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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
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.
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 cells “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 thrombi 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 electropositive 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 section3.3 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. Evidence 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 per-sulfenated, which is a modification with the addition 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 added 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, N-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], \( \alpha \)-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-obclusion 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 mitochondrial, 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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References

[1] Zhou P, Lou Yang X, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020;579. https://doi.org/10.1038/s41586-020-0212-7.
[2] Nosu P, Ibrahim A, Hodroj MH, Bou-Fakhredin R, Taher AT. COVID-19 in benign hematology: emerging challenges and special considerations for healthcare professionals. Expev Rev Hematol 2020;13. https://doi.org/10.1080/17474086.2020.1819785.
[3] Osterman CK, Trigliaños T, Winzelberg GS, Nichols AD, Rodriguez-O’Donnell J, Bigelow SM, et al. Risk stratification and outreach to hematology/oncology patients during the COVID-19 pandemic. Support Care Cancer 2021;29. https://doi.org/10.1007/s00520-020-05744-y.
[4] Kanth Manne B, Denorne F, Middleton EA, Portier I, Roweley JW, Stubben C, et al. Platelet gene expression and function in patients with COVID-19. Blood 2020;136. https://doi.org/10.1182/blood.2020007214.
[5] Hottz ED, Azevedo-Quintanilha IG, Palinhia L, Teixeira L, Barreiro EA, Pio CRR, et al. Platelet activation and platelet-monocyte aggregate formation trigger tissue factor expression in patients with severe COVID-19. Blood 2020;136. https://doi.org/10.1182/blood.2020007252.
[6] Barrett TJ, Lee AH, Xia Y, Lin LJ, Black M, Cotzia P, et al. Platelet and vascular biomarkers associate with thrombosis and death in coronavirus disease. Circ Res 2020;127. https://doi.org/10.1161/CIRCRESAHA.120.317803.
[7] Flaumenhaft R, Enjuvi K, Schmaier AA. Vasculopathy in COVID-19. Blood 2022. https://doi.org/10.1182/blood.2021012250.
[8] Schultz JL, Aschenbrenner AC. COVID-19 and the human innate immune system. Cell 2021;184. https://doi.org/10.1016/j.cell.2021.02.029.
[9] Iba T, Connors JM, Levy JH. The coagulopathy, endotheliopathy, and vasculitis of COVID-19. Inflamm Res 2020;69. https://doi.org/10.1007/s00011-020-01401-6.
[10] Sies H, Jones DP. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. Nat Rev Mol Cell Biol 2020;21. https://doi.org/10.1038/s41580-020-0230-3.
[11] Suhail S, Zajac J, Fossum C, Lowater H, McCracken C, Severson N, et al. Role of oxidative stress on SARS-CoV (SARS) and SARS-CoV-2 (COVID-19) infection: a review. Protein J 2020;39. https://doi.org/10.1007/s10930-020-09935-8.
[12] Bartolini D, Stabile AM, Bastianelli S, Giustarini D, Pierucci S, Busiti C, et al. SARS-CoV2 infection impairs the metabolism and redox function of cellular glutathione. Redox Biol 2021;45. https://doi.org/10.1016/j.redox.2021.102041.
[13] Jackson CB, Farzan M, Chen B, Choe H. Mechanisms of SARS-CoV-2 entry into cells. Nat Rev Mol Cell Biol 2022;23. https://doi.org/10.1038/s41580-021-00418-x.
[14] Shang J, Wan Y, Luo C, Ye G, Geng Q, Auerbach A, et al. Cell entry mechanisms of SARS-CoV-2. Proc Natl Acad Sci U S A 2020;117. https://doi.org/10.1073/pnas.2003138117.
Masso-Silva JA, Moshensky A, Lam MTY, Odish MF, Patel A, Xu L, et al. Increased IL-8, neutrophil activation phenotypes and NETosis in critically ill COVID-19 patients. J Infect Dis 2020;221. https://doi.org/10.1093/infdis/jiaa190.

Luo M, Liu Y, Yuan J, Wen Y, Xu G, Zhao J, et al. Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. Nat Med 2020;26. https://doi.org/10.1038/s41591-020-0901-5.

Guan W, Ni Z, Hu Y, Liang W, Ou C, He J, et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med 2020;382. https://doi.org/10.1056/nejmoa200232.

Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, et al. Dyeregulation of immune response in patients with coronavirus 2019 (COVID-19) in Wuhan, China. Clin Infect Dis 2020;71. https://doi.org/10.1093/cid/ciaa446.

Arcasio A, Loguercio A, Ferraro AD, Celli W, Huang H, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. J Clin Invest 2020;130. https://doi.org/10.1172/JCI137274.

Zeng J, Wang Y, Li K, Meyerholz DK, Allamargot C, Perlmans S. Severe acute respiratory syndrome coronavirus 2-induced immune activation and death of monocyte-derived human macrophages and dendritic cells. J Infect Dis 2021;223. https://doi.org/10.1093/infdis/jiaa573.

Yang D, Chou H, Hou Y, Chai Y, Shuai H, Lee ACY, et al. Attenuated interferon and proinflammatory response in SARS-CoV-2-infected human dendritic cells is associated with viral antagonism of STAT1 phosphorylation. J Infect Dis 2020;222. https://doi.org/10.1093/infdis/jiaa567.

Wang F, Nie J, Wang H, Zhao Q, Xiong Y, Deng L, et al. Characteristics of peripheral lymphocyte subset alteration in covid-19 pneumonia. J Infect Dis 2020;221. https://doi.org/10.1093/infdis/jiaa556.

Liu J, Li S, Liu J, Liang B, Wang X, Wang H, et al. Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. EBioMedicine 2020;55. https://doi.org/10.1016/j.ebiom.2020.102763.

Zhang S, Liu Y, Wang X, Yang L, Li H, Wang Y, et al. SARS-CoV-2 binds platelet ACE2 to enhance thrombosis in COVID-19. J Hematol Oncol 2020;13. https://doi.org/10.1186/s12959-020-00954-7.

Ye Q, Wang B, Mao J. The pathogenesis and treatment of the ‘Cytokine Storm’ in COVID-19. J Infect 2020;80. https://doi.org/10.1016/j.jinf.2020.03.037.

Masso-Silva JA, Moshensky A, Lam MTY, Odish MF, Patel A, Xu L, et al. Increased peripheral blood neutrophil activation phenotypes and neutrophil extracellular trap formation in critically ill coronavirus disease 2019 (COVID-19) patients: a case series and review of the literature. Clin Infect Dis 2022;74. https://doi.org/10.1093/cid/ciab437.

Barnes BJ, Adrover JM, Baxter-Stoltzfus A, Borczuk A, Cools-Lartigue J, Crawford JM, et al. Targeting potential drivers of COVID-19: neutrophil extracellular traps. J Exp Med 2020;217. https://doi.org/10.1084/jem.20200652.

Wu C, Chen X, Cai Y, Xie J, Zhou X, Xue S, et al. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA Intern Med 2020. 180. https://doi.org/10.1001/jamainternalmed.2020.0994.

Skindros P, Mitsios A, Chrysanthopoulou A, Mastellos DC, Metallidis S, Rafaillas P, et al. Complement and tissue factor–enriched neutrophil extracellular traps are key drivers in COVID-19 immunothrombosis. J Clin Invest 2020;130. https://doi.org/10.1172/JCI141374.

Ng H, Haevernik S, Rossell A, Aguilera K, Parv K, Von Meijenfeldt FA, et al. Circulating markers of neutrophil extracellular traps are of prognostic value in patients with COVID-19. Arterioscler Thromb Vasc Biol 2021. https://doi.org/10.1161/ATVBAHA.120.315267.

Arcasio A, Loguercio A, Ferraro AD, Celli W, Huang H, et al. The emerging role of neutrophil extracellular traps. J Exp Med 2020;217. https://doi.org/10.1084/jem.20200652.

Veras FP, Pontelli MC, Silva CM, Toller-Kawahisa JE, de Lima M, Nascimento DC, et al. SARS-CoV-2-triggered neutrophil extracellular traps mediate COVID-19 pathology. J Exp Med 2020;217. https://doi.org/10.1084/jem.202001129.

debont CM, Boelens WC, Pruin GLM. NKT cell: complementation, and coagulation: a triangulation approach. Cell Mol Immunol 2019;16. https://doi.org/10.1038/s41597-018-0024-1.

