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LETTER TO THE EDITOR

Sex differences in COVID-19 mortality: opportunity to develop HSP27 (HSPB1) immunotherapy to treat hyper-inflammation?

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COVID-19, hyper-inflammation, and sex-specific features

Since January 10, 2020, when the first death linked to the severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) occurred in Wuhan, China, more than 530,000 people have died (as of July 4, 2020). Globally, there are 11.2 million people infected with the virus, with 2.9 million in the USA alone and other populous countries surging. While there is a charge to develop a vaccine, as well as means of eliminating the virus from those infected (e.g., remdesivir), neither of these therapeutic strategies will directly address the major life-threatening complications that may occur once infected, namely, the profound upregulation of the innate immune system. Like the severe acute respiratory syndrome coronavirus that emerged in 2002 (SARS-1, caused by SARS-CoV) and the Middle East respiratory syndrome-related coronavirus of 2012 (MERS-CoV), COVID-19 (the disease associated with SARS-CoV-2) is associated with a storm of pro-inflammatory cytokines like IL-1β, IL-6, and TNF. Precisely why some patients evolve to this hyper-inflammation state while others do not remains unclear but is likely due to nonviral factors that are specific to the host, including age and comorbidities (Yang et al. 2020). These cytokines play an important role in various tissue complications with acute respiratory distress syndrome (ARDS), a form of acute lung injury that is without tangible therapeutic options apart from supportive care, of principal concern (Nieto-Torres et al. 2015).

What do we know about sex differences in the COVID-19? The COVID-19 mortality rate in men is double than that of females (Wu and McGoogan 2020). This is not surprising, as clinical and experimental studies with SARS-1 and MERS noted that the infection occurred more readily and was more persistent in males (Channappanavar et al. 2017; Karlberg et al. 2004; Mobaraki and Ahmadzadeh 2019; Zheng et al. 2020). Of note, there are exceptions to the male sex predominance in COVID-19 mortality rate. For example, in Canada, 54% of deaths are in females—however, this appears to be linked to factors other than sex, as 85% of the national deaths occurred in long-term care (LTC) facilities where the vast majority of residents are older females (Estabrooks et al. 2020). For the rest of the world, LTC residences do not represent the hot-bed of COVID-19 deaths (e.g., only 29% of deaths in Australia occurred in LTC). Hence, the global statistics overwhelmingly point to a male predominance of this disease, which has spurred a number of excellent opinions papers (Bischof et al. 2020; Suba 2020). Moreover, based on the assumption that ovarian hormones may be protective, a small clinical trial has begun in men > 18 years and women > 55 years with confirmed or suspected COVID-19, randomizing subjects to a transdermal patch containing estradiol or placebo (Estrogen Patch for COVID-19 Symptoms n.d.). With this in mind, we now postulate that heat shock protein 27 (HSP27), recently recognized to have potent anti-inflammatory effects (Inia and O'Brien 2020), may also have a role in the treatment of COVID-19.

Heat shock protein 27, an estrogen-responsive protein for treating COVID-19?

HSP27 is a member of the small heat shock protein family that is primarily known as an intracellular chaperone and more

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recently for its extracellular, anti-inflammatory roles (Batulan et al. 2016). While looking for estrogen receptor-beta associated proteins that might be involved in modulating hormonal transcription, we discovered HSP27 and began to study the role of this protein in atherosclerosis. Investigations by several laboratories, including our own, highlight that HSP27 arterial expression and blood levels are higher in healthy subjects compared with patients with cardiovascular disease (Lepedda et al. 2009; Liang et al. 2016; Martin-Ventura et al. 2004; Miller et al. 2005; Park et al. 2006). What we also uncovered is a complex relationship between HSP27 and estrogens, as HSP27 acts as a repressor of estrogen-mediated transcription in vitro, yet its expression and extracellular release are also partially regulated by estrogens (e.g., there is an estrogen response element found in the HSP27 promoter) (Miller et al. 2005; Rayner et al. 2009). As well, we recently noted that natural IgG auto-antibodies to HSP27 (AAbs) are detectable in human blood (Chen et al. 2020b) and demonstrated how HSP27 immune complexes (ICs) form, dock at the cell membrane where they engage with Toll-like receptor 4 (TLR4), and compete with LPS to reduce inflammatory signaling (Shi et al. 2020). Interestingly, HSP27 activates the NF-κB pathway—but only modestly—resulting in the expression of both pro- and anti-inflammatory cytokines and proteins (Salari et al. 2013). Boosting HSP27 antibodies via vaccination reduces atherosclerosis and promotes the anti-inflammatory effects of the HSP27 immune complex (Shi et al. 2020). It is the combination of the protein and its antibody that produces the therapeutic benefit—a concept that we refer to as HSP27 **I**mmune **C**omplex **A**ltered **S**ignaling and **T**ransport (or ICAST; transport refers to cellular internalization of HSP27).

