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COVID-19 and Crosstalk between the Hallmarks of Aging

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Abstract

Within the past several decades, the emergence of new viral diseases with more severe health complications and mortality, primarily in older adults with comorbidities, is evidence of an age-dependent, compromised bodily response to abrupt stress with concomitant reduced immunity. The emergence of new infectious coronaviruses such as SARS-CoV-2 has resulted in the coronavirus disease 2019 (COVID-19). The result is increased morbidity and mortality in persons with underlying chronic diseases and among those with compromised defense mechanisms, regardless of age and among older adults who are more likely to fit these categories. COVID-19 appears to be primarily an upper respiratory disease. While SARS-CoV-2 is highly virulent, there is variability in the severity of the disease and its complications in humans. Severe pneumonia, acute respiratory distress syndrome (ARDS), lung fibrosis, cardiac t complication, acute kidney injury, hospitalization, and high mortality have been reported in older adults with COVID-19, that result from pathogen-host interactions. Here, we review potential interactions of the coronavirus and host cellular responses in relation to hallmarks of aging including genomic instability, telomere attrition, impaired autophagy, mitochondrial dysfunction, innate immunosenescence, inflammation and inflammasomes, adaptive immunosenescence, and epigenetic alterations, that likely contribute to the increased pathophysiological responses to SARS-CoV-2 among older adults.

Keywords: COVID-19, pandemic, co-morbidity, aging, hallmarks of aging, anti-aging

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Introduction

The recently emerged coronavirus SARS-CoV-2 is distinguished phylogenetically from other coronaviruses(1), causing more severe upper respiratory tract infections or distress and admissions to intensive care units (ICUs). This often results in mechanical ventilation, as well as mortality(2–5), mainly in persons with comorbidities or compromised immune systems such as diabetes, hypertension, and cancer(6,7). Previous outbreaks of community-acquired pneumonia and severe respiratory disease from coronaviruses were reported in 2003 and 2012, causing severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS-CoV), respectively(8). The latest coronavirus disease 2019 (COVID-19) started in Wuhan, China(9,10) and with its high virulence capacity and fast transmissibility, primarily through aerosol droplets(15), rapidly spread around the world (9,11,12) COVID-19 may appear as asymptomatic or minimally symptomatic with or without fever, cough, shortness of breath, fatigue, and gastrointestinal symptoms, with possible progression to moderate or severe pneumonia, severe symptomatic acute respiratory distress syndrome (ARDS), heart complications, and kidney injury(3,7,13,14), In clinical examinations, most patients had deceased numbers of lymphocytes (lymphocytopenia) and platelets (thrombocytopenia)(3). Increased inflammation and increased blood clots (D-Dimer) were reported in older patients with interstitial pneumonia and ARDS. Computed tomography (CT) depicted multifocal ground-glass opacities and subsegmental areas of consolidation and fibrosis in some cases even without overt clinical symptoms (15).

The silent spread of SARS-CoV-2 via asymptomatic cases likely increases transmission to all individuals but may be especially important for older persons who are at higher risk for more severe complications(5,16–18). Currently, there are no proven treatments, only preventive and

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supportive interventions. However, both the virus and host contribute to COVID-19 initiation, progress, and poor outcomes. Therefore, better understanding both the virus attack process and the subsequent host response is likely to yield better clinical management among older adults. Normal aging includes changes at the cell, tissue and organ levels (the "hallmarks of aging") and these contribute to morbidity and morbidity in the aging population. These hallmarks include genomic instability, telomere attrition, impaired autophagy, mitochondrial dysfunction, innate immunosenescence, inflammation and inflammasomes, adaptive immunosenescence, and epigenetic alterations (Figure 1)(19,20). With the COVID-19 pandemic, consideration of these hallmarks when treating infected older patients may be critical to enhance positive outcomes. Here we focus on some of the hallmarks of aging with their potential roles in the host response to SARS-CoV-2 infection.

SARS-CoV-2 virology

Classified within the *Coronaviridae* family, SARS-CoV-2 shares the main common characteristics of this family. Coronaviruses are enveloped, with large (~30-kb) single stranded, positive-sense RNA(21). Their genome is divided into two parts, 5` two-thirds and 3` one-third, with former including ORF1a and ORF1b that encodes pp1a and pp1ab, two large polyproteins that can be cleaved to smaller non-structural proteins (nsp1 to 16) required for new viral genetic material. The rest of genome includes genes that encode the structural proteins and the accessory genes, which produce virions and play a role in the host response, respectively(22).

