Commentary

Proteasome and reactive oxygen species dysfunction as risk factors for SARS-CoV-2 infection; consider N-acetylcysteine as therapeutic intervention

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Abstract

The new SARS-CoV-2 pandemic is a single stranded RNA virus infecting alveolar epithelial cells among other cells. The virus is spread via droplets, or by indirect spread from contaminated surfaces. The coronavirus pandemic affects us all, when countries attempt to limit the spread by social distancing measures, impacting our daily lives, health and our economy. Upon viral infection by SARS-CoV-2, the virus introduces proteins and RNA into the cytoplasm of the infected cell, to inhibit a rapid IFN response and in preparation of replication and later viral spread. Coronavirus proteins affect mitochondrial integrity, proteasome function and leads to the production of reactive oxygen species (ROS). Innate immune response, cellular redox homeostasis, proteasome function as well as any existing cross-reactive immunity against the virus can affect the outcome of an infection. We propose a mechanism where a vicious circle of accumulation of protein aggregation (viral and endogenous proteins) and ROS formation can hamper cellular functions and impact the pathological response in the lung, especially in individuals with a limited antioxidant capacity. Age, sex, the genetic makeup as well as health status can influence the redox homeostasis in an individual. Published data support that SAS-CoV-2 proteins introduced into the cytoplasm interact with organelles impacting cellular stress responses. Preclinical and clinical activities should aim to understand if existing pharmacological approaches can be used to avoid toxic cytoplasmic protein aggregation or alternative, to boost antioxidant capability using N-acetylcysteine, as a means to dampen the tissue damage seen in SARS-CoV-2 infected patients.

Introduction

More and more data support the role of excessive immune activation as the cause of lung destruction by SARS-CoV-2, the causative virus of the pandemic coronavirus disease 19 (COVID-19). 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[1]. 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 impaired[2]. 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, also with impaired flexibility towards novel antigens, known as inflammaging[2]. This type of inflammation and oxidative stress is further promoted by obesity, which is associated with increased susceptibility to osteoarthritis, Alzheimer’s disease and metabolic syndrome, including type 2 diabetes[3-5]. So far data is limited on the risk of morbidity linked to diabetes and obesity, but some observational data suggest that diabetes or co-morbidities can be a predictor of morbidity when infected by SARS-CoV-2[6].

SARS-CoV-2 infects alveolar epithelial cells via the receptor ACE2[7], triggering innate response mechanisms that alert the immune system. After introduction of viral proteins and RNA into the cytoplasm of the host cells, the virus will replicate and spread via either budding or cell-cell fusion, as a means to avoid antibody-mediated viral neutralization[8]. SARS-CoV proteins are known to interact with the host proteins and affect cell metabolism which can result in stress responses in the infected cells[9, 10]. The SARS-CoV-1 proteins have been shown to interact directly with the NLRP3 inflammasome in macrophages. Cell lacking NLRP3 are instead affected by viral protein aggregation in the cytosol[10], as the coronavirus counteract the cellular anti-viral interferon (IFN) response. This leads to endoplasmic reticulum (ER) stress and mitochondrial dysfunction in infected epithelial cells[10]. Hence, while corona-derived proteins suppress our innate defense toll-like receptor (TLR) induced response, viral proteins that remain in the infected cell cytoplasm, as well as endogenous proteins accumulated in the cytoplasm due to excessive ROS, can over time activate the inflammasome and lead to spiraling cytokine release syndrome [11].

Innate immune responses to SARS-CoV viruses which are positive ssRNA viruses, differ from 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[8, 10, 12]. It will be of great importance to compare innate and adaptive immune responses induced by SARS-CoV with those generated against seasonal influenza strains in different age groups, in order to develop optimal patient care for young and elderly in future pandemics. It should be noted that the severe clinical reaction of SARS-CoV commonly arise a week after the first symptoms, when virus titers commonly decline, perhaps as a result of accumulating cellular protein aggregation over time, leading to acute cellular stress when cellular machineries are overloaded.

ROS production can also hamper cellular functions such as the proteasome, leading to impaired endogenous protein degradation and further negatively influence mitochondrial function[13, 14], this spiral triggered by accumulation of both endogenous and viral proteins in the cytoplasm leading to aggresomes. Mortality from SARS-CoV-1/2 is also linked to risk factors from inflammatory disorders, driven by health style or smoking. Smoking is known to impair the proteasome function in lung epithelial cells[15], and obesity or inflammatory disorders may lead to elevated cellular stress, rendering the infected cells unable to clear toxic protein aggregates. Patients with diabetes, cancer and cardiovascular diseases often show reduced proteasome activity[16] and SARS-COV proteins can interact with the proteasome directly[9].

