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Review Article

Suppressed anti-inflammatory heat shock response in high-risk COVID-19 patients: lessons from basic research (inclusive bats), light on conceivable therapies

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The major risk factors to fatal outcome in COVID-19 patients, i.e., elderliness and pre-existing metabolic and cardiovascular diseases (CVD), share in common the characteristic of being chronic degenerative diseases of inflammatory nature associated with defective heat shock response (HSR). The molecular components of the HSR, the principal metabolic pathway leading to the physiological resolution of inflammation, is an anti-inflammatory biochemical pathway that involves molecular chaperones of the heat shock protein (HSP) family during homeostasis-threatening stressful situations (e.g., thermal, oxidative and metabolic stresses). The entry of SARS coronaviruses in target cells, on the other hand, aggravates the already-jeopardized HSR of this specific group of patients. In addition, cellular counterattack against virus involves interferon (IFN)-mediated inflammatory responses. Therefore, individuals with impaired HSR cannot resolve virus-induced inflammatory burst physiologically, being susceptible to exacerbated forms of inflammation, which leads to a fatal "cytokine storm". Interestingly, some species of bats that are natural reservoirs of zoonotic viruses, including SARS-CoV-2, possess an IFN-based antiviral inflammatory response perpetually activated but do not show any sign of disease or cytokine storm. This is possible because bats present a constitutive HSR that is by far (hundreds of times) more intense and rapid than that of human, being associated with a high core temperature. Similarly in humans, fever is a physiological inducer of HSR while antipyretics, which block the initial phase of inflammation, impair the resolution phase of inflammation through the HSR. These findings offer a rationale for the reevaluation of patient care and fever reduction in SARS, including COVID-19.

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The pandemic and risk of fatal outcome by COVID-19

At the end of 2019, the Wuhan Municipal Health Commission noticed a group of patients with pneumonia of ¨unknown cause" linked to a seafood wholesale market in Wuhan, Hubei Province, China [1]. Bronchoalveolar-lavage samples collected in Wuhan Jinyintan Hospital on December 30, 2019 were analyzed and the etiological agent responsible for the severe acute respiratory syndrome (SARS) observed in the patients was consistent with an RNA virus of the Coronaviridae family [2]. Interestingly, sequencing the genome of this previously unknown betacoronavirus (β-CoV) showed 85 % identity with that of

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a bat SARS-like coronavirus bat-SL-CoVZC45 (MG772933.1) and an overall genome sequence identity of 96.2 % to the bat CoV RATG13 [3]. This novel CoV was named SARS-CoV-2 virus and the WHO thereafter named the novel infectious pneumonia ¨coronavirus disease 2019" or COVID-19 [4].

COVID-19 has proven to be highly contagious. Just to illustrate, by January 21, 2020, it were 278 cases restrict solely to China [5], which, in a period as short as 4 weeks, became a 44,730-case epidemic with 1114 deaths in China and 441 cases outside of China [6]. By March 11, 2020, WHO totalized 118,319 confirmed cases and 4,292 deaths globally and declared COVID-2019 a pandemic [7] that quickly spread out around the world reaching 750,890 cases globally (140,640 in U.S.A.) by March 31, 2020 with 36,405 deaths, being 57,610 new cases and 3,301 new deaths during the previous 24 h period worldwide [8]. Recently, WHO informed a cumulative data of 503,862 deaths being 39,46 deaths during the previous 24 h worldwide by June 30, 2020 [9]. As of July 31, 2020, approximately 17.3 million cases have been confirmed with over 674,000 deaths worldwide: more than 152,000 deceases in U.S.A. and approx 92,000 deaths in Brazil, the latter being the present COVID-19 epicenter in the world [10].

It is remarkable that, even when the outbreak of COVID-19 was whole confined to China, only few of the cases were reported to occur in children, while almost half were observed in adults 60 years of age or older [11,12]. Overall, 31 % of cases, 45 % of hospitalizations, 53 % of intensive care unit (ICU) admissions, and 80 % of deaths associated with COVID-19 were among adults aged 65 years or older with the highest percentage of severe outcomes among persons aged $\geq$85 years [13]. Case-fatality rates among individuals aged 80 years or older are very similar ($\sim$20 %) even when compared populations with disparate features, such as Italy and China [14]. Although children at all ages have been found to be susceptible to COVID-19 [15], severe outcomes and fatalities due to COVID-19 tend to be lower in children than in adults [15,16]. In total, age seems to be the variable showing the highest associated risk of death by COVID-19 with adjusted odds ratio (OR) of at least 18.82 (CI95 %: 7.20–41.55) for those aged 60 years or older [17]. This has been being a reason of perplexity and concern because the most experienced nurses and physicians, which are highly demanded for treating COVID-19 inpatients in U.S.A. (as well as in other parts of the world), are 45 years old or above that age [18].

Intriguingly, a recent age- and sex-corrected meta-analysis based on a genome-wide association study (GWAS) involving 1,980 patients with COVID-19 and severe disease (defined as respiratory failure) at seven hospitals in the Italian and Spanish epicenters of the SARS-CoV-2 pandemic in Europe [19] showed a higher risk of more severe forms of disease among persons with blood group A than among patients with other blood groups (odds ratio, 1.45; 95 % CI, 1.20–1.75; P=1.48 $\times$ 10^{-4}) and a protective effect for blood group O as compared with the other blood groups (odds ratio, 0.65; 95 % CI, 0.53–0.79; P=1.06 $\times$ 10^{-5}). Although the explanations for such finding be unknown as yet, ABO blood groups have also previously been implicated in susceptibility to other SARS, the SARS-CoV-1 infection [20].

It has also been realized [12,21,22] since the first cases reported in China that, besides elderliness, pre-existing comorbidities are strikingly evident in about half of inpatients, hypertension being the most prevalent (30 %), followed by diabetes mellitus (19 %), coronary heart disease (8 %) and 3 % of individuals presenting previous pulmonary condition, such as chronic obstructive lung disease [22]. Similar prevalence was reported in a large cohort study in the region of New York City, which highlights the severity form of COVID-19 in diabetics in comparison with no diabetic subjects [23]. COVID-19 inpatients bearing previous comorbidities were also among those with highest mortality rates, with adjusted OR of 7.42 (95 % CI: 6.33–8.79) for hypertensive patients, 9.03 (95 % CI: 7.39–11.35) for diabetic subjects, 12.83 (95 % CI: 10.27–15.86) for coronary heart disease, while for chronic obstructive lung disease OR is 7.79 (95 % CI: 5.54–10.43) [17].

Finally, it is noteworthy an observation whose prevalence is so impressive as elderliness and comorbidities: the eye-catching discrepancy between males (approximately 60 %) and females at the admission in hospitals. Among non-survivors, this difference is even bigger: approximately 70 % are men [22]. In the Lombardy Region, Italy, these figures are still more impressive: 82 % of the patients admitted in intensive care units (ICU) were older men [24]. While in China such difference has been hypothesized to be related to smoking (higher among men as compared with women), there is yet no concrete association for sex differences encountered in COVID-19 patients and smoking prevalence in men [25,26]. Also, during the peak of the outbreak of Middle East respiratory syndrome (MERS) due to the MERS-CoV in Saudi Arabia between April and May of 2014, ratios were similar: 62 % of the cases were of male sex, mortality rates were higher among men (52 % vs. 23 % in women) along with age-associated hazard, i.e., those older than 60 years were most likely to be infected and die [27]. Almost the same feature was observed during the epidemic of SARS caused by SARS-CoV-1 in China between March and September 2003, when the mortality rates were higher among men than in women [28].

A clue for such disparity between men and women, as well as between young and elderly people comes from mouse studies. C57BL/6 and BALB/c mice infected with lethal doses of mouse-adapted SARS-CoV (MA15) strain show

that young 6-week-old mice, the equivalent of 23-year-old humans [29], are completely resistant to CoV infection but, as mice get older, there is an increasing susceptibility to disease and death [30]. Remarkably, as in SARS-like diseases in humans (e.g., COVID-19), female mice are mostly protected against MA15 CoV even at advanced ages (∼20 months), which is the equivalent of an 81-year-old post-menopausal woman. This sex-specific difference was found to be dependent on estrogen receptor and independent of T- and B-lymphocyte responses [30]. It is intriguing that ovariectomy and estrogen receptor antagonists abolish this protection even in very senescent females, while orchiectomy or anti-androgen treatments do not modify this scenario in male mice at all. Additionally, higher virus titers and increased inflammatory monocytes/macrophages and neutrophil infiltration in the lungs were found to contribute to the disease severity observed in male mice [30].

The above evidence raises questions on the common link between age, sex and the comorbidities that lead to the prevalence of unfavorable outcomes in COVID-19 patients similarly as in other coronavirus diseases. These relevant issues are discussed in the next topics emphasizing conceivable relations with virus-induced inflammation, and the inability of high-risk COVID-19 patients to physiologically resolve inflammation thus avoiding hyperinflammation. The very uncommon pathophysiology of viral infections in bats are also discussed in the last sections. Finally, we suggested novel clinical trials that may help to clarify these main points, as well as to test alternative treatments for COVID-19 patients.

Putative role of ACE2 receptor in cardiovascular disease-bearing patients

SARS-CoV-2, like other coronaviruses, usually employ a receptor-binding domain (RBD) located at their spike (S) proteins to initiate cell infection when binding to host receptors [31], and S protein priming by host cell proteases to complete the invasion [32]. Soon after cloning and analysis of the amino acid sequence of SARS-CoV-2 S-protein structure, molecular docking studies revealed that SARS-CoV-2 RBD S-protein tightly complexes with angiotensin converting enzyme-2 (ACE2) that works as a co-receptor and entry gate of the virus into human cells [33,34], as is the case of its phylogenetically related SARS-CoV-1 [35]. This finding was further confirmed in human cells lacking ACE2 expression in which virus particles cannot enter [3]. Also, SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells [36], which are likely involved in olfactory disturbances and central nervous system (CNS) infection [37], as well as in corneal and intestinal epithelial cells [36]. Moreover, ACE2 mRNA is present in virtually all organs and in arterial and venous endothelial cells, e.g., on lung alveolar epithelial cells, oral mucosa, nasopharynx, stomach, colon, skin, lymph nodes, thymus, bone marrow, spleen, liver, kidney and brain [38].

