SARS-CoV-2 and COVID-19

Subjects: Allergy

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Morbidity and mortality of coronavirus disease 2019 (COVID-19) are due in large part to severe cytokine storm and hypercoagulable state brought on by dysregulated host-inflammatory immune response, ultimately leading to multi-organ failure. Exacerbated oxidative stress caused by increased levels of interleukin (IL)-6 and tumor necrosis factor α (TNF-α) along with decreased levels of interferon α and interferon β (IFN-α, IFN-β) are mainly believed to drive the disease process. Based on the evidence attesting to the ability of glutathione (GSH) to inhibit viral replication and decrease levels of IL-6 in human immunodeficiency virus (HIV) and tuberculosis (TB) patients, as well as beneficial effects of GSH on other pulmonary diseases processes, we believe the use of liposomal GSH could be beneficial in COVID-19 patients.

SARS-CoV-2 COVID-19 cytokines oxidative stress glutathione pathogenesis

1. Introduction

With approximately 11 million confirmed cases and over 525,000 deaths documented, the novel strain of coronavirus, which initially emerged at the end of 2019 in Hubei Province of the People's Republic of China, has been found to precipitate clinical acute respiratory distress syndrome (ARDS) [1]. Coronaviridae are a large family of enveloped RNA viruses with virulent capacity observed across many species. Investigation of this novel emergent strain is ongoing and hence its pathological features have yet to be fully revealed. The first reports of the disease were reported as a cluster of unusual community acquired pneumonia cases concentrated in Hubei Province of the People's Republic of China in December of 2019. On January 7th, 2020 the causative pathogen was identified as a novel coronavirus and provisionally named "2019-nCoVID [1]. One month later, the World Health Organization (WHO) declared the situation to be a public health emergency of international concern. The virus was then officially designated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) for its apparent genetic and zoonotic similarity to SARS-CoV-1, formerly known as SARS-CoV, and Middle East respiratory syndrome coronavirus (MERS-CoV). The disease caused by SARS-CoV-2 is termed COVID-19.

The challenges of responding to this pandemic have been attributed to a myriad of factors including incomplete knowledge of SARS-CoV-2 pathophysiology, the mechanisms of action by which it causes harm, and the immune responses of individuals with comorbidities. Widespread shortages of personal protective equipment and testing systems have hamstrung organized response. Clinical trials are taking place assessing efficacy of the protease inhibitors lopinavir-ritonavir, RNA polymerase inhibitor Remdesivir, the antimalarial hydroxychloroquine, and IFN-1B as possible treatments in adjunct to supportive care [2]. The only long-term solution is seen in a form of vaccine, which is yet to be developed. The role of cytokine dysregulation in COVID-19 pathophysiology has been

documented in multiple studies. This provides the scientific community with the foundation to propose possible mechanisms of pathogenesis and develop treatment modalities to limit morbidity and mortality.

2. SARS-CoV-2 Microbiology and Transmission

2.1. SARS-CoV-2 Microbiology

Genetic sequencing studies have grouped SARS-CoV-2 along with SARS-CoV-1 and MERS-CoV into the genus Betacoronavirus in the family Coronaviridae. SARS-CoV-2 is a spherical, pleomorphic enveloped virus containing a 30 kbp positive-sense single-stranded RNA genome. The family Coronaviridae possess the largest genomes of all known RNA viruses [3].

