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PERSPECTIVE AND REFLECTION ARTICLE

Coronaviruses and stress: from cellular to global

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Abstract

Near the end of 2019, SARS-CoV-2, a novel highly contagious coronavirus phylogenetically related to the SARS virus, entered the human population with lethal consequences. This special issue devoted to the resulting disease COVID-19 was not planned but instead the articles accumulated organically as researchers in the cell stress response field noticed similarities among the pathophysiology of COVID-19 infections and the responses that they studied in contexts unrelated to viral infection. We preface the issue with an introductory article which begins with a brief review of the structure and biology of SARS-CoV-2. As we collected and compared the COVID-19 articles, several shared themes emerged. In the second part of the introduction, each article is summarized briefly and the common themes that link each into a spontaneously arising chain of ideas and hypotheses are emphasized. These themes include growing evidence of molecular mimicry among the viral proteins and the proteins of patients. The realization that much of the consequences of such immune mimicry may play out on the plasma membrane of vascular endothelial cells raised the specter of autoimmune-induced vascular endothelial damage in multiple organs. Proposals of new therapeutic approaches have coalesced around the theme of inducing protection of the vascular endothelium. New chemical treatments that are proposed include stannous chloride, inducers of the gasotransmitter hydrogen sulfide such as sodium thiosulfate and inducers of the cytoprotective stress protein heme oxygenase. Oxygen delivered by ventilators is already in extensive use to provide life support for patients with severe COVID-19. Two articles propose to advance the use of oxygen to the level of a therapeutic treatment early in the detection of the virus in infected patients by delivering oxygen under elevated pressure in hyperbaric chambers. At elevated blood plasma concentrations, hyperbaric oxygen is capable of achieving results far beyond the capability of ventilators as it promotes the activation of transcription factors that control the establishment of inducible cellular defense systems.

Viruses continue to surprise us. While virologists' attention was focused on the Ebola virus outbreak in West Africa and the new highly pathogenic (HPAI) strains of avian influenza, a new type of coronavirus emerged.

Coronaviruses (CoVs), members of the *Coronaviridae* family and the order *Nidovirales*, comprise a large number of enveloped, positive-sense single-stranded RNA viruses causing respiratory, enteric, renal, and neurological diseases of varying severity in domestic and wild animals, as well as in humans (Cui et al. 2019). Coronaviruses have the largest identified RNA genomes (typically ranging from 27 to 32 kb) containing a large replicase-transcriptase gene preceding structural and accessory genes. All CoV genomes are arranged similarly with the replicase locus encoded within the 5′ end and the structural proteins encoded in the 3′ end, with an invariant gene order: 5′- S (spike) - E (envelope) - M (membrane) - N (nucleocapsid)-3′; numerous small open reading frames, encoding accessory proteins, are distributed among the structural genes. Some CoVs also encode an additional structural protein, hemagglutinin esterase (HE) (Fung and Liu 2019).

The N protein complexes with the genomic RNA to form a helical capsid structure surrounded by the viral envelope. Homotrimers of the spike protein are embedded in the envelope and extend beyond the viral surface to bind to host receptors giving the virion its crown-like morphology. M and E proteins are transmembrane proteins, while the HE protein forms smaller spikes in some CoVs.

Other typical CoV features include the expression of many nonstructural genes by ribosomal frameshifting, transcription

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of downstream genes by synthesis of 3′ nested sub-genomic mRNAs, and the presence of several unusual enzymatic activities encoded within the replicase-transcriptase polyprotein. The unique replicative mechanism of CoV involves noncontiguous transcription of the genome, leading to a high rate of recombination for coronaviruses, which may play a role in viral evolution and interspecies infections (Weiss 2020).