ACE2, on the other hand, is responsible for generating angiotensin 1–7 (Ang 1–7) from angiotensin II (Ang II), thus counteracting the vasoconstrictor, endothelial and vascular wall remodeling effect of Ang II that can damage the cardiovascular system if chronically elevated in the plasma [39]. Because a considerable proportion of COVID-19 patients are hypertensive or have pre-existing coronary heart disease (above), there was a concern that the use of anti-hypertensive drugs, such as ACE inhibitors (ACEI) or Ang II receptor blockers (ARB), could influence the evolution of COVID-19 in such patients [33,34]. A 12-year follow-up study of patients recovered from SARS-CoV-1 infection found that 68 % of them had hyperlipidemia, 44 % had cardiovascular system abnormalities and 60 % had disorders of glucose metabolism while similar patterns were observed in patients recovered from SARS-MERS-CoV infection [34]. A recent meta-analysis on 700 lung transcriptomes found that ACE2 is expressed at higher levels in patients with COVID-19-associated comorbidities that could be related to epigenomic modifications [40]. In addition and remarkably, Ziegler and co-workers discovered that ACE2 is also a human interferon (IFN)-stimulated gene in human (but not mouse) lung type II pneumocytes, ileal absorptive enterocytes and nasal goblet secretory cells [41]. Since IFN pathways are strongly activated in response to viral infections (detailed below), one cannot rule out the possibility that antiviral IFN-based response in COVID-19 patients could indeed enhance SARS-CoV-2 infection. However, there is yet no clinical or basic-science evidence to suggest that treatment with ACEI or ARB should be discontinued because of the SARS-CoV-2 infection or its severity [33,34]. Moreover, it is estimated that only a tiny fraction of patients with COVID-19, at least in China, have been previously treated with renin–angiotensin–aldosterone system (RAAS) inhibitors [42].

If RAAS inhibitors could increase the chance of SARS-CoV-2 to enter the cells on account of "possibly" enhanced expression of ACE2 receptor, elevated ACE2 expression is, on the other hand, known to be protective to pulmonary and cardiovascular system at all [33,43]. Indeed, the counter-regulatory action of ACE2/Ang 1-7 system is important to preserve ejection fraction impeding heart failure [39], which is a common complication observed in COVID-19 patients. Because of this, the present understanding is that RAAS inhibitor treatments **should not** be added to or removed from at-risk COVID-19 subject therapeutics [44]. Therefore, it remains elusive why patients with pre-existing

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cardiovascular diseases (CVD) are so prone to develop fatal forms of COVID-19. The same is true in relation to diabetes mellitus, although studies on human pancreatic β-cells and liver organoids had shown a particularly high expression of ACE2 that is associated with high permissiveness to SARS-CoV-2 infection [45] and that SARS-CoV-2 infection might induce new-onset diabetes [46].

Chronic suppression of the physiological resolution of inflammation via heat shock proteins (HSPs) due to deficient heat shock response (HSR)

One possibility to explain the connection between age, male sex and the specific pre-existing comorbidities in COVID-19 is a deficiency in HSR, which physiologically resolves inflammation. The lack of a robust HSR is likely to be the underlying reason for the unfavorable prognosis in many (if not all) chronic inflammatory diseases, such as cardiovascular and obesity-related illnesses [47]. The same can be suspected in groups at high-risk for COVID-19. For example, the discrepancy in fatal outcomes between males and females can be associated with a reduced HSR in males, as estrogen is a powerful inducer of HSR [48] and blockade of estrogen receptor signaling abolish protection against SARS-CoV-1 in females [30]. Additionally, although male cardiomyocytes do respond to estrogen and progesterone increasing heat shock factor 1 (HSF1) activation and 70 kDa heat shock proteins (HSP70) expression, they do not have androgen receptors that could promote the same protective effect [49]. That is, males do not have the cardioprotection centered at estrogen-HSR axis (please, see an illustrative summary of the anti-inflammatory HSR in Figure 1). Thence, it is very likely that males with pre-existing comorbidities of inflammatory nature must be at higher risk of further inflammation-related complications. On the other hand, females seem to be constitutively protected against pro-inflammatory senescence-associated cytokines [50], which might render women less susceptible of developing most of the severe forms of COVID-19, as we shall discuss next. Interestingly, although the virus-induced direct cytopathic effects can play a role in the deleterious clinical manifestations of SARS, the exuberant innate immune response against virus is thought to be responsible for the high rates of mortality involving exacerbated inflammatory response [30]. In this regard, depletion of lung-infiltrating inflammatory monocyte/macrophages partially protected mice from lethal SARS [30]. Data obtained from Wuhan patients during the outbreak of 2019 also suggest that COVID-19 mortality can be due to virus-activated "cytokine storm syndrome (a.k.a. cytokine release syndrome)" as well as fulminant myocarditis [21].

"Cytokine storm" is a life-threatening situation that can occur even in young subjects in the absence of contaminating pathogens, endotoxin or any underlying disease [51]. Importantly, cytokine storm was found to be primary triggering factor of mortality in SARS-CoV-1 epidemic between 2002 and 2003 [52]. Cytokine storm is again common in patients with COVID-19, and elevated serum levels of interleukin 6 (IL-6) as well of IL-6-elicited C-reactive protein (CRP) correlate with respiratory failure, acute respiratory distress syndrome (ARDS) and poor clinical outcomes [53]. In line with rapid and explosive inflammatory syndrome is the fact that 100 % of COVID-19 non-survivors has had sepsis [22].

Inflammation associated with a cytokine storm, particularly if virus-induced and IFN-initiated, usually begins at a specific site and spreads throughout the body via the systemic circulation [52]. Cytokine storm is best exemplified by severe lung infections, in which local inflammation spills over into the systemic circulation, producing systemic sepsis, as defined by persistent hypotension, hyper- or hypothermia, leukocytosis or leukopenia, and often thrombocytopenia [52]. Additionally, recent findings from necropsy samples of COVID-19 patients whose death involved SARS as a result of a cytokine storm showed the involvement of neutrophil extracellular traps (NETs) [54]. That is, in the lungs, NETs drive the accumulation of mucus in cystic fibrosis patients' airways whereas NETs in the vascular system drive atherosclerosis and aortic aneurysms, as well as thrombosis (particularly microthrombosis), with devastating effects on organ function [54]. From thence, it would be expected that individuals with pre-existing complications in resolving inflammation could be more susceptible to pro-inflammatory cytokine storm.

Heat Shock Response (HSR), fever and the physiological resolution of inflammation

Inflammation was selected during the evolution of animals to be a rapid and self-resolving response that protects the whole organism against pathogens and stimulates the repair of injured tissues, whether after sterile or pathogen-elicited insults. Accordingly, after detection of such injuries, cells of the innate immune system are recruited within minutes (neutrophils) to hours (monocytes/macrophages) to the site of injury/invasion. In response to such stimuli, a finely orchestrated expression of inducible proteins centered at nuclear transcription factors of

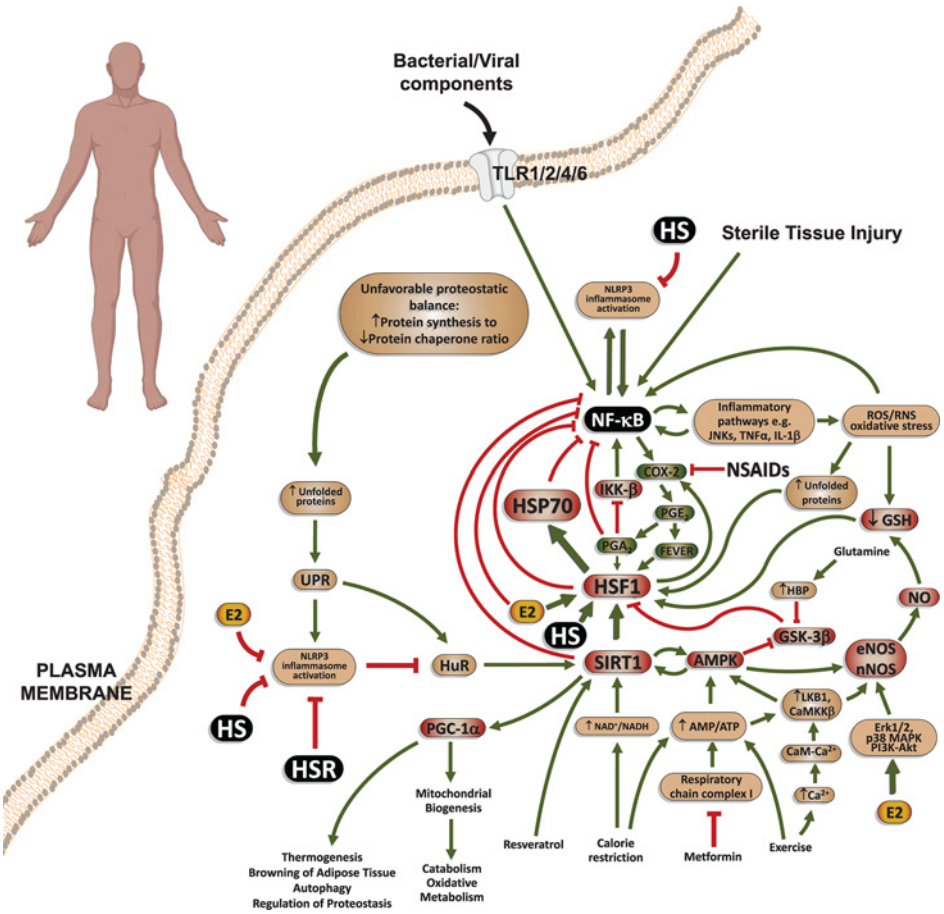

Figure 1. The anti-inflammatory branch of the heat shock response (HSR) biochemical pathway

HSR is a biochemical route mainly centered at the heat shock transcription factor-1 (HSF1) that leads to massive production of the 70 kDa family of heat shock proteins (HSP70). HSF1 may be directly activated by PGE_2-induced rise in temperature (fever), by heat shock (HS), by estrogen (E2) and by the antiviral cyclopentenone prostaglandins (cyPGs), such as the PGE_2-derivative PGA_2, whose physiological production is enhanced at late stages of inflammation. At this time during an inflammatory response (approximately 48 h after stimuli), a second-wave cyclo-oxygenase-2 (COX-2) expression takes place and the production of pro-inflammatory PGs is replaced with that of cyPGs. Since various components of the HSR, including HSP70 and HSF1, both directly and indirectly blocks the activation and transcribing activity of NF-κB, which is the master inflammatory nuclear factor, HSR is anti-inflammatory *per se*. On the other hand, the use of antipyretics/nonsteroidal anti-inflammatory drugs (NSAIDs), while alleviating inflammation-related discomfort, severely blunts the resolution phase of inflammation via HSR. Of note, HSF1 depresses the production of pro-inflammatory cytokines (e.g. IL-6, IL-1β, TNFα) at gene regulatory level (not shown herein). Conformational changes in unfolded proteins can be relied to HSF1 either directly (e.g. following oxidative stress and the formation of reactive oxygen and nitrogen species, ROS/RNS), via alterations in glutathione (GSH)/protein sulphydryl redox status or, finally, after the activation of the unfolded protein response (UPR) protocol that activates HSF1 through sirtuin-1 (SIRT1) route. This branch of the HSR takes place through UPR-mediated activation of HuR (also known as ELAV1), an RNA-binding protein that stabilizes SIRT1 mRNA and, consequently, its expression. SIRT1 downstream signals can also be conveyed to the HSR pathway via metabolic alterations (e.g. ↑NAD^+/NADH or ↑AMP/ATP ratios) and the consequent activation of 5′-AMP kinase (AMPK). Glycogen synthase kinase-3β (GSK-3β), which constitutively inhibits HSF1 and the HSR, may be inhibited by AMPK and glutamine (via the hexosamine biochemical pathway, HBP), thus increasing anti-inflammatory HSR under energy-restriction situations, exercise or pharmacologically via the antidiabetic drug metformin. As HSP70 are protein chaperones that impede the formation of protein aggregates, the HSR is critical to avoid chronic activation of NLRP3 inflammasome and, consequently, low-grade chronic inflammatory diseases. Other abbreviations: NF-κB, nuclear factor transcription factors of the kappa light chain enhancer of activated B cells (κB) family; NLRP3 inflammasome, NLR [nucleotide-binding oligomerization domain (NOD)-leucine-rich repeat- and pyrin-domain (LRP) containing protein]-3 inflammasome; NOS, nitric oxide synthase; PGC-1α, peroxisome proliferator-activated receptor-1 alpha subtype; TLR, Toll-like receptors. Green arrows indicate direct stimulatory pathways while red lines indicate inhibition. Thicknesses of lines indicate their relative degree of stimulation/inhibition as compared to the other pathways.