Borella R, De Biasi S, Paolini A, Boraldi F, Lo Tartaro D, Mattioli M, et al. Metabolic reprogramming shapes neutrophil functions in severe COVID-19. Eur J Immunol 2020;52.484-502. https://doi.org/10.1002/eji.201949841.

Lundqvist H, Follin P, Khalfan L, Dahlen C. Phorbol myristate acetate-induced NADPH oxidase activity in human neutrophils: only half the story has been done. Cell 2020;181. https://doi.org/10.1016/j.cell.2020.05.015.

Peckham H, de Gruijter NM, Raine C, Radziszewska A, Ciurtin C, Wedderburn LR, et al. Male sex identified by global COVID-19 meta-analysis as a risk factor for severe acute respiratory syndrome coronavirus 2 (COVID-19) disease and unexposed individuals. Cell 2020;181. https://doi.org/10.1016/j.cell.2020.05.015.

Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. J Thromb Haemostasis 2020;18. https://doi.org/10.1111/jth.14768.

Chua RI, Lukassen S, Trump S, Hennig BP, Wendisch D, Port F, et al. COVID-19 severity correlates with airway epithelium–immune cell interactions identified by single-cell analysis. Nat Biotechnol 2020;38. https://doi.org/10.1038/s41597-020-0604-2.


Hati S, Bhattacharyya S. Impact of thiol-disulfide balance on the binding of covid-19 spike protein with angiotensin-converting enzyme 2 receptor. ACS Omega

Jana S, Heaven MR, Alayash AI. Cell-free hemoglobin does not attenuate the effects of sars-cov-2 spike protein s1 subunit in pulmonary endothelial cells. Int J

Kim ES, Jeon MT, Kim KS, Lee S, Kim S, Kim DG. Spike proteins of sars-cov-2 induce pathological changes in molecular delivery and metabolic function in the brain endothelial cells. Viruses 2021;13.10.3390/v13100221.

Jana S, Heaven MR, Alayash AI. Cell-free hemoglobin does not attenuate the effects of sars-cov-2 spike protein s1 subunit in pulmonary endothelial cells. Int J Mol Sci 2021;22.10.3390/ijms21269041.

Venkat Gupta, C. Sulfenic acid chemistry, detection and cellular lifetime. Biochim Biophys Acta 2014:1840:847-75.10.1016/j.bbagen.2013.05.040.

Paulzen CE, Carroll KS. Orchestrating redox signaling networks through regulatory cysteine switches. ACS Chem Biol 2010;5:47–62.10.1021/acschembio.10b00582.

Hatí S, Bhattacharyya S. Impact of thiol-disulfide balance on the binding of covid-19 spike protein with angiotensin-converting enzyme 2 receptor. ACS Omega

Wang Q, Zhang Y, Wu L, Niu S, Song C, Zhang Z, et al. Structural and functional basis of SARS-CoV-2 entry by using human ACE2. Cell 2020;185.10.1016/j.cell.2020.03.045.

Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science 2020:367.10.1126/science.abb2762.

Yin R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science 2020:367.10.1126/science.abb2762.

Singh J, Dhindsa RS, Misra V, Singh B. SARS-CoV2 spike protein is potentially modulated by host redox status. Comput Struct Biotechnol J 2020;18.10.1016/j.csbj.2020.10.016.

Puthenveetil R, Lun CM, Murphy RE, Healy LB, Vilmen G, Christenson ET, et al. S-acylation of SARS-CoV-2 spike protein: mechanistic dissection, in vitro and in vivo. J Biol Chem 2020;295.10.1074/jbc.201908088.

Ramadan AA, Mayilsamy K, McGill AR, Ghosh A, Giuliani MT, Donow HM, et al. Identification of SARS-CoV-2 spike palmitoylation inhibitors that results in release of attenuated virus with reduced infectivity. Viruses 2022;14.10.3390/v14030531.

Abdulrahman DA, Meng X, Veit M. S-acylation of proteins of coronavirus and influenza virus: conservation of acylation sites in animal viruses and dhcr7 enzymes in their animal reservoirs. Pathogens 2021;10.1002/pathogen.100666.