HSP27 and COVID-19-related endothelial dysfunction

Clinical evidence of endothelial dysfunction in COVID-19 patients is striking, ranging from vascular thrombosis and altered microvascular function (toes, fingers) to large artery strokes in relatively young individuals (Teuwen et al. 2020). Indeed, the presence of frank thrombosis in the lungs and to a lesser extent the microvessels of the heart are turning out to be the hallmarks of this clinical entity. With the pulmonary air space already compromised due to the development of ARDS, no amount of supplemental oxygen therapy (e.g., with ventilators) can overcome the effects of pulmonary circulatory obstruction that further impairs gas exchange. What can be done to improve the endothelium, and why are (pre-menopausal) women enjoying relative protection from COVID-19? Currently, there is no answer for that question; regardless, the acuity and magnitude of COVID-19 pandemic have prompted a "think now – do now" attitude to exploring a variety of therapeutic options. Indeed, Perdrizet and Hightower recently proposed that treatments with stannous chloride and hyperbaric oxygen (HBOT) may offer protection to the vascular endothelium that could reduce COVID-19 damage and invoke HSP70 as a biomarker for the cytoprotected state (Perdrizet and Hightower 2020). In contrast, we now propose that vaccination with HSP27 (or alternatively passive immunization with anti-HSP27 antibodies) may be worth exploring for the treatment of the inflammatory complications of COVID-19—including the important vascular effects (Table 1).

NLRP3 inflammasome activation and COVID-19

Elevated plasma levels of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF seem to drive SARS-CoV-2 pathogenicity in COVID-19 patients and is associated with adverse outcomes and poor prognosis (Huang et al. 2020). Blood levels of lactate dehydrogenase (LDH) levels are also highly elevated in patients with severe COVID-19 (Chen et al. 2020a). LDH is a cytosolic enzyme released from cells undergoing pyroptosis, an inflammatory form of cell death, possibly triggered by the activation of the NLRP3 inflammasome. The NLRP3 inflammasome is an innate immune sensor that is under tight regulation and requires two signals for full activation (Broz and Dixit 2016). The first signal is received from the binding of the virus to toll-like receptors on host cells leading to the NF-κB-mediated transcription of pro-IL-18 and pro-IL-1β. The secondary signals are received in the form

Table 1 Potential therapeutic benefit of HSP27 immunotherapy for managing the inflammatory complications of COVID-19

Beneficial effects	Implications for COVID-19 pathophysiology
Estrogens augment synthesis and extracellular secretion (Rayner et al. 2009; Shi et al. 2019; Sun et al. 2011)	Higher levels of HSP27 that may attenuate inflammation
Competing for TLR4 and directing NF-κB activation towards anti-inflammatory mediators (Rayner et al. 2008; Shi et al. 2020)	Reduced IL-1β and increased IL-10
Upregulating GM-CSF (Pulakazhi Venu et al. 2017; Salari et al. 2013)	GM-CSF maintains alveolar epithelial and macrophage health—useful for treating COVID-19 ARDS
Promoting endothelial repair and regrowth by upregulated VEGF (Ma et al. 2014)	Critical for prevention of COVID-19 vascular complications like pulmonary emboli and stroke