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the kappa light chain enhancer of activated B cells (κB) family (NF-κB) drives inflammation during the initial phase [55] at the same time that arms its resolution approximately 48 h later [47,56,57]. One of these inducible proteins is cyclooxygenase-2 (COX-2), responsible for the production of proinflammatory arachidonic acid-derived prostaglandins (PGs) as well as other lipid mediators and vasoactive compounds that increase vascular permeability and allow the arrival and activation of inflammatory cells and tissue repair [58].

Depending on the nature of injuring stimuli and the amount of cytokines (e.g., interleukin 1 beta, IL-1β) systemically produced during this phase (approximately 2 h after the stimuli), COX-2-derived prostaglandin E_2 (PGE_2) induces fever (Figure 1) by blocking the processing of thermosensory information at the preoptic area in the anterior hypothalamus, which leads to the activation of coordinated sympathetic/parasympathetic heat-sparing mechanisms to elevate core temperature [48]. The rise in core temperature of approximately 2–3 °C initiates the HSR [59], which is an anti-inflammatory program mainly centered in heat shock factor-1 (HSF1)-dependent expression of HSPs and other anti-aggregative protein chaperones [60,61]. This avoids protein denaturation that otherwise would be imposed to the organism due to "supraphysiological" temperatures. Structural changes in the plasma membrane during the establishment of fever also participates in HSF1 activation [62].

HSF1 activation not only drives transcription of HSPs but also regulates expression of pivotal cytokines and early response genes [63] in a way that provides a reciprocal regulation of inflammatory cytokine expression by HSF1 and of anti-inflammatory HSPs by cytokine transcription factors [47]. Moreover, heat-activated HSF1 directly controls COX-2 transcription thus allowing for high throughput PGE_2 production (Figure 1) during the mounting of inflammation [64]. At a later phase, however, HSR potently resolves acute inflammation by shutting off nuclear factor κB (NF-κB) and other downstream pro-inflammatory signals [47,56,57].

It is noticeable that, along the course of an inflammatory response, the same fever-inducing PGE_2 may undergo dehydration into PGA_2, an α,β-unsaturated cyclopentenone PG (cyPG), which is highly electrophilic and strong activator of HSF1 and, consequently, of the HSR [65]. In addition, 48 h after inflammation-eliciting stimuli, there is a second peak of COX-2 expression, which is now 350 % greater than that one at 2 h, although associated with minimum PGE_2 production [56]. At late stages, the production of PGE_2 is replaced with that of cyPGs, which are anti-inflammatory *per se* by virtue of directly inhibiting NF-κB activation [66] thus assisting in the physiological resolution of inflammation (Figure 1). Also, the HSP70 produced in response to HSF1 activation associates with the complex formed by NF-κB and its inhibitor (IκB) to impede NF-κB translocation into the nucleus [67]. Thence, the HSP70 family proteins (i.e., HSP72, HSP73, HSP78) are anti-inflammatory chaperones, and the pharmacological exploitation of HSP70-inducing anti-inflammatory cyPGs has proven to ameliorate a series of inflammatory conditions [68,69]. As a consequence, at the beginning of an inflammatory response, both selective COX-2 inhibitors (COXIBs) and the traditional dual COX-1/COX-2 nonsteroidal anti-inflammatory drugs (NSAID) are able to inhibit the progress of this early phase (Figure 1). However, such inhibitors strongly exacerbate inflammation at late stages by preventing the resolution phase of inflammation [57]. This scenario tends to perpetuate the inflammatory state and aggravate severe acute inflammatory reactions that cannot thus be resolved physiologically. As a corollary, fever, which is the major physiological inducer of the HSR, is anti-inflammatory in its very nature and pharmacologically suppressing fever with NSAIDs may be very inappropriate in some acute inflammations.

Fever (in its "broad sense") may have evolved in modern animals about 600 million years ago [59] and is not a privilege of homeothermic mammals and birds, as many poikilothermic animals, including lower vertebrates, arthropods, and annelids, also increase their core temperature in response to infection or injury [63]. Nevertheless, generating fever is a complex response and is very costly in metabolic terms. Accordingly, in humans, fever may require a 6-fold rise in metabolic rate, while it is estimated that maintaining core temperature at febrile levels demands about a 12 % rise in metabolic rate per 1 °C increase [59]. However, this simplifying approach does not take into account the possible benefits of fever. If fever (or maintenance of hyperthermia) can be closely monitored in a way that does not lead to deleterious conditions (associated with low HSR capacity) or heart imperilment, fever may be the last resource and the natural therapeutic tool capable of blocking exacerbated cytokine production in critically septic patients.

The use of antipyretics in ICU is controversial, but fever is commonly combated with antipyretics in critically ill patients [70–72]. Indeed, treatment with NSAIDs or acetaminophen independently increase 28-day mortality for septic patients (adjusted odds ratio: NSAIDs: 2.61, P=0.028; acetaminophen: 2.05, P=0.01), but not for non-septic patients [71]. Indeed, the absence of a robust febrile response may be associated with greater risk of mortality in patients with bacteremia [72]. It has long been recognized that heat-induced HSR conspicuously reduces mortality rate and organ damage in sepsis-induced acute lung injury in rats [73]. A meta-analysis of a systematic review has shown that, at least in experimental animals (humans' studies have not reached inclusion criteria), treatment with aspirin, paracetamol and diclofenac increases the risk of mortality in influenza infections [74]. Importantly, as asserted

by Oxford COVID-19 Evidence Service [75], current evidence does not support routine antipyretic administration to treat fever in acute respiratory infections and COVID-19.

In COVID-19 patients of Wuhan, fever was not correlated with fatal outcome (P=0.94), as 94 % of all incoming subjects (180 out of 191) had fever (temperature $\geq$ 37.3 °C) while 74 % of patients that evolved to death (51 out of 69) and 94 % (129 out of 137) of survivors also had fever [22]. However, no one of the recent studies on COVID-19 patients, as of July 2020, provided detailed information about the use of antipyretics, supposedly employed in the large majority of inpatients arriving at the hospitals. To the best of our knowledge, only one paper [76] published online May 25, 2020 raised the possibility that over-the-counter use of ibuprofen as an antipyretic for COVID-19 could worsen disease outcome because fever is antiviral. If so, which would be the outcome of COVID-19 patients having the appropriate care but not antipyretic therapy? This is particularly relevant because not only hyperthermia could be beneficial for being antiviral, but also because the vast majority of severe COVID-19 patients present chronic diseases of inflammatory nature. In other words, if the inpatient faces difficulties to physiologically resolve background chronic inflammation, then anti-inflammatory medicines would worsen the situation. While there is no controlled clinical trial to definitely warrant the use of antipyretics in humans, the mainstream thought that "fever is noxious" [77] continues to be prevalent.

As stated above, one of the main causes of fatal outcome in SARS-CoV infections is virus-induced cytokine storm [52,78]. HSP70, in contrast, is a fundamental anti-inflammatory chaperone of the HSR that may inhibit cytokine storm induced by SARS viruses through binding/degrading p65 subunit of NF-κB [79], which is needed for a complete inflammatory response and, eventually, a cytokine storm. On the other hand, Chionh and colleagues [80] have shown that a huge basal expression of HSPs in bats (discussed in detail below) is associated with cell survival to prolonged heat treatment and defense against oxidative stress. From the other point of view, bats are capable of mounting a perpetually activated IFN response [81] without suffering from any hyperinflammation-induced cytokine storm. In support for a protective role of the HSR against hyperinflammation in viral infection is the fact that IFN-γ is able of enhancing HSP70 production and export from cells [82]. Moreover, a recent preprint [83] with the analysis of dataset of patients with SARS during the 2003 outbreak in a platform to conduct drug repositioning suggested that 90 kDa heat shock proteins (HSP90) inhibitors, mainly geldanamycin and its derivatives, could be useful in treating COVID-19 patients. This observation might appear to be in contrast with the vast amount of literature describing inhibition of virus replication under conditions where HSPs synthesis is enhanced. This contradiction, however, is only apparent because geldanamycin and other HSP90 inhibitors can activate an HSF1-dependent HSR and their administration leads to increased levels of HSP70 as well as HSP90 itself [84]. Therefore, inducers of the HSR, whether physiological (e.g., fever, hot tub) or pharmacological (e.g., cyPGs), are envisaged to counterbalance COVID-19 cytokine storm by imposing a physiological resolution of inflammation, as previously reported for other viruses [84]. Unfortunately, there has been no controlled randomized clinical trial (RCT) addressing the HSR in persons affected by MERS-CoV, SARS-CoV-1 or SARS-CoV-2 as yet.

Particularly in the case of COVID-19 patients who are susceptible to develop a severe lung-born systemic inflammation, it would be important that a placebo-controlled clinical trial (blinded at least to data collectors, outcome adjudicators and/or data analysts) could be conducted to check the validity of antipyretic therapy soon after SARS-CoV-2-positive patient present the first signals of COVID-19, including fever. It is plausible that COVID-19 patients not undergoing antipyretic therapy might have a better response due to a preserved HSR than the opposite. Indeed, the same might be predicted for the less severe forms of this disease affecting the general population.

Cellular senescence and suppression of HSR in chronic-degenerative diseases of low-grade inflammatory nature

Although some patients were reportedly healthy before presenting COVID-19 severe symptoms, the first COVID-19 cases reported in China [21,22] described the high incidence of comorbidities found in patients admitted in hospitals (approximately 50%) as well as the great mortality rates among those bearing such comorbidities (approximately 70 %). Pre-existing CVD and diabetes mellitus were the most frequent findings. Obesity-related diseases would account for the differences in mortality between China and Italy, because, in the latter, the prevalence of older and obese adults is higher [85]. Severe obesity and diabetes mellitus are recurrently found as being related to enhanced fatality in all types of SARS caused by coronaviruses [86]. In common, these comorbidities share chronic inflammation underlying their physiopathology [47]. This point deserves further details aiming to understand the occurrence of lesions in high-risk COVID-19 patients and the potential role of HSR.