Virally induced ROS production in the cytoplasm will modify proteins as well as DNA in the cell. The nicotinamide adenine dinucleotide (NAD)+ is an important factor to maintain cellular homeostasis. 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, crucial for DNA
repair. Coronaviruses reportedly have the capability to reverse ADP ribosylation driven by PARP, thereby counteracting the host-virus defense system[17]. NAD+, generation takes place via the 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 IFNγ release, in both immune and non-immune cells at local inflammation sites and many of the PARP-family members are regulated by IFN, driven cellular responses[17]. The half-life of NAD+ is 15min-15h 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[18]. Thus, a dysfunctional replenish of NAD+ can impact our ability to maintain cellular redox homeostasis during a SARS-CoV-2 infection upon toxic protein accumulation. It is known that age affect redox homeostasis[19]. In an experimental influenza model, female mice had less severe lung damage caused by the infection, than male mice. This was attributed to a better systemic antioxidant power[20]. If gender-based antioxidant capabilities exist in elderly, remains to be investigated. Sex-based differences in natural IgM levels, where men have been reported to have significantly lower IgM levels than women, can also play a role in the immune defense against SARS-CoV infections[21]. The presence of circulating natural IgM levels has been suggested to mediate neutralization of neoepitopes generated by oxidation[22].

In addition to the oxidative stress by virally induced ROS production, ROS have been reported to activate the STAT/IL-6 axis[23], spiraling cytokine release and immune cell infiltration in the lung as a result. Genetic factors can also impact the antioxidant capability in patients. Influenza, a negative (-) ssRNA virus, can also display age related mortality, however the mortality can also, for some influenza pandemics affect a younger population. Previous immunity, and potential cross-reactive antibodies to a virus, can explain why mortality is skewed in a population[24] however, it is not known whether exposure to less virulent coronaviruses has induced SARS-CoV-2 relevant cross-immunity in our population, and whether this could be sex-skewed.

Anecdotal warnings of ibuprofen causing rapid deterioration in SARS-CoV-2 patients have led to initial recommend of using paracetamol instead of ibuprofen for patients with SARS-CoV-2 infections, however, regulatory agencies have not found any scientific evidence establishing a link between ibuprofen and worsening of COVID-19, the agencies continues monitoring and investigations will follow[25, 26]. What has so far been lacking in this discussion and in the published literature, is how nonsteroidal anti-inflammatory drugs (NSAIDs) can trigger mitochondrial decoupling in epithelial cells, leading to increased ROS production[27]. The production of which would start in the intestine, but then could lead to a systemic antioxidant consumption, thereby depleting systemic precursor substrates for cellular NAD+ production. If ROS generation is a cause of coronavirus induced tissue damage, any type of drug-induced ROS release may risk a consumption of the systemically available antioxidant precursor buffering capacity, rendering the host less able to control redox homeostasis in the lungs thereby worsening the disease. As SARS-CoV-2 negatively impact cellular homeostasis it is also of importance to understand how drugs that affect mitochondrial function, such as antibiotics[28], would impact any type of virus driven cellular dysfunction to avoid aggravated tissue damage.

A possible accessible pharmacological intervention to manage ROS induced lung toxicity could the evaluation of N-acetylcysteine (NAC) administration as a means to avoid ROS induced cell damage by SARS-CoV-2. NAC is a synthetic precursor of intracellular cysteine and glutathione (GSH) and can be an important pharmacological intervention to maintain the antioxidant capability for individuals with increased oxidative stress due to a SARS-CoV-2 infection. If successful, NAC could also minimize the need for more
advanced biological therapies such as anti-IL6R intervention for cytokine release syndrome. A therapy that is also not available in all countries and may also be limited due to scale-up limitations in production. Interestingly, dietary NAC has been shown to have reversed the enteropathogenic effects of the coronavirus porcine epidemic diarrhea virus (PEDV)[29], 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 H2O2 levels[29].

Conclusive remarks and future directives

There is an urgent medical need for measures against the devastating pandemic COVID-19 with respect to both antiviral and immune modulating treatment of the disease, and the further spread of SARS-CoV–2. With respect to the latter, major efforts involving companies and academic institutes are currently ongoing to develop vaccines[30], but it may still take a while before clinical studies and production have reached a stage of global vaccine coverage. Herein, we propose that SARS-CoV infections can lead to viral and endogenous cytoplasmic protein accumulation that leads to excessive ROS production and toxic responses in patients. To handle the toxic ROS response it would be of value to evaluate the administration of NAC in preclinical and clinical trials as a potentially cost-effective intervention for virus-infected patients with symptoms of lung dysfunction, with the aim to normalize the redox-homeostasis. The administration route should also be evaluated prior a trial start, as both oral infusion as well as inhalation administration routes are available. NAC has also been show to interact and inhibit proteasome inhibitors[31], it remains to be shown if NAC or other pharmacological agents can affect virally released proteasome binding proteins and thereby aid proteasome function and by this, prevent tissue damage.

References


