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Reactive oxygen species as an initiator of toxic innate immune responses in retort to SARS-CoV-2 in an ageing population, consider N-acetylcysteine as early therapeutic intervention

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ABSTRACT

During the current COVID-19 pandemic, a need for evaluation of already available drugs for treatment of the disease is crucial. Hereby, based on literature review from the current pandemic and previous outbreaks with corona viruses we analyze the impact of the virus infection on cell stress responses and redox balance. High levels of mortality are noticed in elderly individuals infected with SARS-CoV2 and during the previous SARS-CoV1 outbreak. Elderly individuals maintain a chronic low level of inflammation which is associated with oxidative stress and inflammatory cytokine production, a condition that increases the severity of viral infections in this population. Coronavirus infections can lead to alterations of redox balance in infected cells through modulation of NAD + biosynthesis, PARP function along with altering proteasome and mitochondrial function in the cell thereby leading to enhanced cell stress responses which further exacerbate inflammation. ROS production can increase IL-6 production and lipid peroxidation resulting in cell damage. Therefore, early treatment with anti-oxidants such as NAC during COVID-19 can be a way to bypass the excessive inflammation and cell damage that lead to severe infection, thus early NAC as intervention should be evaluated in a clinical trial setting.

Coronaviruses are tiny viruses with diameter ranging between 65 and 125 nm, whose name derives from the spikes projected from their surface; in transmission electron microscopy they resemble to solar corona. Their genetic material is single-stranded RNA and they are divided into 4 subgroups, namely alpha (α), beta (β), gamma (γ) and delta (δ). The first two typically infect humans and the others mostly birds [1]. Coronaviruses mainly cause infections of respiratory tract that may be either mild or lethal. Two recent outbreaks in several countries worldwide due to coronaviruses have been reported. The first was observed in 2002 because of the severe acute respiratory syndrome coronavirus (i.e., SARS-CoV or SARS-CoV-1) and the second ten years later caused by the Middle East respiratory syndrome coronavirus (i.e., MERS-CoV). The third and most recent outbreak for which the severe acute respiratory syndrome coronavirus 2 (i.e., SARS-CoV-2) is responsible, is ongoing throughout the world. It started in Wuhan, the capital city of Hubei province in China, with patients reporting symptoms of an atypical pneumonia, in December 2019. This outbreak has now reached a global fatality rate of over 400 000 reported deaths, likely an underestimated number, and fatalities rise rapidly and continuously. Although the virus was initially named as 2019-novel coronavirus (2019-nCoV) by WHO, it has been renamed as SARS-CoV-2 virus and the disease as the coronavirus disease 2019 (COVID-19).

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More and more data support the role of excessive immune activation as the cause of lung destruction by SARS-CoV-2 [2–4]. Although there is no sex-based skewing to contract the viral infection, there appears to be a skewed mortality towards elderly men with underlying diseases [5] in the current pandemic. It is known that pulmonary immunity in elderly persons is diminished/impaired, with inadequate innate and adaptive cellular immune responses and reduced function of the lung itself. However, that does not mean that the innate cellular sensing machinery of infected lung epithelial cells is decreased [8]. In fact, while overall innate immune responses may decline with age, inflammatory cytokines such as IL-6 and TNF-a, and acute phase reactants such as C-reactive protein have been shown to be elevated in elderly, maintaining a low level of chronic inflammation, known as inflammaging which is also accompanied by immunosenescence [8,9]. Inflammaging is associated with increased levels of oxidative stress which drive the sustained levels of inflammation [10,11] and impairment of T cell activation in aged individuals, resulting in increased severity of viral infections in elderly individuals. This phenomenon was particularly apparent in previous outbreak of SARS with a mortality of > 50 % in individuals > 65 years of age compared to 100 % survival in individuals younger than 24 years old [12].