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In this regard, all chronic degenerative diseases of low-grade inflammatory are characterized by a conspicuous suppression of the HSR. In fact, the HSF1-HSPs axis, which is crucial for the establishment of an anti-inflammatory HSR, is progressively blunted due to a gradual suppression of the expressions of both HSP70 and HSF1 in metabolic tissues (Figure 2). In diabetes mellitus and obesity, decreased HSF1 and HSP70 expression is always observed in the adipose tissue [87], liver [87], skeletal muscle [88,89] and vascular beds of patients [90,91]. This drawback is also found in older adults presenting neurodegenerative diseases [92] and in menopause-related metabolic dysfunctions [48,93]. The same can be encountered in rodent models of obesity and insulin resistance [94–96]. In these cases, physiological, pharmacological or transgenic induction of HSP70 reverts the insulin resistance [47,94].

Suppression of the HSR pathway is strongly correlated with the degree of enhancement of c-Jun N-terminal kinase (JNK1) and JNK2 expression in adipose tissue [47,87]. This is followed by similar rises in the amount of Thr183/Tyr185-diphosphorylated, which are the activated and pro-inflammatory forms of JNKs (p-JNK1 and p-JNK2). Stress-induced HSP70 inhibits JNK-dependent pro-inflammatory signal transduction [97] so that weakened HSR makes it difficult to resolving chronic inflammation. When this occurs, how could the suppression of the HSR in these chronic diseases be explained? Cellular senescence and senescence-associated secretory phenotype (SASP) might be the answer to this issue.

When the organism faces a positive energy imbalance (e.g., obesity and type 2 diabetes mellitus) and/or continuous lipid input and turbulent shear stress (e.g., in atherosclerotic CVD and hypertension), there is an overburden of utilization of the cellular endoplasmic reticulum (ER) and ER stress [98–105]. Continued ER stress was found to play a causal role in the development of insulin resistance, nonalcoholic fatty liver disease (NAFLD) and pancreatic β-cell dysfunction [102]. Since ER stress is not resolved because noxious stimuli do not disappear (Figure 2), as is the case of inappropriate diet, low physical activity and uncontrolled hypertension [48], ER stress evolves to the unfolded protein response (UPR) [106]. This is a cellular strategy to stop cellular functions while increasing chaperone synthesis and to avoid the accumulation of protein aggregates with the consequent apoptotic cell death and tissue injury [106]. However, UPR activates various potentially pro-inflammatory pathways so that, if the ER stress-triggering factors are not removed, UPR becomes indefinitely inflammatory [48]. Under an endless UPR, cells continuously signal to the activation of the nucleotide-binding oligomerization domain leucine-rich repeat- and pyrin domain-containing protein-3 (NLRP3) inflammasome, which mediates the cleavage of inactive procaspase-1 and pro-interleukins into their active forms [107]. This activation, alongside of oxidative stress and the consequent DNA damage, gives rise to SASP and cellular senescence [47,48]. SASP comprises a set of pro-inflammatory factors, including interleukins (e.g., IL1β, IL6, IL8, IL18), which can interfere locally in tissue function thus spreading inflammatory signals systemically [47,108].

One of the consequences of persistent NLRP3 inflammasome activation is the NLRP3-dependent caspase-1-mediated cleavage of an RNA-binding protein (HuR/ELAVL1) that is responsible for maintaining high intracellular expression and activity of HSF1 via the sirtuin-1 (SIRT1) route [47,48]. SIRT1 enhances both the expression and the transcribing activity of HSF1, thus supporting a robust HSR [47,109,110]. That is, long-term increased NLRP3 and SASP gradually suppress the HSR, disseminating cellular senescence and low-grade inflammation throughout the body (Figure 2). This condition is dramatically aggravated by the presence of viruses that trigger NLRP3 inflammasome activation, which is the case of some coronaviruses [111]. Moreover, doxorubicin, a pro-senescent DNA damage-inducing drug employed in cancer therapy, increases the expression of chemokines in adipose tissue, promotes infiltration of pro-inflammatory macrophages and neutrophils, as well as induces adipocyte insulin resistance [112]. This may provide another connection between cancer-bearing COVID-19 patients and mortality due to cytokine storm in a condition of depressed HSR.

Senescent cell accumulation is found in a variety of dysfunctional conditions, such as age-related chronic diseases, oxidative stress, changes in hormonal milieu and developmental factors, chronic viral infection, some cancer chemotherapeutics, HIV protease inhibitors and exposure to ionizing radiation [113]. Effects are dual. Cellular senescence is an alternative response to the UPR that avoids apoptosis [47]. On the other hand, it leads to an irreversible growth arrest and resistance to the elimination of such cells by apoptosis in response to cell stress [114–116].

Senescent cells have up-regulated pro-survival pathways, which protect them from their own pro-apoptotic SASP [116,117]. Because they are resistant to apoptosis, senescent cells perpetuate inflammation-related cell dysfunction and SASP [47]. Furthermore, SASP observed in radiation-induced senescent preadipocytes resembles that one of endogenous senescent cells after aging and in idiopathic pulmonary fibrosis [118].

In obesity, SASP spreads to all the tissues commencing from adipose tissue and then progressively reaching the liver, pancreatic islets of Langerhans, skeletal muscle and blood vessels to arrive at other metabolic tissues and lastly the brain [92]. Senescence of adipose tissue can occur even before insulin resistance and glucose intolerance [112]. In rodents, even a relatively small number of senescent cells can cause long-lasting cellular dysfunction and spread to other

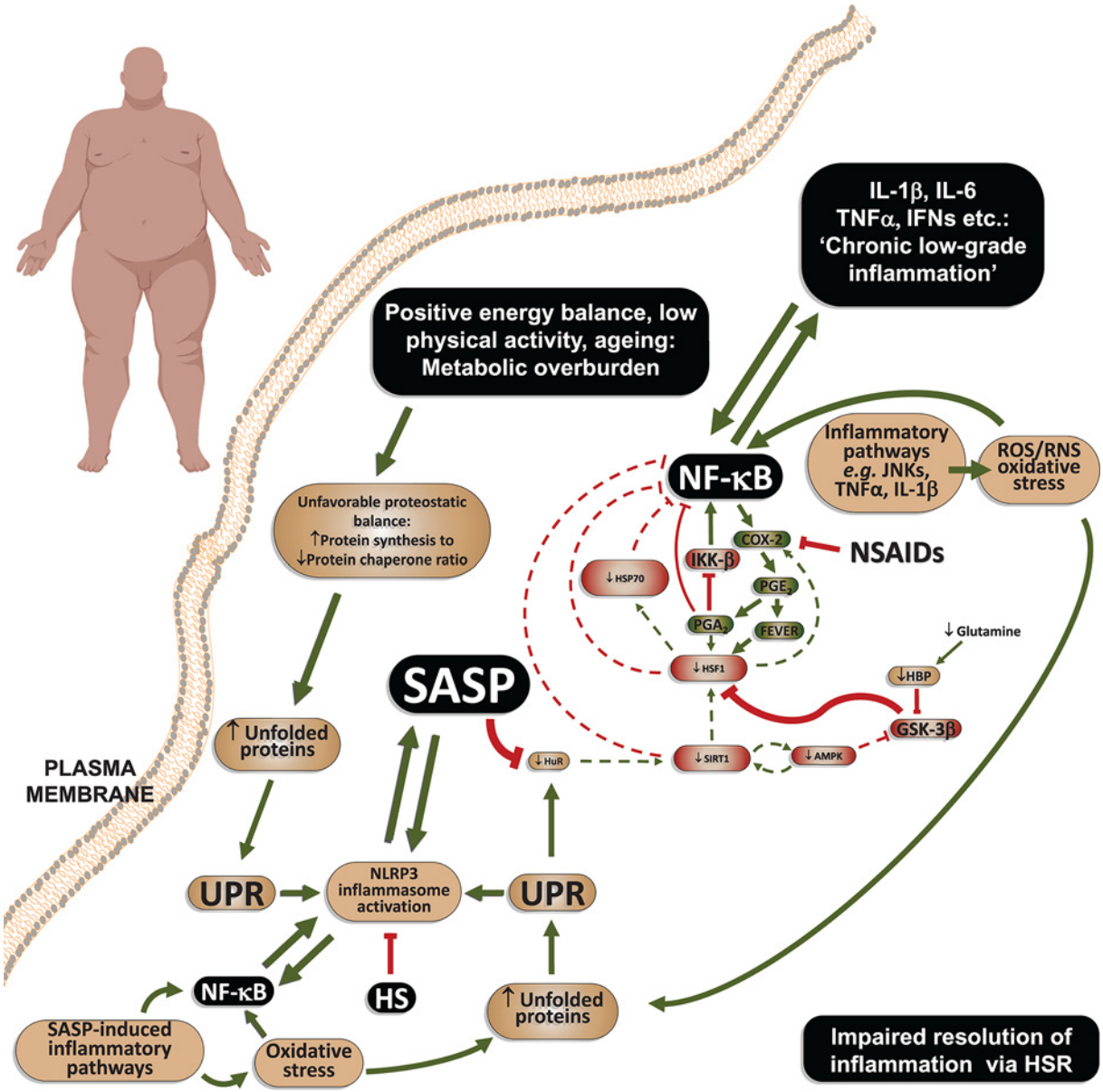

Figure 2. Suppression of HSR in chronic inflammatory diseases

There is a common feature to all chronic degenerative diseases of inflammatory nature (e.g., obesity, type 2 diabetes mellitus, low-estrogen states, neurodegenerative and cardiovascular diseases) that is an unfavorable proteostatic balance caused by excess demand for protein synthesis in the face of low protein chaperone availability. This imbalance is caused by a combination of different factors, including low physical activity, positive energy balance, high plasma levels of lipids, arterial hypertension and ageing. As a consequence, there is accumulation of unfolded proteins in target tissues leading to the unfolded protein response (UPR), a cellular strategy to halt protein synthesis while increasing the expression of protein chaperones, such as HSP70. However, UPR has an inflammatory branch that leads to the activation of NF-κB and NLRP3 inflammasomes in such a way that if injuring stimuli (e.g., hyperlipemia, hypertension) are not removed, NLRP3 inflammasome activation does not cease and inflammation becomes perpetual with massive production of inflammatory cytokines leading to a condition known as senescence-associated secretory phenotype (SASP). On the other hand, SASP leads to the impairment HuR-SIRT1-HSF1 axis so that SASP blocks the HSR and the physiological resolution of inflammation thereby imposing a state of chronic inflammation. Conversely, heat shock (HS) is able to disassemble NLRP3 inflammasomes. Abbreviations and notations are as in the legend of Figure 1.