On the basis of their phylogenetic relationships and genomic structures, CoVs are subdivided into four genera: *Alpha-*, *Beta-*, *Gamma-*, and *Delta-coronavirus*; among these, alpha- and beta-CoVs infect only mammals, whereas gamma- and delta-CoVs infect birds and only occasionally can infect mammals. Human coronaviruses (HCoVs) were discovered in the 1960s and historically include four viruses (HCoV-NL63, HCoV-229E, HCoV-OC43, and HCoV-HKU1) that are globally distributed and cause only mild upper respiratory diseases (10–30% of all common colds) in immunocompetent hosts. In some cases, they have been reported to cause severe infections in infants, young children, and elderly individuals (Lim et al. 2016). However, since the beginning of this century, three highly pathogenic HCoVs have emerged (Cui et al. 2019, Tang et al. 2020). SARS-CoV (severe acute respiratory syndrome coronavirus), a beta-CoV, emerged in China and Hong Kong in 2002–2003, causing more than 8000 cases worldwide and a death rate of approximately 10% (WHO; https://www.who.int/csr/sars/country/table2004_04_21/en/); MERS-CoV (Middle East Respiratory Syndrome coronavirus), also a beta-CoV, emerged in 2012 in the Arabian Peninsula, causing over 2500 confirmed cases and a fatality rate of 34–36% (WHO; https://www.who.int/csr/don/24-february-2020-mers-saudi-arabia/en/). SARS and MERS coronaviruses likely originated in bats and have been transmitted directly to humans from market civets (SARS) and dromedary camels (MERS). Infection with both SARS-CoV and MERS-CoV can result in acute respiratory distress syndrome (ARDS), which may lead to long-term reduction in lung function and death.

Near the end of 2019, the seventh coronavirus known to infect humans, phylogenetically in the SARS-CoV clade, emerged in Wuhan, China (Tang et al. 2020). The novel HCoV was named SARS-CoV-2, while the disease associated with it is referred to as COVID-19 (coronavirus disease 19).

SARS-CoV-2 turned out to be a far more serious threat to public health than SARS and MERS HCoVs because of its ability to spread more efficiently, making it extremely difficult to contain worldwide with a reported 25,602,665 confirmed cases and 852,758 deaths as of 2 September 2020 according to The World Health Organization.

COVID-19 clinical features vary, ranging from asymptomatic state to respiratory symptoms that, in a subset of patients, may continue to deteriorate and may progress to pneumonia, ARDS (often requiring mechanical respiration), multi organ dysfunction, and death (Wiersinga et al. 2020). This progression is associated with an extreme rise in pro-inflammatory cytokines and chemokines, including IL6, IL-2, IL-7, IL-10, macrophage inflammatory protein 1α (MIP1α), granulocyte colony-stimulating factor (G-CSF), IP-10, MCP1, and tumor necrosis factor α (TNF α) (Tay et al. 2020).

Efforts to defeat COVID-19 are hampered by lack of information on several important aspects of this new virus, ranging from SARS-CoV-2 biology to its interaction with the host response.

SARS-CoV-2 replication cycle is initiated by the binding of the spike S protein to the cell surface receptor, which, similarly to SARS-CoV, is the human angiotensin-converting enzyme 2 (hACE2) (Wiersinga et al. 2020). SARS-CoV-2 S protein is an extensively glycosylated, trimeric, class I fusion protein; each monomer is composed of two functional subunits: S1 responsible for receptor binding and the membrane-anchored S2 that contains the fusion machinery. Like for SARS-CoV, activation of S fusion ability requires sequential cleavage at distinct sites; however, in the case of SARS-CoV-2, there was recently identified an unexpected polybasic furin-like cleavage site located at the S1/S2 boundary, which is processed during S protein biogenesis and is considered to be responsible for S protein "priming" (Coutard et al. 2020; Hoffmann et al. 2020; Walls et al. 2020). Importantly, the acquisition of similar cleavage sites is associated with increased pathogenicity in other viruses such as avian influenza viruses, and it is suggested to be responsible for SARS-CoV-2 high infectivity and transmissibility (Coutard et al. 2020; Hoffmann et al. 2020; Walls et al. 2020).

After entry, SARS-CoV-2 replication is mediated by a multi-subunit replication/transcription machinery, containing several non-structural viral proteins (nsp). The key component of this complex is the RNA-dependent RNA polymerase (RdRp), also known as nsp12. It contains the canonical viral RdRp motifs in its C-terminal part, and employing a primer-dependent initiation mechanism, RdRp catalyzes the synthesis of viral RNA. In order to function effectively, nsp12 requires accessory factors including nsp7 and nsp8 that increase RdRp template binding and processivity (Kim et al. 2020; Gao et al. 2020; Weiss 2020). Whereas the nsp12-nsp7-nsp8 complex represents the minimal complex required for nucleotide polymerization, it is likely, however, that additional viral nsp subunits are necessary to carry out the full repertoire of transcription/replication activities. Being distinct from the host transcriptional machinery, SARS-CoV-2 RdRp is considered a primary target for therapy. It should be noted that development of nucleoside-based therapeutics for CoV infections has been hampered by the presence of an exoribonuclease (ExoN, nsp14) that acts as a "proofreading" enzyme correcting errors in the RNA sequence and potentially limiting the effects of analogues; however, remdesivir, the first antiviral drug that the Food and Drug Administration

(FDA) has made available under an emergency use authorization for the treatment of adults and children with severe COVID-19 disease in the USA (https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-issues-emergency-use-authorization-potential-covid-19-treatment), was found to be able to evade this proofreading (Agostini et al. 2018).