Kneller DW, Phillips G, O’Neill LM, Tan K, Joachimiak A, Coates L, et al. Mitigation of the replication of SARS-CoV-2 by nitric oxide in vitro. Redox Biol 2020;22.10.1016/j.redox.2020.101734.

Davis DA, Bulut H, Shrestha P, Yaparla A, Jaeger HK, Hattori SI, et al. Regulation of the dimerization and activity of SARS-CoV-2 main protease through reversible glutathionylation of cysteine 300. mBio 2021;12.10.1128/mBio.02094-21.

Smith BC, Marletta MA. Mechanisms of S-nitrosothiol formation and selectivity in nitric oxide signaling. Curr Opin Chem Biol 2012;16:498–506.10.1016/j.cbpa.2012.10.010.

Akbarei D, Krambrich J, Ling J, Luni C, Heinzenstaerna J, Jarlth JD, et al. Mitigation of the replication of SARS-CoV-2 by nitric oxide in vitro. Redox Biol 2020;8.10.1016/j.redox.2020.101734.

Gehring M, Lafer SA. Emerging and Re-emerging warheads for targeted covalent inhibitors: applications in medicinal chemistry and chemical biology. J Med Chem 2019;62.10.1021/acs.jmedchem.8b01153.

Yamane D, Onitsuka S, Re S, Isogai H, Hamada R, Hiramoto T, et al. Selective covalent targeting of SARS-CoV-2 main protease by enantiopure difluorocacetamide. Chem Sci 2022;3:4494-507.10.1039/d2sc000160a.

Zeng XT, Yu XT, Chen W. The interactions of ZDHHC5/GOLGA7 with SARS-CoV-2 spike (S) protein and their effects on S protein’s cellular localization, palmitoylation and pseudovirus entry. Viral J 2021;12.10.1128/viralj.02172-w2.

Gehringer M, Laufer SA. Emerging and Re-emerging warheads for targeted covalent inhibitors: applications in medicinal chemistry and chemical biology. J Med Chem 2019;62.10.1021/acs.jmedchem.8b01153.

Ramadan AA, Mayilsamy K, McGill AR, Ghosh A, Giuliani MT, Donow HM, et al. Identification of SARS-CoV-2 spike palmitoylation inhibitors that results in release of attenuated virus with reduced infectivity. Viruses 2022;14.10.3390/v14030531.

Abdulrahman DA, Meng X, Veit M. S-acylation of proteins of coronavirus and influenza virus: conservation of acylation sites in animal viruses and dhcr7 enzymes in their animal reservoirs. Pathogens 2021;10.1002/pathogen.100666.

Kneller DW, Phillips G, O’Neill LM, Tan K, Joachimiak A, Coates L, et al. Mitigation of the replication of SARS-CoV-2 by nitric oxide in vitro. Redox Biol 2020;22.10.1016/j.redox.2020.101734.

Davis DA, Bulut H, Shrestha P, Yaparla A, Jaeger HK, Hattori SI, et al. Regulation of the dimerization and activity of SARS-CoV-2 main protease through reversible glutathionylation of cysteine 300. mBio 2021;12.10.1128/mBio.02094-21.

Smith BC, Marletta MA. Mechanisms of S-nitrosothiol formation and selectivity in nitric oxide signaling. Curr Opin Chem Biol 2012;16:498–506.10.1016/j.cbpa.2012.10.010.

Akbarei D, Krambrich J, Ling J, Luni C, Heinzenstaerna J, Jarlth JD, et al. Mitigation of the replication of SARS-CoV-2 by nitric oxide in vitro. Redox Biol 2020;8.10.1016/j.redox.2020.101734.
[93] Rut W, Groborz K, Zhang L, Sun X, Zmudzinski M, Pawlik B, et al. SARS-CoV-2 Mpro inhibitors and activity-based probes for patient-sample imaging. Nat Chem Biol 2021;17. https://doi.org/10.1038/s41589-020-00689-z.

[94] Breidenbach J, Lemke C, Pillaiyar T, Schakel L, Al Hamwi G, Diett M, et al. Targeting the main protease of SARS-CoV-2: from the establishment of high throughput screening to the design of tailored inhibitors. Angew Chem Int Ed 2021;60. https://doi.org/10.1002/anie.202016961.