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tissues [118]. In humans, obesity triggers an early senescence program in adipose tissue-derived mesenchymal stromal/stem cells [119]. Moreover, both aging and diabetes mellitus share ER-stress-dependent senescence-associated dysfunctions such as atherosclerosis, coronary artery disease, insulin resistance and related metabolic anomalies, NAFLD, neurodegenerative diseases, osteoporosis, chronic kidney disease, peripheral vascular disease, periodontitis, impaired immune responses, frailty and sarcopenia [87,113,120,121].

Importantly, accumulation of senescent cells is indistinguishable when compared tissues from an older human with those of a (young or elderly) human bearing chronic inflammatory diseases. Immunosenescence associated with both innate and adaptive immune responses may also play a role in aging and age-related diseases in which immune imbalances are observed [122–124]. Indeed, cellular senescence dampens the HSR needed for the proper function of immune cells [125–128].

In the context of SARS-CoV-2-infected subjects, cellular senescence and SASP were identified in high-risk COVID-19 patients with elevated circulating levels of IL-6 and pre-existing chronic inflammatory diseases [22]. In line with the association between ER stress, UPR and senescent-associated chronic inflammatory comorbidities in COVID-19 patients, there is a decreased expression of TRIB3 (a.k.a. SKIP3) in ACE2-expressing alveolar epithelial cells form the lungs of older men, but not in women [129]. Furthermore, TRIB3 provides a HuR-mediated negative feedback onto NF-κB during UPR [130]. The reduced TRIB3 expression in elderly men might help to explain why these patients are more susceptible to severe forms of COVID-19. In conjunction, these findings would explain, at least in part, the strong association between chronic inflammatory comorbidities and the unpoised immunoinflammatory status in COVID-19 patients (Figure 3) that can eventually lead to death.

Virus-induced ER stress and suppression of HSR

Viruses not only alter host cell metabolism, but they do hijack both cellular signaling pathways and transcriptional machinery thereby controlling them in virus favor [131]. Herpes simplex virus, for example, subverts host NF-κB downstream pathways and directs NF-κB-dependent transcription for viral genes [132]. The control of host protein chaperoning, UPR and translational machinery are powerful tools for the invading virus to manipulate the fate of the host cell [84]. RNA viruses, as the positive-sense single-strand RNA SARS-CoV-2, evolved several strategies to control the translational apparatus of the host cell and usually provoke a dramatic shut-off of host cell protein synthesis [84]. Induction of ER stress is also a common feature of cells infected with different coronaviruses [133], but the way ER stress impacts an UPR to favor virus replication or the host protection depends on the coronavirus.

We do not yet have a complete panorama of how SARS-CoV-2 takes over the host protein machinery. In general, the production of viral proteins by coronaviruses (e.g., SARS-CoV-1, MERS) triggers an ER response that tends to be threatening to the viruses, as UPR evolve to halt protein synthesis in the cell. However, coronaviruses can subvert UPR to overcome translational shutdown ensuring proper viral protein synthesis [134]. For example, SARS-CoV-1 S-protein induces a conspicuous ER stress followed by UPR via PERK pathway to facilitate virus replication [135], while infectious bronchitis coronavirus envelop (E) protein induces an ER stress associated with induction of pro-inflammatory cytokines and enhanced pathogenicity of the virus [136]. Murine hepatitis coronavirus triggers ER stress and modifies UPR to sustain shutdown of host protein synthesis at the same time that it enhances translation of viral proteins [137]. On the other hand, transmissible gastroenteritis virus (an α-coronavirus) induces ER stress and a strong UPR in infected cells, both *in vitro* and *in vivo*, but the PERK arm of UPR triggers NF-κB activation and allows for the production of type I interferons (IFN-α and IFN-β) [133], which are pyrogenic and trigger powerful autocrine as well as paracrine antiviral response [138].

Since the different antiviral strategies of human cells, including those based on IFNs, can trigger a strong inflammatory response, the presence of a previous chronic low-grade inflammatory disease might exacerbate virus-elicited inflammation due to a defective HSR needed to resolve inflammation. This would contribute to the aggravation of the clinical state of COVID-19 patients presenting senescent comorbidities when virus-induced inflammation adds to the pre-existing SASP-mediated chronic inflammation. A potentiated condition like this would lead to an inescapable cytokine storm (Figure 3).

The physiological antisenescent program in women

Female physiology developed a hormonally-mediated antisenescent program different from that of males. For example, SASP components are produced during the phases of pregnancy, including the inflammation-like driven parturition response [50,139]. Nevertheless, this does not influence fetus health or labor and there are no signs of cellular senescence or SASP-related chronic inflammatory disease in females after parturition [50]. On the contrary, cellular senescence plays an important role in placental and fetal development as a beneficial process, ensuring proper

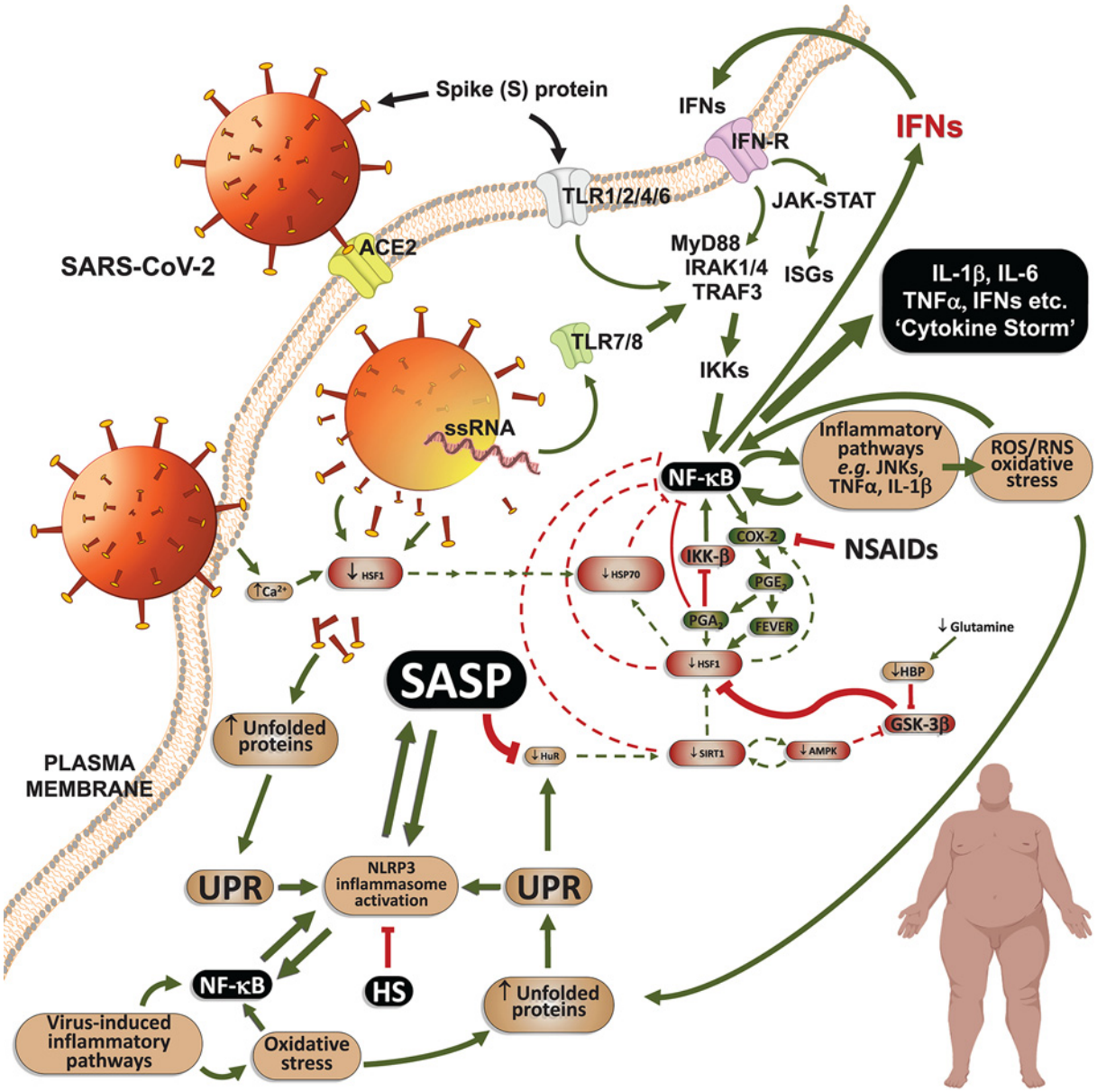

Figure 3. The origin of cytokine storm in high-risk COVID-19 individuals presenting SASP-related low-grade inflammation

Cellular processing of SARS-CoV-2 particles and their components produces an exuberant inflammatory response with several loops of positive feedback routes that exacerbate the production of interferons (IFNs) and other NF-κB-dependent cytokines. Since persons presenting SASP-mediated chronic inflammatory diseases (Figure 2) are unable to resolve inflammation physiologically through the HSR, a life-threatening cytokine storm takes place that may be fatal to this high-risk COVID-19 patients. In this sense, antipyretics, and NSAIDs in general, may worsen the scenario by impeding the resolution of inflammation via HSR. Low bodily levels of glutamine, very common in ICU patients, may aggravate this situation because glutamine-associated hexosamine biochemical pathway (HBP) depress GSK-3β activity that constitutively inhibits the HSR. Abbreviations and notations are as in the legend of Figure 1.

homeostasis during pregnancy [139]. Along the reproductive age, females are protected against chronic inflammation by estrogen antisenescent actions [48]. The estrogenic protective actions rely on the ability to sustain a strong HSR (Figure 1) by interrupting the vicious cycle that decreases HSF1 availability caused by cellular senescence [48]. However, only estrogen cannot explain the disproportionately lower prevalence of severe outcomes related to cytokine storm in reproductively senescent females (including post-menopausal women) in comparison with males,

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as observed in SARS-CoV-infected mice [30] and coronavirus-infected women with MERS [27] or in COVID-19 cases [22]. This indicates an additional and pre-existing defense apparatus against the potentially detrimental effects of chronic proliferative senescence in females and lower prevalence of women among critical COVID-19 patients, as mentioned above. How the female homeostasis opposes cellular senescence and SASP in order to preserve the HSR and avoid chronic inflammatory diseases is an open avenue for further focused research.

Bypassing chronic suppression of HSR and re-arming the resolution of inflammation

Recently, a class of drugs that preferentially target senescent cells ("senolytics") have entered clinical trials. Senolytics selectively eliminate senescent cells by inducing apoptosis [113,117] and aiming to stop the noxious ER stress-SASP-inflammation vicious cycle and re-establishing the physiological resolution of inflammation via HSR. While senescent cells can be dysfunctional and decrease the survival even in young mice, senolytics can enhance the lifespan in old mice [118]. Combinations of FDA-approved senolytic drugs, such as quercetin and azithromycin, were in fact proposed for treating COVID-19 patients [140].