In the perspective of the innate immune response towards coronavirus infections we must understand the virus life cycle. Coronaviruses are ssRNA viruses and SARS-CoV-2 infects alveolar epithelial cells via the receptor ACE2 [13], leading to the triggering of innate response mechanisms. After introduction of viral proteins and RNA into the cytoplasm of the host cells, components known to interact with the host proteins and affect cell metabolism are inserted to the cytoplasm which can lead to induced stress responses [14]. As most viruses, SARS-CoV-1 have been shown to modulate the cellular antiviral interferon (IFN) responses. Viral proteins can either interact directly with the NLRP3 inflammasome in macrophages or, in cells lacking NLRP3, the viral sensing machinery can be modulated by viral protein aggregates in the cytosol [15,16]. There is also a recent report of a dysfunctional interferon response in critically ill patients during the current pandemic [18]. It has been shown that corona-derived viral protein deposits lead to endoplasmic reticulum (ER) stress and mitochondrial dysfunction in the affected epithelial cells [15]. Hence, while the viral-derived proteins suppress innate sensing machineries such as toll-like receptor (TLR) induced innate responses, the inflammasome can independently trigger a stress-induced reactive oxygen species (ROS) production via the redox-homeostasis sensing machinery and mitochondrial machinery leading to for example IL-6 production [19]. In support of a potential role of oxidative stress and inflammaging to the pathology of SARS-CoV2, previous studies have indicated that increased chronic oxidative stress through lipid peroxidation lead to enhancement of PLA2G2D expression, a secretory phospholipase A2 precursor. PLA2G2D expression was shown to be increased in the lungs of middle aged mice, resulting in decreased survival and impaired T cell responses upon infection with SARS-CoV1 [20]. Interestingly, NAC administration to aged mice, diminished PLAG2D expression in both lung cells and CD11c + DCs. In addition, increased levels of oxidized phospholipids are a common feature associated with acute respiratory distress syndrome (ARDS) caused by viruses including SARS and H5N1. Notably, in an experimental mouse system that mimics the initial phases of ARDS using inactivated H5N1 lung challenging, ROS levels were increased in alveolar macrophages. This led to the formation of oxidized phospholipids and activation of TLR4 that exacerbated IL-6 production [20,21] and lung injury. Lung injury in response to H5N1 was impaired by usage of mice that lack Ncf1, a major component of NADPH oxidase.

To maintain normal homeostasis and counteract ROS responses described above, cells rely on a balanced redox status together with an effective DNA damage repair machinery. The nicotinamide adenine dinucleotide $(NAD)^+$ is a crucial electron transporter in mitochondrial respiration and oxidative phosphorylation, and is also the sole substrate for poly (ADP-ribose) polymerase (PARP), responsible for ADP ribosylation, an important step in DNA repair. Upon viral infection, cells sense the ssRNA virus and host-viral interactions occur in the cytoplasm of the infected cell that also affect the DNA repair system. NAD^+ is generated from ingested tryptophan by the kynurenine pathway and there is a hepatic (tryptophan 2,3-dioxygenase (TDO)) and extra-hepatic (indoleamine 2,3-dioxygenase (IDO)) NAD^+ generating pathway. The IDO driven pathway can be triggered by immune activation via IFN_γ release, in both immune and non-immune cells at local inflammation sites. Also, many of the PARP-family members are regulated by IFN_γ driven cellular responses [22]. The half-life of NAD^+ is 15min-15 h depending on the tissue, and the liver secretes the precursor nicotinamide (NAM), which is taken up by the organs and transformed into NAD^+ in the cytoplasm [23]. NAD^+ levels are decreasing during increased oxidative stress conditions including aging [24]. Coronaviruses reportedly have the capability to reverse ADP ribosylation driven by PARP, thereby counteracting the host-virus defense system [22]. As discussed above, the viral infection will also lead to mitochondrial stress inside the alveolar epithelial cells, which increase ROS accumulation and skew the redox balance in the exposed cells. In a recent not yet peer reviewed transciptomic analysis, lungs of a diseased individual from COVID-19 showed that both specific members of the PARP superfamily and the NAD biosynthetic pathways are modulated by the infection [25]. Interestingly, the nsp10 protein of SARS-CoV was shown to directly interact with the enzymes of the oxido-reductase system and result in loss of inner mitochondrial potential which regulates release of ROS [26,27]. If genetic or environmental factors can impact the antioxidant power and if this has an impact on the SARS-CoV-2 mortality remains to be shown.

In addition to the oxidative stress induced by increasing ROS production, ROS have also been reported to activate the STAT/IL-6 axis [28,29], spiraling cytokine release and immune cell infiltration in the lung as a result. Young healthy individuals most likely have a redox homeostasis in balance [30], which is better equipped to respond to a coronavirus infection. Some will experience signs of ARDS, but severe cases are few [5,31]. We hypothesize that with age, the gradual decrease in the capability of maintaining redox homeostasis will increase the risk of excessive immune activation and lung damage in response to a viral infection as has been documented now with SARS-CoV-1/2 infections [31].