The main structural components of SARS-CoV-2 are the protein spike (S), membrane (M), envelope (E), and nucleocapsid (N). The M glycoprotein is the most abundant structural protein and is responsible for the intracellular host-assembly of viral particles [3]. The S protein is a club-shaped glycoprotein found on both SARS-CoV-1 and SARS-CoV-2 that binds to angiotensin-converting enzyme 2 (ACE-2) on potential host cells [4]. Viral entry is demonstrated to require the activity of the cysteine proteases cathepsin B and cathepsin L (CatB/L) as well as the serine protease TMPRSS2 for S protein priming. Aloxistatin and camostat mesylate, which inhibit CatB/L and TMPRSS2, respectively, are shown to prevent entry of SARS-CoV-2 into human cells when used in combination [5]. These medications are being investigated as a potential treatment modality for COVID-19 [5]. Incubation of SARS-CoV-2 in the presence of tunicamycin results in non-infectious virus particles with absent S protein [4][6]. The S protein is also the inducer of neutralizing antibodies in the host, which makes the S protein an ideal target for the vaccines [4][6].

A study conducted on SARS-CoV-1, the strain of Coronaviridae attributed to the 2002–2004 SARS outbreak, found that intracellular response to the virus is mediated by RNA-activated protein kinase (PKR) in human cells expressing ACE-2. The molecular mechanism of this response involves phosphorylation of the translational initiator eukaryotic initiation factor 2-α) at Ser51 residues. Phosphorylation of eIF2-α results in global inhibition of mRNA translation. However, this inhibition of eIF2-α was not found to significantly decrease viral replication. It has been proposed that this mechanism is a viral adaptation to limit host-immune response by inhibition of translation of host-response proteins [7].

2.2. Transmission

Early data suggest that horseshoe bats and pangolins are likely mammalian reservoirs for SARS-CoV-2. However, the intermediate host through which direct interspecies transmission to humans occurred remains uncertain. Transmission of SARS-CoV-2 between human hosts is mediated by aerosolized respiratory droplets expectorated by an infected host. Transmission can also occur during aerosol-generating procedures including endotracheal intubation and administration of nebulizer therapy. Human-to-human transmission by asymptomatic carriers makes containment of SARS-CoV-2 difficult, as 40–50% of cases are caused by transmission from asymptomatic carriers

[8][9]. The R_0 of SARS-CoV-2 is estimated to be 3.28 in a recent review article, meaning that each infected host is expected to transmit the infection to approximately three healthy individuals. Data estimates for R_0 vary across the literature and will likely continue to evolve as the pandemic progresses [10].

3. Covid-19 Addendum

In Part I of "Liposomal Glutathione Supplementation As An Adjunctive Therapy in COVID19" [11], we discussed the rationale for support of GSH and particularly liposomal glutathione as an adjunct for treatment of Covid-19. In this addendum we present additional information explaining why Covid-19 can lead to a rapid progressive loss of the reduced form of glutathione (GSH). The loss of GSH appears initially in the lung and may progress to a systemic depletion of glutathione.

The rapidity and severity of the illness associated with Covid-19 has slowed researchers' ability to access and process blood specimens from individuals with acute illness. Additionally, measurement of the reduced form of serum GSH is not available as a standard laboratory test, even in most university hospitals.

As infection with Covid-19 progresses, information has been reported that allows a more complete understanding of the oxidation stress and loss of GSH that accompanies this illness. The purpose of this paper is to add additional information explaining the mechanism of the loss of GSH during Covid-19 infection. During Covid-19, GSH appears to be lost due to the combination of oxidation stress decreasing GSH and loss of normal formation of GSH. The normal cell construction of GSH using the building block amino acids, glutamate, cysteine and glycine may be impaired by the down regulation of the enzymes needed to efficiently utilize the GSH building blocks, which occurs, for example, when TGFβ is increased. Information on the construction of GSH is reviewed in Silvagno et al [12] and Checconi et al [13]. In the presence of blocks in the enzymes needed to form GSH, as seen in individuals with HIV, a liposomal formulation of reduced glutathione has been shown to be useful in restoring GSH [14][15].