Despite the large amount of literature exploring the relationship between viruses and the heat shock response (HSR) (Santoro et al. 2010) and the consequent link between the HSR and inflammatory responses (Morimoto and Santoro 1998, Santoro et al. 2003), very little is known in the case of coronavirus infection. It has been recently pointed out that, in addition to age, major risk factors to fatal outcome in COVID-19 patients, i.e., preexisting metabolic and cardiovascular diseases, share in common the characteristic of being chronic degenerative diseases of inflammatory nature associated with defective HSR (Heck et al. 2020). Therefore, there is an urgent need to understand the HSR-pathogen biology of COVID-19 in order to gain important insights translatable into effective treatment and management of the disease.

This special issue about severe COVID-19 is unusual in that the articles accumulated organically as researchers in the cell stress response field began to notice similarities among the pathophysiology of COVID-19 infections and characteristics of responses that they were studying in completely unrelated contexts. They submitted Letters, Perspective articles, and Short Communications to share their observations and hypotheses with colleagues in their field as well as other interested readers. As the numbers of articles increased in the version of *Cell Stress & Chaperones* in Springer Online First, it became clear to the editors that a special issue is warranted. As we compared the articles, several shared themes began to emerge.

Our first COVID-19 article published in *Cell Stress & Chaperones*, a Letter to the Editor from Francesco Cappello, has appeared (Cappello 2020). Therein, the author asks the question "Is COVID-19 a proteiform disease inducing also molecular mimicry phenomena?" The multiplicity of organ involvement and the range of patient-specific symptoms suggested the term proteiform, derived from Proteus, a sea god able to change shape to escape capture, a metaphor for the ways in which SARS-CoV-2 escapes the hands of the physicians attempting to treat COVID-19 patients. Cappello proposed that the virus employs molecular mimicry to trick patients' immune systems to produce antibodies against viral protein epitopes capable of cross-reacting to those of self-proteins. Given the clues that have emerged implicating the blood vessel walls, he suggested that the setting for this molecular sleight of hand is the vascular plasma membranes of stressed endothelial cells. Such autoimmune reactions may explain the thrombosis, disseminated intravascular coagulation, systemic inflammation, and eventual multiple organ system failure experienced by some patients. Gammazza and co-authors including Francesco Cappello expanded on this question and began providing answers in a Short Communication published in this issue (Marino Gammazza et al. 2020). They searched a protein database for contiguous segments of SARS-CoV-2 proteins having an exact match to human protein segments with a minimum length of six amino acids. They found 17 molecular chaperones with shared peptides also found in the viral proteins. Significantly, all of these peptides are found among the immunogenic epitopes predicted using the Immune Epitope Database and analysis resource for either B or T lymphocytes. In their discussion of this molecular mimicry, they focused on those chaperones localized on endothelial cell plasma membranes. Thus, we are back to the common theme of autoimmune-induced vascular endothelial damage.

In an independent study, Lucchese and Flöel searched a library of human proteins linked to acute and chronic immune-mediated neuropathies (Lucchese and Flöel 2020). They found that SARS-CoV-2 shares two hexapeptides with HSP90B, HSP90B2, and HSP60, respectively. Epitopes of these human heat shock proteins, the historic name for this group of cellular stress response proteins, are associated with Guillain-Barré syndrome and other autoimmune diseases. The authors emphasized the critical observation that these shared peptides are located within the immunoreactive epitopes experimentally validated in humans. They proposed molecular mimicry with human HSPs as a possible pathogenic mechanism behind the association of SARS-CoV-2 and Guillain-Barré syndrome.