Besides drugs and phytochemicals targeting the anti-apoptotic pathways in senescent cells, anti-senescent antibodies against SASP interleukins (e.g., IL-1β, IL-6) or their receptors (e.g., tocilizumab) have been suggested to improve SASP and to block NF-κB-dependent pro-inflammatory pathways that determine low-grade inflammation [117,141]. Although acute treatment with SASP antagonists was suggested for treating SARS [53], possible consequences and undesirable side-effects of such strategies on immune surveillance have not been completely studied in clinical trials until the moment. Importantly, IL-6 plays a critical role in initiating preliminary responses against virus infection by promoting neutrophil-mediated viral clearance [142].

On the other hand, whole body heat treatment or just the maintenance of core temperature at fever-range levels are also feasible and involves physiological antisenescent HSR-inducing strategies. These approaches can be carried out even in an ICU setting and in critical patients, having the advantage of not interfering in immunoinflammatory balance, as is the case of antibodies against particular pro-inflammatory components [117]. Actually, heat shock inhibits both NLRP3 inflammasome activation and caspase-1 activity in mouse macrophages [143]. HSP70 *per se* inhibits NLRP3 inflammasome activation *in vitro* and in peritonitis *in vivo*, while HSP70 deficiency worsens NLRP3-dependent peritonitis in mice [144]. The HSR role in resolving acute inflammatory response can be linked to the effects of glutamine in critically ill patients [145–147]. Accordingly, glutamine potentializes the HSR via the hexosamine biochemical pathway (HBP) that increases HSF1 transcribing activity and, thus, blocks NF-κB-dependent pathways and prevents acute respiratory distress syndrome (ARDS) following sepsis [145–147]. This would explain the well-known beneficial effects of glutamine for the attenuation of lung injury and for improving survival in septic patients involving the integrated role of HSP70 and the HSR [92,148–150].

Based on these data, we are led to consider that the lack of a vigorous HSR in COVID-19 patients bearing pre-existing SASP-related chronic inflammatory comorbidities tends to be much more endangering for health and related outcomes. As a corollary, heat treatment at fever-range temperature is antisenescent and able of physiologically resolving inflammation, as mentioned above. Since one major hazard for poor outcome in COVID-19 patients is a chronic low-grade inflammation that hampers the resolution of inflammation, heat treatment could induce anti-inflammatory effects taking place even independently of HSF1 levels, which are severely reduced in the tissues of these chronic disease patients [48]. Controlled heat disassembles NLRP3 inflammasome, rescues HuR-SIRT1-HSF1 axis and resumes the resolution of inflammation [143,144]. This explains why chronic heat treatment is effective in relieving atherosclerotic lesions and stopping death in mouse models [151], while improving metabolic status of ovariectomized rats [152] and obese mice with insulin resistance [94]. For this reason, it is intriguing to perceive that, after two decades since the seminal report that 30 min sessions of hot-tub therapy (37.8–41.0 °C) for just 3 weeks is able to reduce plasma glucose and HbA_{1c} levels in diabetic patients [153], no clinical trial was conducted to outline the whole scenario of the HSR in diabetic patients under hyperthermic therapy. This is even more surprising in view that increased frequency of sauna bathing, a common practice in some cultures, is inversely correlated with fatal outcomes in CVD patients as well as with all-cause mortality events [154].

Although the mechanisms involved in the multiple beneficial effects of hyperthermic treatment in chronic inflammatory diseases have not been fully elucidated, it has been hypothesized that elevating body temperature in humans up to about 38–39 °C (by sauna or hot-tub bathing) can exert anti-inflammatory effects due to nitric oxide (NO)-based improvement of endothelial function as well as chronic NO-elicited HSP70 expression [155]. Indeed, the restoration of immunoinflammatory balance through chronic hyperthermic treatment has also been suggested in handling chronic autoimmune diseases without the need of immunosuppressive approaches [156]. The paucity of long-term

studies in humans, however, makes it difficult the establishment of the right protocols for each inflammatory disease [157] and to control for increased side responses, if they occur.

These findings reinforce the necessity of re-evaluation and caution at the time of prescribing antipyretics to COVID-19 patients. On the other hand, passive elevation (or maintenance) of core temperature at fever-range level is a possibility that has never been tried in critically ill patients, particularly in those presenting ARDS and SARS. COVID-19 patients would benefit from anti-inflammatory approaches to avoid inflammation causing "storm" effects. The moment and how to interfere is crucial when taking into account that the use of steroids has delayed coronavirus clearance in MERS-CoV- and SARS-CoV-1-infected patients [158]. Therefore, the effects of passive heat treatment (or fever) should be investigated in randomized controlled clinical trials (RCT) even in the midst of COVID-19 pandemic. Adaptive trial designs, which tend to minimize harm to the participants, could indeed accelerate the evaluation of adjunctive COVID-19 therapies [159].

Antiviral effects of HSR and HSR inducers

The evolution of viruses was forged by sophisticated strategies viruses developed *pari passu* with the mechanisms that cellular organisms have to avoid viruses taking control of protein synthesis and chaperone machinery. In this sense, the components of the HSR might have also evolved functions that serve as antiviral. In fact, cells "created" several ingenious mechanisms to arm an HSR in response to many different clues for the presence of viruses or viral components reaching cells or inside them. As described above, a prompt reaction to the presence of viral components is the triggering of ER stress that progresses toward UPR, which, in turn, halts cellular (and viral) protein synthesis at the same time that augments protein chaperone circuitry, including the anti-inflammatory HSR. Viruses can be detected at the membrane level as well as in all cell compartments. This culminates in NF-κB activation of an inflammatory response that is later headed to an anti-inflammatory status via HSR [84]. Viral components can be detected at the level of Toll-like receptors (TLR) present in plasma membrane (TLR1/2/4/6) or in endosomes (TRL3/7/8/9) and activate IFN production via MyD88-NF-κB pathways [160,161]. Whatever the evolutionary reason, virus infection induces inflammation that mounts the HSR, which, in turn, acts as an anti-inflammatory negative feedback [48]. In other words, viral infection is paralleled by HSR whereas HSR inducers, including fever, are prominently antiviral [84].

Hyperthermic treatment, the most efficient HSR inducer, is effective in protecting humans against common colds and in inhibiting HIV1 transcription in AIDS patients [84]. Indeed, it has long been known that rabies virus-infected mice survive for long time if maintained in an ambient temperature of 35 °C, as compared with the high mortality and morbidity found in animals housed at commonly preconized 20–21 °C ones [162]. The same was described for different virus types *in vitro* in an HSP70-dependent way [163,164]. In addition, fever-range hyperthermia activates a dendritic cell-$CD4^+$ T-cell interacting circuit, which is responsible for maintaining a homeostatic antigen-independent memory in an HSP70-dependent fashion that is centered at T-central memory subset of T lymphocytes [165]. This is absolutely noteworthy because approximately 75 % of COVID-19 patients who died presented lymphocytopenia [22] and more than 80 % of COVID-19 patients presented T-cell exhaustion markers in both $CD4^+$ and $CD8^+$ lymphocytes [166].

While the engraving of immunological memory by the HSR is evident, it is particularly significant in COVID-19 because we do not yet know the capacity of convalescent people to form a long-term immunological memory against SARS-CoV-2. This concern is reinforced by the fact that the titers of antibodies in the plasma of convalescent patients are not always maintained at a high level [167]. Importantly, fever-range hyperthermia reverts the impairment of mitogen stimulation in human mononuclear cells infected with influenza virus [168], thus strengthening the notion that the HSR is able to equilibrate immunoinflammatory status. Indeed the evolutionarily conserved parallelism between virus-induced inflammation and the anti-inflammatory HSR serves to poise viral clearance with tissue protection against escalating inflammatory responses.

Related to heat effects, the cyPGs produced during the resolution phase of inflammation (e.g., PGA_1, PGA_2, PGJ_2, 15-deoxy-$\Delta^{12,14}$-PGJ_2) are also powerful antiviral against several DNA and RNA viruses so far tested, including HIV1 [168–172]. In all these cases, the antiviral effect was found to be dependent not only on cyPG-elicited activation of the HSF1, but also on the HSR that is triggered for full inhibitory actions [173–175]. Inasmuch as many viruses utilize NF-κB-dependent machinery of the host cell to replicate, as is the case of HIV1, cyPGs are antiviral by directly inhibiting NF-κB activation [66,174] and also because HSP70 mediates degradation of p65 subunit of NF-κB [79]. PGD_2, the immediate precursor of J-type cyPGs, is also implicated in the suppression of coronavirus-induced NLRP3 inflammasome activation [111]. Nevertheless, cyPGs have never been tested against SARS-CoV-2 infection.

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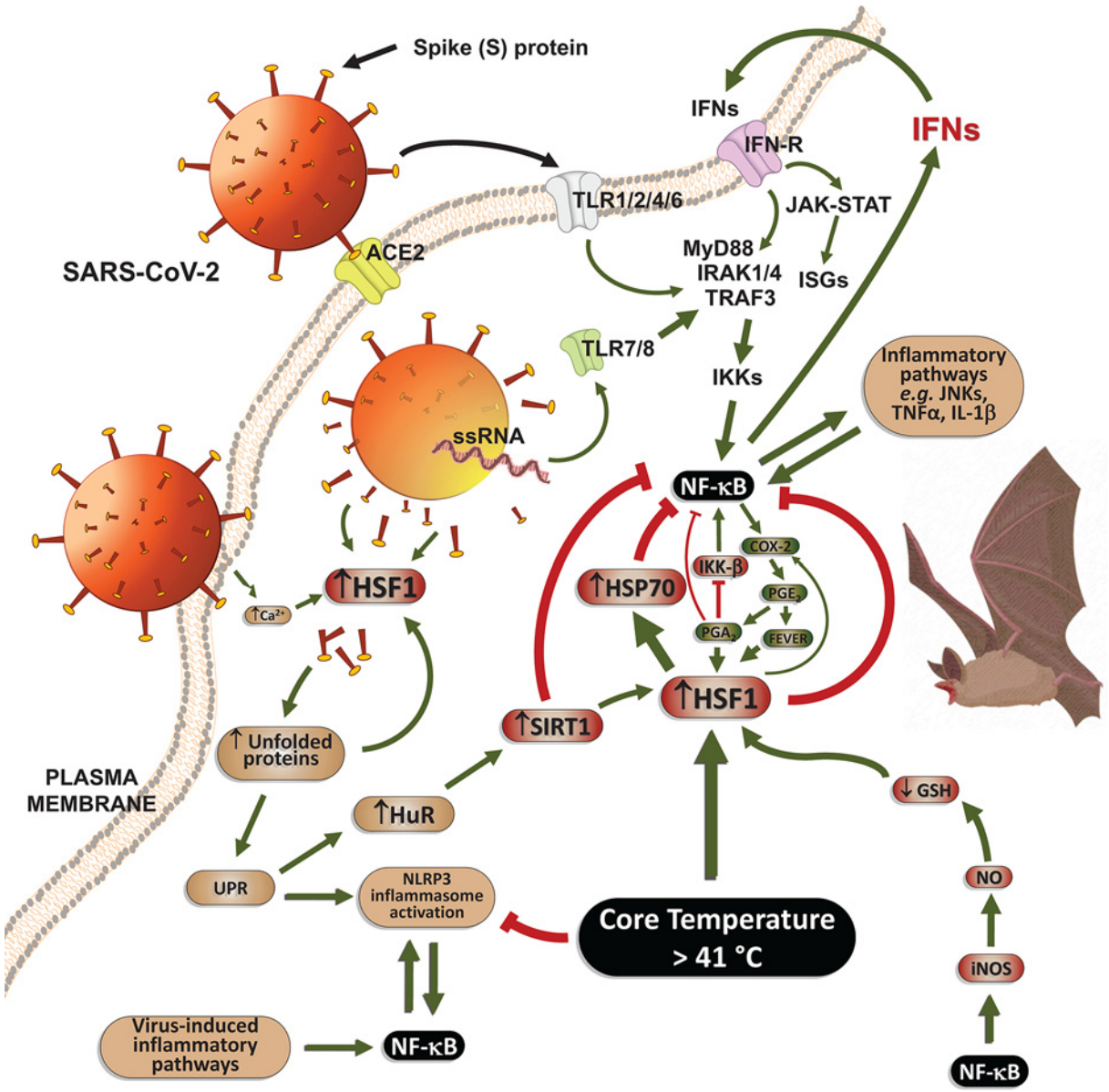