3.1. COVID-19 Infection Associated with Impaired Redox Homeostasis

Silvagno et al have stated, "The pathology of respiratory viruses causing severe illness appears to be related to depletion of glutathione during an inflammatory response [12]. Checconi et al report that different viruses break the redox balance between oxidant and antioxidant species and induce an oxidative stress that in turn facilitates specific steps of the virus lifecycle and activates an inflammatory response [13]. A common denominator in all conditions associated with COVID-19 appears to be the impaired redox homeostasis responsible for reactive oxygen species (ROS) accumulation; therefore, levels of glutathione (GSH), the key anti-oxidant guardian in all tissues, could be critical in extinguishing the exacerbated inflammation that triggers organ failure in COVID-19 [12]. These concepts explain the association of a decreased amount of glutathione in the reduced form, GSH, and more severe symptoms of Covid-19 reported in a small series of patients in May, 2020 [16].

3.2. Loss of GSH begins with the Binding of Covid-19

In the article on “Glutathione Supplementation as an Adjunctive Therapy in COVID-19” [11] it was speculated that the binding of the S protein of SARS-CoV-2 to angiotensin converting enzyme 2 (ACE-2) on host cell stimulates an oxidation reaction similar to reports of the binding of other viruses like influenza to cells [13]. Covid19 binds to cells at ACE2 receptors, which begins the depletion of GSH.

Infection of cells by SARS viruses that bind ACE2 results in two effects: inhibition of ACE2 activity and decrease of ACE2 expression in infected cells and leads to toxic overaccumulation of AGNII [12]. The increased ANGII, through binding to AT1R, activates NADPH oxidases that transfer an electron from NADPH to O_2 generating several radical species, which are scavenged by and deplete GSH [12]. As GSH is depleted, ROS-mediated oxidation increases.

ROS-mediated oxidation can initiate transcription factors leading to the formation of NF-kB, whose role in inflammation in severe acute respiratory syndrome (SARS) has been demonstrated in SARS-CoV-infected cultured cells and mice [17]. Nuclear Factor (NF)-kB is involved in inflammation through multiple mechanisms [18].

Antioxidant therapies including N-acetyl-cysteine (NAC) [19] and glutathione have been reported to regulate NF-κB signaling [18] and downregulate NF-κB [20].

3.3. Coronavirus N Protein Programs for the Formation of IL-6 and TGF-β, and both Deplete GSH.

The main structural components of SARS-CoV-2 are the proteins spike (S), membrane (M), envelope (E), and nucleocapsid (N) [21]. The M glycoprotein is the most abundant structural protein and is responsible for the intracellular host-assembly of viral particles [22]. The S protein is a club-shaped glycoprotein found on both SARS-CoV-1 and SARS-CoV-2 that binds to angiotensin-converting enzyme 2 (ACE-2) on potential host cells [23]. E protein plays a multifunctional role in the pathogenesis, assembly, and release of the virus [24]. The N protein of coronavirus is multipurpose. Among several functions, it plays a role in complex formation with the viral genome, facilitates M protein interaction needed during virion assembly, and enhances the transcription efficiency of the virus [25][26].

N protein activates the IL-6 promoter and subsequent IL-6 transcription as demonstrated in A549 human lung cells [27]. It has been shown that SARS-CoV-1 N protein interacts with the host transcriptional factor NF-κB in a dose-dependent manner to regulate IL-6 expression [27][28].

3.4. Severe Covid-19 Associated with Increased Serum IL-6 and TGF-β

Patients with more severe presentation of Covid-19 had higher serum concentration of IL-6 and TGF-β in their peripheral blood than patients with less severe presentation [29]. It has been reported that plasma levels of IL-6 increase with severity of the illness [30][31].

The article “Glutathione Supplementation as an Adjunctive Therapy in COVID-19” [11] pointed out that GSH could be diminished by IL-6. It has been shown that IL-6 induced a dose-dependent decrease in intracellular GSH levels

in a number of human cell lines, including lung cells [32]. This would begin to explain the loss of GSH seen in individuals with Covid-19 [16].