[95] Owen DR, Allerton CMN, Anderson AS, Aschenbrenner L, Avery M, Berritt S, et al. An oral SARS-CoV-2 Mpro inhibitor clinical candidate for the treatment of COVID-19. Science 2021;80(374):374. https://doi.org/10.1126/science.abc784.

[96] Dai W, Zhang B, Jiang XM, Su H, Li J, Zhao Y, et al. Structure-based design of antiviral drug candidates targeting the SARS-CoV-2 main protease. Science 2020;368. https://doi.org/10.1126/science.abb4689.

[97] Qiao J, Li Y-S, Zeng R, Liu F-L, Lu R-H, Huang C, et al. SARS-CoV-2 Mpro inhibitors with antiviral activity in a transgenic mouse model. Science 2021;371. https://doi.org/10.1126/science.abf1611.

[98] Yang KS, Ma XR, Ma Y, Alugubelli YR, Scott DA, Vatansever EC, et al. A quick route to multiple highly potent SARS-CoV-2 main protease inhibitors. ChemMedChem 2021;16. https://doi.org/10.1002/cmdc.202000924.

[99] Shi Y, Zeida A, Edwards CE, Mallory ML, Sastre S, Machado MR, et al. Thiol-based chemical probes exhibit antiviral activity against SARS-CoV-2 via allosteric disulfide disruption in the spike glycoprotein. Proc Natl Acad Sci U S A 2022;119. https://doi.org/10.1073/pnas.2120419119.

[100] De Flora S, Balansky R, La Maestra S. Rationale for the use of N-acetylcysteine in both prevention and adjuvant therapy of COVID-19. Faseb J 2020;34. https://doi.org/10.1096/fj.202001807.

[101] Poe FL, Corn J. N-Acetylcysteine: a potential therapeutic agent for SARS-CoV-2. Med Hypotheses 2020;143. https://doi.org/10.1016/j.mehy.2020.109862.

[102] Fratta Pasini AM, Stranieri C, Cominacini L, Mozzini C. Potential role of antioxidant and anti-inflammatory therapies to prevent severe sars-cov-2 complications. Antioxidants 2021;10. https://doi.org/10.3390/antiox10020272.

[103] Menéndez CA, Bylén F, Perez-Lemus GR, Alvarado W, de Pablo JJ. Molecular characterization of ebselen binding activity to SARS-CoV-2 main protease. Sci Adv 2020;6. https://doi.org/10.1126/sciadv.abb3045.

[104] Ma C, Hu Y, Townsend JA, Lagarias PJ, Marty MT, Kolocouris A, et al. Ebselen, disulfiram, carmofur, PX-12, tideglsib, and shikonin are nonspecific promiscuous SARS-CoV-2 main protease inhibitors. ACS Pharmacol Transl Sci 2020;3. https://doi.org/10.1021/acsptsci.6b00130.

[105] Jo S, Kim S, Kim DY, Kim MS, Shin DH. Flavonoids with inhibitory activity against SARS-CoV-2 3CLpro. J Enzym Inhib Med Chem 2020;35. https://doi.org/10.1080/14756366.2020.1810172.

[106] Aroa S, Lohiya G, Moharir R, Shah S, Yende S. Identification of potential flavonoid inhibitors of the SARS-CoV-2 main protease 6YNQ: a molecular docking study. Digit Chinese Med 2020;3. https://doi.org/10.1016/j.dcm.2020.12.003.

[107] Hoogenboom WS, Alamuni TT, McMahon DM, Balanchivadze N, Dahak V, Mitchell WB, et al. Clinical outcomes of COVID-19 in patients with sickle cell disease and sickle cell trait: a critical appraisal of the literature. Blood Rev 2022;53. https://doi.org/10.1016/j.brr.2021.100911.

[108] Singh A, Brandow AM, Panepinto JA. COVID-19 in individuals with sickle cell disease/trait compared with other Black individuals. Blood Adv 2021;5. https://doi.org/10.1182/BLOODADVANCES.2020003741.

[109] Minniti CP, Zaidi AU, Nouraie M, Manwani D, Crouch GD, Crouch AS, et al. Clinical predictors of poor outcomes in patients with sickle cell disease and COVID-19 infection. Blood Adv 2021;5. https://doi.org/10.1182/bloodadvances.2020003456.