Innate immune responses to SARS-CoV viruses as positive ssRNA viruses differ to negative ssRNA based influenza viruses in how they trigger IFN pathways, how the viruses replicate in the cell, accumulation of protein aggregates inside the cell, as well as their mechanism to avoid IFN activation [14,15,32]. It will be of great importance to compare innate and adaptive immune responses of SARS-CoV and the seasonal influenza strains, to develop optimal patient care for young and elderly in future pandemics. It should be noted that the severe clinical symptoms of SARS-CoV commonly arise first after a week after onset of symptoms, or even later, when virus titers commonly decline. A theoretical explanation for this is that the virus with time build up toxic protein aggregates within the cells [15]. This overload of aggregates could be the reason for the toxic stress response in the epithelial cells and acute ROS release. Of interest is that ROS production hamper the proteasome function, which leads to impaired protein degradation and further negatively influence mitochondrial function [33–36], this negative spiral triggered by accumulation of viral proteins in the cytoplasm, leading to aggresomes, that can over time become toxic to the cell.

There is an urgent need to further understand the redox homeostasis in a corona viremia. We propose that in-depth investigations for the therapeutic use of anti-oxidant pharmaceutical interventions, such as N-acetylcysteine (NAC), or other applicable antioxidant therapies, as early pharmacological interventions. It may be equally important to consider how use of other drugs, such as for example antibiotics that are used to treat secondary infections, can affect mitochondrial integrity [37] or

proteasome function to further aggravate ROS induced tissue damage. In countries with an overuse of antibiotics, an increase mortality of SARS-CoV-2 may be linked to antibiotic-induced cellular dysfunction, which may be relieved by introducing N-acetylcysteine in the care of SARS-CoV-2 patients [37].

A number of studies have analyzed of the use of NAC as preventive or therapeutic intervention for respiratory tract infections, and while there is not clear effect on mortality, several reports indicate a shortening of the duration of intensive care unit days along with support of use in a disease preventive manner [16,38–40]. Future trial designs should consider early use of NAC in risk groups, or alternative patient selection strategies, to assess if NAC can reduce ARDS incidences in risk groups or patients with predisposing risks of developing ARDS. Interestingly, dietary NAC has been shown to reverse the enteropathogenic effects of the coronavirus porcine epidemic diarrhea virus (PEDV) [16], a disease that causes large economic losses through the high mortality of the affected pigs. In this study there were also indications of therapy reduced systemic oxidative stress symptoms, as indicated by decrease in plasma and mucosal H_2O_2 levels [43]. In another study, NAC could inhibit H5N1 infection of lung epithelial cells in vitro and production of pro-inflammatory mediators [44].

There is clearly an urgent medical need for measures against the devastating pandemic COVID-19 with respect to both treatment of the disease and the further dissemination of SARS-CoV–2. With respect to the latter, major efforts involving many companies and academic institutes are currently ongoing to develop vaccines [45], but it may still take a while before clinical studies and production have reached a stage of global vaccine coverage. Here, we suggest that early treatment in the form of NAC should be evaluated a cost-effective intervention for virus-infected patients with symptoms of lung dysfunction, to provide each patient with the best protection against excessive ROS production which negatively impact lung integrity, but also to handle drug-induced ROS during severe viremia. Pharmacological intervention using NAC dosing aims to normalize the redox-homeostasis, as ROS production is also part of the normal innate anti-viral host response, when in balance. The administration route should also be evaluated as both oral, infusion as well as inhalation administration are available.

CRediT authorship contribution statement

Aikaterini Nasi: Conceptualization, Methodology, Data curation, Writing - original draft, Writing - review & editing. **Stephanie McArdle:** Data curation, Writing - original draft, Writing - review & editing. **Gustav Gaudernack:** Data curation, Writing - original draft, Writing - review & editing. **Gabriel Westman:** Data curation, Writing - original draft, Writing - review & editing. **Cornelis Melief:** Data curation, Writing - original draft, Writing - review & editing. **Johan Rockberg:** . **Ramon Arens:** Data curation, Writing - original draft, Writing - review & editing. **Demetrios Kouretas:** Methodology, Data curation, Writing - original draft, Writing - review & editing. **Jan Sjölin:** Methodology, Data curation, Writing - original draft, Writing - review & editing. **Sara Mangsbo:** Conceptualization, Methodology, Data curation, Writing - original draft, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare no conflict of interest.

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