Unexpectedly, individuals with Covid-19 have been shown to have lower levels of IL-10 compared to those with severe community-acquired pneumonia requiring ICU support (CAP_{ICU} patients) (20). The CAP_{ICU} patients exhibited markedly increased IL-10 levels in response to inflammation when compared with $COVID_{ICU}$ patients, indicating that in addition to the rise in proinflammatory mediators, concomitant loss of anti-inflammatory protection may also be clinically relevant [30].

3.5. Elevated TGF-β Predicts Covid-19 Severity

A clinical study has shown that Transforming growth factor beta (TGF-β) can be used as a predictor of disease severity in patients with COVID-19 [33]. Notably, high levels of Monocyte Chemoattractant Protein-1 (MCP-1) and TGF-β1 were identified in SARS-CoV infected lung cells at autopsy [34].

It has been shown that TGF-β suppresses glutamate cysteine ligase (GCL) gene expression and induces oxidative stress in a lung fibrosis model [35]. GCL is a controlling factor in intracellular GSH formation [36]. The elevation of TGF-β will add additional suppression of GSH formation during Covid-19. High levels of TGF-β1 may trigger fibrotic changes and may account for the typical early CT features of COVID-19 pneumonia of ground-glass opacity [37]. Fibrosis is a major complication related to Covid-19 [38] and is related to TGF-β elevation [35].

The high levels of MCP-1 explains the findings of a mononuclear inflammatory infiltrate with lymphocytes and CD68+ macrophages in the lungs of Covid-19 patients [39][40][41].

3.6. Thrombosis and Covid-19

The platelet membrane contains sulfhydryl groups which are essential for normal platelet function. Reduced glutathione (GSH) and other thiols such as cysteine and 6-mercaptopurine were found to inhibit human platelet aggregation induced by adenosine diphosphate (ADP), collagen and arachidonic acid [42].

Oxidative stress has been described in many of the disorders including atherosclerosis, diabetes mellitus, hypertension, obesity, and cancer [43]. Many of these conditions are associated with complications following Covid-19. Increase oxidative burden in the circulation exposes platelets to a pro-activatory milieu, responsible for platelet pro-adhesive and pro-aggregatory phenotype, which in turn lead to thromboembolic events, which is a common characteristic of these conditions [44]. It has been shown that oxidative stress-activated platelets are a source of ROS, which further contribute to the circulating oxidative stress. This process generates a vicious circle capable to affect other cell types, that can contribute to the progression and complication of other diseases [44].

3.7. Pulmonary Thrombosis appears to be Common in COVID-19 Pneumonia and Takes Two Forms, Proximal Pulmonary Emboli and/or Distal Thrombosis [45].

It has been shown that platelets express ACE2, a host cell receptor for SARS-CoV-2, and TMPRSS2, a serine protease for Spike protein priming. Detectable SARS-CoV-2 RNA in the blood stream was associated with platelet hyperactivity in critically ill patients. SARS-CoV-2 and its Spike protein directly enhanced platelet activation such as platelet aggregation. SARS-CoV-2 and its Spike protein directly stimulated platelets to facilitate the release of coagulation factors, the secretion of inflammatory factors, and the formation of leukocyte–platelet aggregates leading to pulmonary thrombus formation in Coid-19 patients [46]. As discussed previously, the binding of Covid-19 spike protein induces NADPH oxidases to transfer an electron from NADPH to O_2 generating several radical species, which are scavenged by and deplete GSH [12]. Thus, it seems likely that the decrease of GSH plays a role in the pulmonary thrombosis seen in Covid-19.

3.8. Coinfection with RSV

Part 1 of “Glutathione Supplementation as an Adjunctive Therapy in COVID-19” (1) discussed the mechanism of loss of GSH during infection with Respiratory Syncytial Virus (RSV). In An autopsy study, 9 patients were tested for respiratory syncytial virus. Three of nine (33.3%) patients tested positive for respiratory syncytial virus [47].