Figure 4. Pro-inflammatory interferon pathways and anti-inflammatory heat shock response (HSR) are constitutively activated in bats

Differently from humans in which virus-induced interferon (IFN) pathways are activated on demand, many bats evolved conspicuous antiviral pro-inflammatory responses including constitutively activated IFN pathways that allow them to deal with the most lethal viruses. At the same time, bats present constitutively high expression of anti-inflammatory HSPs so that these animals do not suffer from cytokine storm because they are able of resolving inflammation through the HSR. Constitutive anti-inflammatory responses of bats are centered at both HSF1-dependent and -independent routes and are chiefly associated with high core temperature of bats that oscillates above 41°C. Abbreviations and notations are as in the legend of Figure 1.

As summarized in Figure 1, cells evolved many forms of activation of the HSR. For example, NF-κB-elicited inflammatory pathways to trigger the production of reactive oxygen and nitrogen species (ROS/RNS), which leads to the depletion of intracellular glutathione (GSH) contents, increases oxidation of protein thiols and the amount of unfolded proteins, thus activating HSF1 and the HSR [61,176,177]. The presence of highly conserved (chemically reactive) cysteines at positions 35 and 105 of HSF1 makes it prone to redox-regulation [176]. This is also the reason why nitric oxide (NO), which is massively produced in response to estrogen via endothelial (encoded by *NOS3*

gene) and neuronal (*NOS1*) NO synthases (NOS) or by the inducible- and NF-κB-dependent iNOS (*NOS2*) during an inflammatory response, is also anti-inflammatory.

As long as NO is a powerful physiological inducer of the HSR [178], NO shows cytoprotective and anti-inflammatory actions. For example, NO refrains tumor necrosis factor-alpha (TNFα) from being cytotoxic to hepatocytes [179]. Studies in rodent models have shown that NO induces rapid accumulation of HSP70 both *in vitro* and *in vivo* [180]. Conversely, whole-body heat treatment of awake rats (41 °C, 15 min) induces NO-dependent HSP70 expression in different organs [181]. Moreover, the associated HSP70 expression and NO production is thought to mediate the adaption of blood pressure to chronic heat treatment to avoid hypotension [182].

NO is a very reactive free radical that has long been recognized as a microbicidal agent produced by inflammatory phagocytes [183]. Besides its effects on bacteria and protozoa, NO is also antiviral and stimulates innate immune response independently of type-I IFNs, although NO and INFs have additive effects on virus infections [183,184]. NF-κB and IFN regulatory factor-1 interact to increase the expression of iNOS and the amount of NO produced [184]. NO is also antiviral by covalently modifying critical cysteines of viral proteases via *S*-nitrosylation [185], and the NO-donor *S*-nitroso-*N*-acetylpenicillamine (SNAP) is an effective antiviral against SARS-CoV-1 [186]. Again, there is no current clinical report in humans assessing the role of NO-mediated HSR in different viral infections, including COVID-19.

The HSP70 related to virus-induced ER stress or NO can potentiate IFN production along the antiviral inflammatory response [187], assisting in virus elimination before the HSR-associated resolution of inflammation. Interestingly, after enough NO had been produced during the course of an inflammatory antiviral response, NO suppresses Th1 cell activity while increasing that of Th2 [183]. This occurs at the same time that anti-inflammatory HSR takes place, thus avoiding IFN-activated NF-κB-dependent superinflammation [79]. In addition, NO regulates the switch between adaptive and apoptotic UPR signaling during ER-stress induced by pro-inflammatory cytokines, including IL-1β, TNFα and IFN-γ [188]. Finally, apart from its HSR-inducing role, NO is anti-aggregating to platelets, which may be critical to COVID-19 patients because half of non-survivors present coagulopathies [22], including disseminated intravascular coagulation that is directly correlated with IL-6 plasma levels [189]. In conclusion, provided that NO-mediated oxidative stress and hemodynamics are maintained under control, its powerful antiviral effects combined with HSR-inducing and antiplatelet activities might be exploited in COVID-19 patients, including those with indication for inhaled NO therapy and pulmonary vasodilation [190], as has already employed to treat SARS [191].

Extracellular-to-intracellular HSP70 ratio and immunoinflammatory status

It is important to consider another relevant point regarding the HSR. The same metabolically stressful situations that trigger the HSR within the intracellular milieu are able to activate the release of exosomes containing HSP70 by non-canonical secretory mechanisms [192–194]. Once secreted, extracellular HSP70 (eHSP70) can bind to Toll-like and other membrane receptors bringing about pro-inflammatory cytokine-like signals toward all tissues for the presence of homeostasis-threatening conditions [128,195,196]. Extracellular HSPs can also be taken up by different cell types [196] and, once in the intracellular space, work as authentic anti-inflammatory chaperones to influence cellular functions, especially in the central nervous system [126,197].

The ability of releasing eHSP70 in response to heat treatment is a measure of HSR integrity [198], and is impaired in aged diabetic people compared with middle-aged or healthy old adults [198]. Elevated levels of eHSP70 are found in the plasma of obese diabetic persons [89] whose excessive eHSP70 is positively correlated with insulin resistance and pancreatic β-cell failure [199]. High plasma levels of eHSP70 are predictors of the development of atherosclerotic disease in hypertensive subjects [200], and increased extracellular HSP60 is associated with enhanced risk of coronary heart disease [201]. High levels eHSP72 are found in the plasma of septic patients and are related with fatal outcome if associated with a concomitant high plasma oxidative profile [202], which probably involves loss of eHSP72 immune signaling property after oxidation [203]. However, a single dose of non-oxidized (native) recombinant mouse eHSP72 attenuates sepsis severity and enhances survival rate in mice, perhaps because eHSP72 may be taken up by immune cells leading to a new immunoinflammatory poise [204]. Otherwise, intracellular HSP72 (iHSP72) expression in monocytes and polymorphonuclear cells are repressed and related to decreased CD14/HLA-DR expression and mortality in critically ill patients with sepsis [205], while anti-inflammatory HSR would be increased by glutamine administration in septic patients [92,150].

There is a consensus on the underlying role of suppressed HSR in chronic low-grade inflammatory diseases [206] associated with disruption in HSP chaperone balance [207]. The immunoinflammatory imbalance favors the excess of pro-inflammatory extracellular HSP70 (eHSP70) compared to the anti-inflammatory intracellular HSP70

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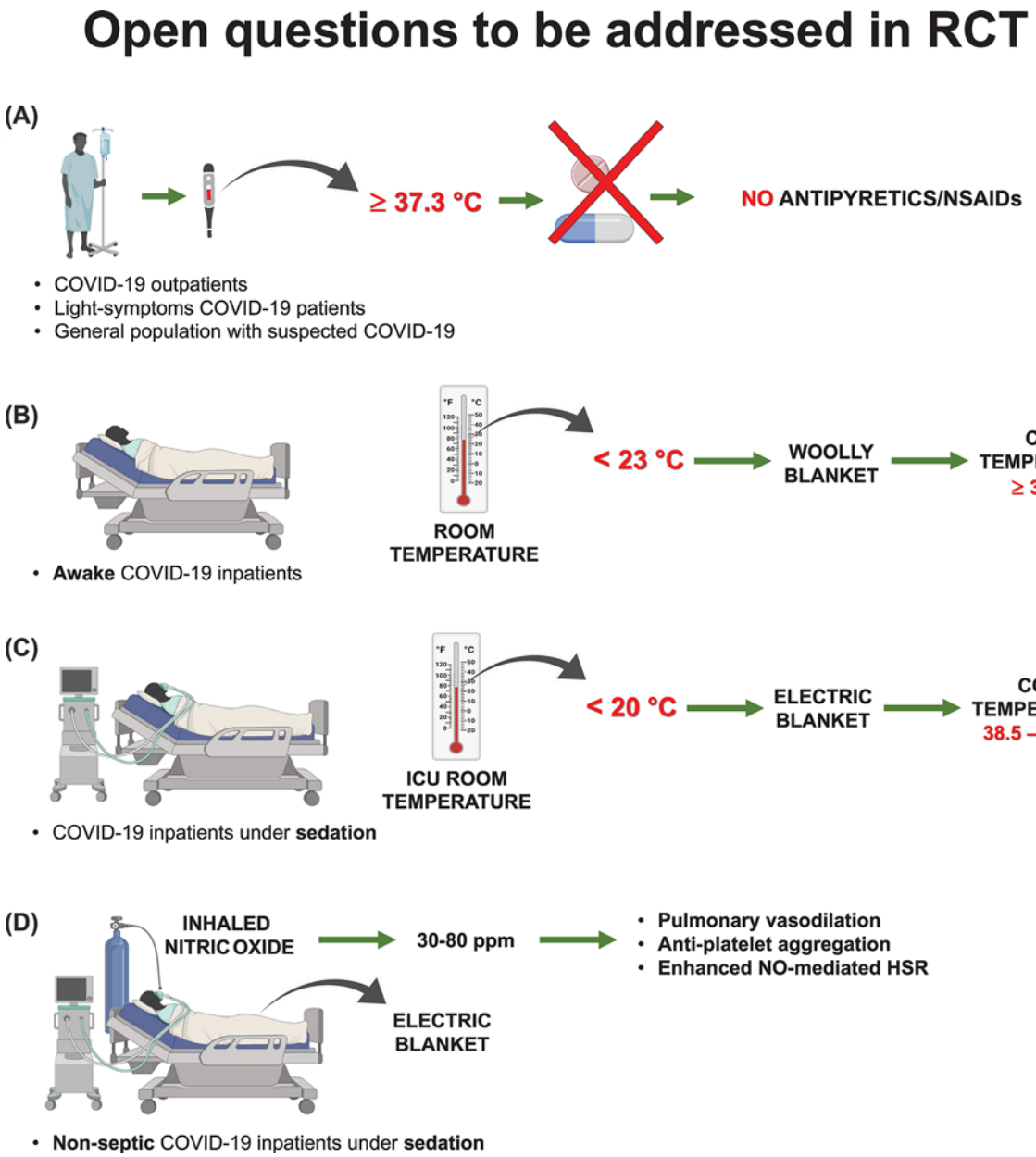