O'Brien and Sandhu advanced the themes of immunotherapy to treat COVID-19-related hyper-inflammation and vascular endothelial dysfunction (O'Brien and Sandhu 2020). They noticed the data on sex differences among COVID-19 patients where the mortality rates in men are twice that of women. It has been known that HSP27 is an estrogen-responsive protein practically from its discovery. This suggested to them that it could be useful for treating COVID-19. Previously the O'Brien lab found that the combination of the HSP27 protein and its antibody produces the therapeutic benefit of reducing atherosclerosis and promoting anti-inflammatory effects. There is clinical evidence of endothelial dysfunction in COVID-19 patients. These conditions include vascular thrombosis, reduced circulation in fingers and toes, and also large artery strokes in surprisingly young patients. They proposed that these vascular effects and other complications of inflammatory responses may be reduced by vaccination with HSP27 or by passive immunization with anti-HSP27 antibodies.

Continuing the theme of protecting the vascular endothelium, Perdrizet and Hightower proposed that treating COVID-19 patients early with stannous chloride and hyperbaric oxygen (HBOT) could reduce damage to the vascular endothelium (Perdrizet and Hightower 2020). They emphasized the need to apply systemic therapeutic approaches such as

HBOT and intravenous stannous chloride against systemic infections that trigger overwhelming immune responses and associated systemic inflammatory responses characteristic of severe COVID-19. Both of these suggested that treatments induce cytoprotection in a variety of tissues including vascular endothelium. A bonus result found in earlier studies is that both of these treatments induce HSPA1A, which serves as a biomarker for the state of acquired cytoprotection in tissues.

De Maio and colleagues also noticed the similarities among COVID-19 symptoms and those of patients with inflammatory responses such as acute respiratory distress syndrome (ARDS). They realized that the incidence of ARDS and its progression into systemic inflammatory responses and ultimately multiple organ system dysfunction is not unique to respiratory viral infections. While firmly entrenched in the cell stress and chaperones field, this laboratory also studies sepsis and wound responses to traumatic injury. In the article by De Maio and Hightower, they elaborate on the idea that it should be possible to learn more about the etiology of COVID-19 and its treatment from our current understanding of sepsis and injury (De Maio and Hightower 2020). ARDS is a component of the development of sepsis and septic shock. This hypothesis logically leads to HBOT, which is already an FDA-approved treatment of patients with chronic nonhealing wounds. Experimental studies in animal models demonstrate that an initial treatment with HBOT dramatically improves the outcome from sepsis. De Maio and Hightower make the case that HBOT could be a helpful tool to shorten the hospital stays and need for ventilators by COVID-19 patients, especially when the HBOT application is done early.

In their Letter to the Editor, Evgen'ev and Frenkel remind readers that the hydrogen sulfide (H_2S) gasotransmitter is an evolutionarily ancient biological mediator and the defects in H_2S synthetic enzymes are associated with a number of human diseases (Evgen'ev and Frenkel 2020). At low concentrations, it causes production of reactive oxygen species (ROS) as a cell signal to induce cytoprotection primarily through the activation of the transcription factors Hsf1 and Nrf-2. Again, we return to common themes, cytoprotection of vulnerable tissues and systemic treatments to counter systemic inflammation. They conclude with the suggestion that sodium thiosulfate (a well-tolerated H_2S donor) be used to treat patients in any stage of COVID-19. This could be administered via aerosol inhalation, intravenous injections, or both routes as needed.

It is highly appropriate to end with the theme of the cytoprotective properties of heme oxygenase (HO-1) introduced in the article by Philip Hooper (Hooper 2020). There is evidence that the inhibition of heme production in patients with COVID-19 may lower the cytoprotective properties of HO-1 as most of these are the result of its enzymatic cleavage of heme into biliverdin, ferrous iron, and carbon monoxide. These products protect tissues by reducing inflammation and oxidative stress. The conditions that increase a person's vulnerability to SARS-CoV-2 infection and the resulting COVID-19 illnesses include metabolic syndrome, old age, and the male gender, all conditions that share the characteristic of reduction in levels of stress response proteins. These individuals have the greatest morbidity and mortality. These stress proteins include HO-1. Hooper also pointed out that controlled trials with HO-1 inducers, and there is a substantial list of them, are needed in order to prevent or treat COVID-19 disease. In addition to limiting the damage of infection, the cytoprotective and anti-inflammatory properties of HO-1 should reduce ventilator-induced inflammation as well.

Weaving its way through the articles is the unifying concept that systemic illnesses like severe COVID-19 require systemic therapies to achieve successful treatments. Important lessons are being learned at all levels of our healthcare systems that hopefully will prepare us better to combat the next viral pandemic.

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