RSV has been shown to cause increased oxidation stress. Initially, Respiratory Syncytial Virus (RSV) was shown to use NOX2 as an essential regulator of RSV-induced NF-κB activation, which likely led to excessive NF-κB-mediated inflammatory gene expression [48][49]. A later study found that RSV infection down-regulates NF-E2-related factor 2 (NRF2) expression in airway epithelial cells and a decrease in the expression of airway antioxidant enzymes, which led to additional oxidative stress [50]. NRF2 is an important redox-responsive protein that helps protect the cells from oxidative stress and injury [51][52]. RSV infection induces a progressive reduction in nuclear and total cellular level of the transcription factor NF-E2-related factor 2 (NRF2), resulting in decreased binding to endogenous antioxidant element (AOE) gene promoters and decreased antioxidant enzyme expression [50]. The decrease in NRF2 binding resulted in a decrease in a number of Nrf2 target antioxidant genes including glutamate cysteine ligase (GCL), which is a controlling factor in intracellular GSH formation [36]. The RSV-induced inhibition of NRF2 activation, due to deacetylation and proteasomal degradation was shown to occur both *in vitro and in vivo*, in animal studies [50].

3.9. Deficient GSH appears to be Associated with Complications of Covid-19

The pathology of Covid-19, may begin as a mild infection in the upper respiratory tract, which will be self limiting especially in a young individuals with robust antioxidant defenses, but is a risk for severe infection in older individuals who often have decreased GSH production, even when they appear healthy [53]. Individuals in our society with lower GSH production may be at risk because of a decreased capacity to maintain many metabolic and detoxification reactions mediated by glutathione.

Risk of mortality associated with Covid-19 is associated with a number of factors such as diabetes, hypertension and obesity [54]. Conditions with decreased GSH that may be predictive of complications for more severe course of illness with Covid-19 include:

Age, CDC - older adults at highest risk- GSH decreases with age [53].

Hypertension with its high representation of ACE2 receptors.

Diabetes Mellitus is a low GSH condition [55] with increased IL-6 [55].

Obesity associated with oxidation stress [56]

Cardiac: decline of GSH predicts mortality in coronary artery disease [57].

Cognitive decline predicted by low GSH [58].

Fibrosis is a major complication related to Covid-19 [38] and is related to TGF-β elevation [35].

4. Summary

This entry adds additional explanation for the loss of GSH during infection with Covid-19. Combining the additional information with Part I suggests that a number of mechanisms conspire to deplete GSH as Covid-19 infection progresses in an individual. It has been shown that Covid-19 increases oxidation stress during attachment of the Spike protein, the N protein programs for the production of IL-6 and TGFβ, both of which deplete GSH. Co-infection with RSV will add additional stress onto the system designed to defend against oxidation stress. It is likely that oxidation stress related to loss of GSH contributes to thrombosis, which often accompanies more severe Covid-19.

This information suggests that the ability to maintain adequate GSH by endogenous or exogenous means during infection with Covid-19 may add significantly to the ability of an individual to defend against this infection.

COVID-19 represents a historic challenge to the fields of research, infectious disease, and international healthcare. The need for detailed analysis of its pathogenesis and clinical course is readily apparent. The unprecedented acuity of a rapidly spreading pandemic presents an opportunity to advance international collaboration in the scientific community. While vaccine trials remain ongoing, physicians have been compelled to apply various treatments with established efficacy in similar viral or bacterial illnesses that also lead to bilateral pneumonia and ARDS. Here we present the antioxidant GSH as a potential untapped avenue for further investigation as intervention for COVID-19. We propose to use a formulation that contains a predominately reduced form of glutathione in the formulation rather than oxidized. In a patient that is burdened with cytokine storm, the best thing for the immune system would be to supply it with reduced glutathione such that it is already able to supply reducing equivalents from its thiol group. Our work with HIV, TB, and other pulmonary or immunosuppressive illnesses demonstrates the value of GSH as an adjunct treatment for SARS-CoV-2 infection.

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