain, heart, lungs, and kidney, and the presence of pulmonary hypertension) have also been published [109]. At the molecular level, the etiology that pre-disposes sickle cell disease patients to severe COVID-19 is not very well-understood.

Oxidative stress in sickle cell disease contributes to the risk for vaso-occlusion and could be a mechanism of crosstalk between the two diseases. As with COVID-19, platelets, leukocytes, endothelial cells, and red cells all contribute to a network of ROS generation in sickle cell disease. Importantly, hemolysis of sickled red cells releases hemoglobin where free heme could propagate ROS in the vasculature. Furthermore, older sickle cell patients have higher hospitalization rate with COVID-19 compared to younger sickle cell patients [107]. In this regard, aging is a contributing oxidative co-morbidity to both diseases. It is also likely that other redox driven co-morbidities of sickle cell disease (*e.g.* dyslipidemia, diabetes mellitus) contribute to an increased risk for COVID-19 severity [15]. As antioxidative treatments are in clinical trials for sickle cell disease, it would be interesting to see if the same antioxidants could limit vaso-occlusion in hemoglobinopathy while reducing COVID-19 disease severity.

5. Summary

The generation of ROS is important for cellular signaling and is a natural byproduct of metabolism. Certain species of oxidants promote vascular homeostasis (*e.g.* the inhibitory role of nitric oxide in vasodilation and on platelet activation). In some instances, oxidant generation is protective and helps destroy pathogens. Yet in pathophysiologic conditions, such as in COVID-19, excess ROS generation overwhelms the anti-oxidative buffering capacity of the environment and thus modifies many components of the cells. Excess oxidants could be detrimental and cause cell death. In summary, the sources of oxidants from hematopoietic (leukocytes, platelets, and red blood cells) and non-hematopoietic vascular cells (endothelial cells) were identified. NADPH oxidase, the mitochondria, a dysregulated metabolism, and a dysfunctional eNOS could contribute to ROS generation from these cells. In addition, SARS-CoV-2 contains reactive cysteines that are targets of ROS, thus forming oxidative cysteine modification. These cysteines have been targets of much interest to prevent viral infection and replication. Lastly, some hematologic disorders manifest as a pre-existing prothrombotic redox condition (*e.g.* sickle cell disease) and thus complicates treatment strategies for COVID-19. In this context, a further understanding of the redox stress associated with COVID-19 as well as the development of potent and selective inhibitors for SARS-CoV-2 would improve treatment strategies for patients with hematologic disorders.

Practice points

- Oxidative stress is a component of many hematologic disorders and COVID-19 is a disease associated with oxidative stress.
- Oxidative stress promotes adverse thrombotic events.
- Patients with hematologic disorders (e.g. sickle cell disease) infected with SARS-CoV-2 should be followed closely as their conditions could increase the risk for severe COVID-19.

Research agenda

- During SARS-CoV-2 infection, reactive oxygen species is generated from both hematopoietic and non-hematopoietic cells and contribute to oxidative stress.
- Cysteines on SARS-CoV-2 are sensitive to oxidative modification.
- Reactive cysteines are attractive therapeutic targets to prevent SARS-CoV-2 infection.
- Hematologic disorders with a pre-existing prothrombotic redox condition (*e.g.* sickle cell disease) have increase risk for severe COVID-19.

Declaration of competing interest

The author declares no conflict of interest.

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