Figure 5. Open questions to be addressed in randomized clinical trials

(**A**) What would happen if antipyretics/anti-inflammatory drugs were initially avoided and patients could be clinically monitored on the evolution of the acute phase of antiviral inflammation? The same arm of this RCT could be subdivided to embrace a study with general population with suspected COVID-19 under or not under antipyretic/NSAID treatment. (**B**) What if heat treatment would be tested for patients together with supportive therapies in next RCTs? (**C**) As ICUs are rarely warm or hot places, would the use of electric blanket (particularly in patients under sedation) be carried out to maintain core temperature at higher values without compromising the metabolic state of the patient or requiring additional respiratory efforts? (**D**) Would NO inhalation therapy be used in those patients without hemodynamic instability since NO is a natural antiviral and strong inducer of the anti-inflammatory HSR, besides being anticoagulant?

(iHSP70) and HSR [128,208–210]. In this regard, the comparison of the ratios between extracellular to intracellular HSP70 (a.k.a. Heck index or H-index of HSP70 ratios) in different clinical conditions provides an index of overall immunoinflammatory balance [128,211] Accordingly, H-index is proportional to IL-2-to-IL-10 ratios and its value is

directly correlated with ultrasensitive C-reactive protein and HOMA-IR values [89, 128]. The H-index can be calculated from results obtained from a small blood sample, provided eHSP70 can be assessed in the plasma and iHSP70 in blood cells, regardless of the technique employed for the measurements [48]. In conjunction, the H-index can be a valuable tool to monitor the evolution of viral infections and alert for severe immunoinflammatory imbalances that can be catastrophic, as in COVID-19 cytokine storm.

Constitutively armed heat shock response: lessons from bats

Bats have recently attracted much attention for being the host reservoirs of highly lethal viral zoonoses, including rabies, Ebola and SARS coronaviruses [212,213], including the COVID-19 etiological agent SARS-CoV-2. This raised the question on how a mammal, not so dissimilar to humans [214,215], is able to support long-term virus infections without signs of apparent disease.

After detection and in order to destroy viruses, human cells employ "on demand" type-I IFNs (*e.g.*, IFN-α, IFN-β, IFN-ε, IFN-κ, IFN-ω) mainly produced by epithelial cells, fibroblasts, monocytes and plasmacytoid dendritic cells [216]. This deflagrates both autocrine and paracrine signals that advise surrounding and immune cells about the presence of viruses [81]. IL-12-activated cytotoxic T cells and Th1 lymphocytes produce type-II IFN-γ to attack virus-infected cells [161]. Humans need a finely tuned immunoinflammatory balance to promote inflammation-based viral clearance at the same time that must quickly initiate the resolution of such inflammatory response to avoid a cytokine storm.

Bats, on the other hand, have a plethora of antiviral defenses that keep the amount of virus in check without facing any signal of inflammation-elicited injury. Some bats have an antiviral immune response named as the *interferon pathway perpetually switched on* [81]. Even in this condition, bats do not suffer from excess cytokine production. As IFN responses are all pro-inflammatory via JAK-STAT and NF-κB pathways [216], the maintenance of a persistent and systemic inflammation would be intolerable to humans and other non-volant mammals. Bats, otherwise, evolved with anti-inflammatory features that protect them from such hyper-vigilant immune responses [81], allowing them to arm exuberant IFN-mediated inflammation devoid of any form of systemic inflammatory syndrome (SIRS) or sepsis (Figure 4).

Analogous to birds [217–219], wing muscles of bats generate a large amount of heat during flight and the bat core temperature can oscillate within 41.1–42.1 °C [220]! Not surprisingly, bats evolved a disproportionately higher thermotolerance when compared to other mammals. Bat (but not bird) cells survive and proliferate in culture at 40 °C for days, an HSP-dependent effect [80]. Indeed, basal expression of HSP70 (*HSPA1A* gene) in bat cells can be at least 10-fold as high as that found in human cells, while the cognate form of HSP70 (*HSPA8* gene) is almost 200 times higher in bat than in human cells [80]. Basal expression of ER chaperone GRP78 (*HSPA5* gene) is more than 400 times more abundant in bat than in human cells, in an HSF1-dependent and -independent fashion. In addition, heat-induced expression of HSP70s is remarkably higher in bat cells than in bird, mouse or human ones. These features might explain the impressive proteostasis, intrinsic resistance to oxidative stress and enhanced longevity of bats (*ca.* 34 years!) as compared to mammals of similar size and surface-to-volume ratios, such as mice that live approximately 2–3 years [80].

Bats have an unequalled HSP-based anti-inflammatory HSR and do not develop chronic degenerative diseases nor cytokine storm [221], regardless of being under constant virus-induced inflammatory response (Figure 4). For such high anti-inflammatory response, bat immune cells have strongly suppressed NLRP3 inflammasome activation in response to TLR ligands and sterile stimuli [221]. The same occurs for different viruses, including MERS coronavirus, without influencing bat cells' ability to defeat viruses [221]. High anti-inflammatory responses also include the finding that HSP70 potentiates antiviral IFN-γ production [187] whereas IFNs regulate HSP expression at two levels: by enhancing the transcription rate of the heat-shock genes and by increasing the stability of mRNA coding for HSPs [222]. IFN-α also enhances cyPG-induced HSP70 synthesis in virus-infected cells [223], and IFN-γ increases the synthesis and release of HSP70 towards the extracellular space via the exosome pathway, where eHSP70 influences naïve dendritic cells [82]. The constitutive HSR provides bats with at least three clear advantages over the other animals: quick proteostatic response upon virus-induced ER stress, strength of INF antiviral activity and rapid resolution of inflammation to avoid tissue damage (Figure 4).

Although being different species, comparative physiology and the similarity of bats' antiviral and anti-inflammatory protective pathways involving HSR can direct some clues on how to avoid or treat cytokine storm in SARS patients. It reinforces the need to reconsider the general, uncontrolled use of antipyretics. Furthermore, it addresses the discussion on the maintenance of patients' body temperature within fever-range levels to amplify the

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anti-inflammatory HSR as an adjunctive care in infected patients, particularly in those with chronic inflammatory comorbidities. These important issues deserve further evaluation.

Concluding remarks and suggestions

Despite many efforts to find effective COVID-19 therapies, including ongoing RCTs with repurposed drugs [224–226], no successful therapy has been shown convincing to date, unfortunately. Particularly for the group of patients who are at higher risk of severe illness, the suppressed HSR associated with previous, chronic inflammatory diseases can be too much for the body to deal with a virus infection and more inflammation. A dual condition can be occurring: a strong inflammatory response is the only resource capable of defeating SARS-CoV-2, but exacerbated inflammation can cause fatal cytokine storm.

While these findings offer a rationale for the reevaluation of SARS patient care and fever reduction in next RTCs, there are many open questions, as depicted in Figure 5. What is the actual percentual of patients that use their natural response to the virus and are able to rebalance the immunoinflammatory status while reaching disease remission? What would happen if anti-inflammatory drugs were initially avoided and patients could be clinically monitored on the evolution of the acute phase of antiviral inflammation? For the general population with suspected COVID-19, should not the over-the-counter use of antipyretics/NSAIDs be discouraged (Figure 5A)? Do (and when) the high-risk patients need anti-inflammatory treatment? Could heat treatment be tested for patients together with supportive therapies in next RCTs?

As the use of corticosteroids in COVID-19 is still controversial [142,224], hyperthermic treatment, whether by maintaining fever or by passive heating could be considered as a feasible procedure. An important concern is that ICUs are rarely warm or hot places so that care should be taken to not allow patient's temperature to drop, especially if the inpatient is to be sedated. Would the use of electric blanket (Figure 5B–D) be done to maintain core temperature at higher values without compromising the metabolic state of the patient or requiring additional respiratory efforts? Would NO inhalation therapy be used in those patients (Figure 5D) without hemodynamic instability since NO is a natural antiviral and strong inducer of the anti-inflammatory HSR, besides being anticoagulant? Moreover, can vasopressor therapy in these patients induce higher HSR considering that α_1-adrenergics are powerful inducers of expression and release of HSP70?

Proper RCTs can answer these relevant issues. Perhaps allowing patients to elaborate a natural and own resolution of inflammation by having high core temperature, instead of trying to avoid it with antipyretics, would be a measure as simple and positive as handwashing heralded by Dr Ignaz Philipp Semmelweis at the end of May 1847 in Vienna in order to eliminate the poisonous '*subter ungues*' cadaveric matter [227]. This may also suggest that the perceived Gordian knot (an intractable problem that may be solved by 'thinking outside the box') of allowing COVID-19 patients to develop inflammation, but resolve it by the HSR without having cytokine storm, especially those bearing chronic inflammatory diseases, may be about to be untied.

"*Quæ medicamenta non sanat, æ ferrum sanat. Quæ ferrum non sanat, æ ignis sanat. Quæ vero ignis non sanat, æ insanabilia existimare oportet.*

That which drugs fail to cure, the scalpel can cure. That which the scalpel fails to cure, heat can cure. If heat cannot cure, it must be determined to be incurable."

(Aphorisms of Hippocrates, by Elias Marks, from the Latin version of Verhoofd, Collins & Co., New York, 1817).

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Author Contribution

All the authors actively contributed to the review of literature and co-wrote the paper giving key inputs. P.I.H.B.J. prepared the figures. The authors had approved submitted and published versions of the manuscript.

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We would like to apologize for not mentioning important primary studies due to space constraints and to allow a better flow of information throughout the text. Therefore, whenever possible, we presented some mechanisms by citing review articles to which the reader is kindly requested to refer. Figure 5 was prepared by using icons from Biorender.com (available at https://app.biorender.com/).

Abbreviations

LRP, leucine-rich repeat- and pyrin-domain; NLRP3, nucleotide-binding oligomerization domain LRP-containing protein type 3; NF-κB, nuclear factor transcription factors of the kappa light chain enhancer of activated B cells (κB) family; NOS, nitric oxide synthase; PGC-1α, peroxisome proliferator-activated receptor-1 alpha subtype; TLR, Toll-like receptors.

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