of endogenous or exogenous ATP, reactive oxygen species, or lysosomal proteases released from damaged or dying cells leading to NLRP3 inflammasome assembly, activation of caspase-1, and subsequent secretion of IL-18 and IL-1β, a mediator of fever, lung inflamamtion, and fibrosis (Shrivastava et al. 2016). Under normal physiological conditions, NLRP3-triggered responses lead to the death of infected cells, critical in limiting viral spread. However, over-activation of the NLRP3 inflammasome causes the hyper-inflammatory responses seen in COVID-19 patients, resulting in a vicious cycle of release of pro-inflammatory cytokines, pyroptosis, and infiltration of the lungs with inflammatory cells leading to ARDS, multi-organ failure and even death (De Nardo et al. 2014; Grailer et al. 2014). The elderly, who are already suffering from age-related low-grade inflammation (Franceschi et al. 2000) and a decline in their immune systems, are particularly susceptible due to their inability to mount type I and type III interferon responses to clear the viral infection (Molony et al. 2017). Hence a dysfunctional NLRP3 inflammasome and the impaired ability to clear viral infections are a perfect storm for COVID-19.

SARS-CoV encodes ion-channel viroporins, namely, protein E, ORF3a, and ORF8a, which are known to induce NLRP3 inflammasome activity and IL-1β production by altering intracellular ionic concentrations (Chen et al. 2019; Siu et al. 2019). Similarly, treatment of macrophages derived from COVID-19 patients with SARS-CoV-2 spike protein and nigericin activated the NLRP-3 inflammasome, resulting in IL-1β production (S.J. Theobald et al. 2020). Incubation of these cultures with MCC950, a small-molecule NLRP3 inhibitor, blocks IL-1β secretion and therefore highlights the potential value of anti-inflammatory therapeutics for managing hyper-inflammation in COVID-19 patients. Accordingly, a number of clinical trials are ongoing, testing the potential therapeutic effect of individually or simultaneously blocking IL-6 and IL-1 cytokines using tocilizumab, canakinumab, siltuximab, and anakinra in patients with severe COVID-19. In a phase 3 clinical study, anakinra reduced mortality and the need for supplemental oxygen therapy (mechanical ventilation) in critically ill patients with COVID-19, thereby pointing to the need for further controlled studies (Huet et al. 2020). While these antibodies block the release of pro-inflammatory cytokines IL-6 and IL-1, they do not affect the secretion of other cytokines such as IL-18 and TNF; hence, they may be unsuccessful in breaking the vicious cycle of inflammation and tissue damage. Other synergistic or more broadly acting strategies may be needed.

HSP27 and NLRP3 inflammasome activation

Growing evidence suggests that HSPs play an important role in regulating the NLRP3 inflammasome activation. Although many HSPs act as alarmins and promote inflammation, HSP27 and HSP70 have been shown to inhibit activation, supporting their role as modulators of inflammation (Batulan et al. 2016; Martine and Rebe 2019). Treatment of macrophages with exogenous recombinant HSP27 reduces the uptake of modified low-density lipoprotein (LDL), lowers IL-1β levels, and increases levels of an anti-inflammatory cytokine, IL-10 (Miller-Graziano et al. 2008; Rayner et al. 2008). Additionally, extracellular recombinant HSP27 inhibits modified LDL uptake by competing for scavenger receptors, SR-A and CD36 (Shi et al. 2020). Scavenger receptors bind modified LDL and intiate both signals 1 and 2 for complete NLRP3 inflammasome activation. Thus, the competition between HSP27 and scavenger receptors might down-regulate inflammation by preventing lysosomal disruption and subequent inflammasome activation (Duewell et al. 2010; Sheedy et al. 2013). Similarly, in a rat model of skeletal muscle disuse atrophy, prophylactic application of HSP27 attenuated NF-κB activation and skeletal muscle disuse atrophy (Dodd et al. 2009), possibly by inhibiting the priming step of inflammasome activation. These studies support the concept that extracellular HSP27 (or perhaps HSP27 ICAST) competes for the receptors that recognize the danger signals and targets pathways upstream of NF-κB and the inflammasome to dampen the cytokine storm and prevent the tissue damage characteristic of critically ill COVID-19 patients. Further study of the anti-inflammatory potential of HSP27 immuno-therapeutics for COVID-19 and other inflammatory disease states is ongoing, with a plan of moving from the bench to the clinic soon.

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