Structural proteins include the Spike (S) glycoprotein known for its pathogenicity and having two functional subunits, S1 as the receptor binding domain and S2 for mediating fusion between the virus envelope and host cell membrane; nucleocapsid (N), shown to be involved in genome replication; a membrane (M) protein originated from host organelles such as endoplasmic

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reticulum or Golgi and responsible for virus assembly; and the envelope protein (E) (Figure 2, Table1). Replication commences by the binding of the S protein to angiotensin-converting enzyme 2 (ACE2), a cell surface receptor, in a manner similar to that of SARS-CoV, but with stronger affinity (23, 24,28). ACE2 is expressed in the vascular endothelia, lung, kidney, small intestine epithelial cells, immune cells, and testis (28,29). The virus enters the cell through either an endosome (in acidic environments) or by host cell protease cleavage via Furin or TMPRSS2, a serine protease shown to contribute to the immunopathology and lung illness of SARS-CoV(25–27). Using their own RNA polymerase, coronaviruses replicate in the host cell cytoplasm using the host ribosome machinery to produce proteins. Subsequently, particle assembly occurs in the host endoplasmic reticulum-Golgi intermediate complex (ERGIC), and mature virions are released through a secretory mechanism in smooth-walled vesicles. The host and virus factors interact in all processes, including entry into the cell, translation of replicase and replication transcription assembly, genome replication and transcription, translation of structural proteins, virion assembly, and release. (Figure 2). SARS-CoV-2 highly resembles SARS-CoV, with 77% similarity of residual amino acids of the S protein to that of SARS-CoV. Also, the similarity of N, M and 3a proteins in SARS-CoV and SARS-CoV-2 implies a similar pathogenic pathway (28).

Hallmarks of Aging and COVID-19

Genomic instability

With aging, somatic mutations accumulate in cells and tissues leading to altered gene expression, and with diminished DNA repair capacity, results in genomic instability(29). Collectively, adverse cellular responses to DNA damage, such as programmed cell death (apoptosis), cellular senescence, proteomic changes, and the effects on the error-prone mitochondrial genome have

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been reported as a reflection of genome instability; a putative common denominator of aging and the decline in general bodily function(30). Genetic instability via age related accumulation of somatic mutations has been reported for all cells, especially among cells of the immune system.

Diminished DNA repair capacity is decreased with aging. The DNA repair machinery involves p53 functions, with mild and transient activation protecting cells from oxidative damage in response to low stress. Conversely, in high levels of oxidative stress, persistent activation of p53 and increased mitochondrial outer membrane permeability results in apoptosis (Figure 2)(31). Also, with aging, it has been shown that p53-mediated reduction in stress-induced p38 mitogen-activated protein kinase (p38MAPK) can diminish senescence-associated secretory phenotype (SASP). Additionally, p38MAPK is shown to regulate SASP independent of the canonical DNA damage response (DDR)(32). Overall, DDR can play a role(s) in the pathogenesis of RNA viruses through apoptosis induction, deleterious somatic mutations, and excessive stimulation of inflammatory immune responses (Figure 3)(33).

Coronaviruses can induce cell arrest(34), but p53, through regulation of the cell cycle, can downregulate coronavirus replication(35). However, the coronavirus papain-like protease in SARS-CoV degrades p53 and interferes with interferon type I signaling, one of the first steps in the innate immune response to viral infection(36). Increased p53 phosphorylation was induced by both SARS-CoV's M and 3a expression in mammalian cells, leading to cell cycle arrest at G1(37). Additionally, the N protein, with a possible pathogenic role, can inhibit the activity of the cyclin-dependent protein complex, resulting in hypophosphorylation of retinoblastoma, a cell cycle check and tumor suppressor protein, and down-regulation in E2F1-mediated transactivation, which results in the cell cycle arrest at the S phase(38,39).

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Of note, the 7a protein was shown to mediate apoptosis, interfering with Bcl-X (an anti-apoptotic protein). Moreover, S, E, M, N, and accessory proteins 3a and 9b were also shown to modulate apoptosis by activating the ER stress response and p38MAPK pathway (Figure 2)(40,41).

Together, genomic instability and DNA repair dysfunction are thus possible denominators of various hallmarks of aging; not only are they likely to be a risk of poor outcomes, but their impact is likely to be amplified with coronavirus infection as well as other pathogens whose impact is greatest in the older population.

Telomere attrition

Telomeres are repetitive nucleotides (TTAGGG)n at the ends of each chromosome. The telomere repetitive elements are motifs for shelterin, a protective protein complex, maintaining genome stability. The telomere repeats can be transcribed to non-coding RNAs, called TERRA (telomere repeat-containing RNA) that regulate telomeric structure and function. In addition, there are regions near telomeres, called subtelomeres, containing particular CG-enriched genes that regulate innate immunity(42). These genes, affected by telomere length, regulate TERRA transcription and can be elevated in response to viral infection. Of these genes, interferon-stimulated gene (ISG)-15 is the most significantly activated ISG in response to viral infection. This suggests that telomere length, TERRA, and subtelomeric regions are linked with innate immunity. Immune cells with diverse telomere lengths, often observed with aging, may underlie differential viral immune responses(42). Another consequence of telomere attrition is the premature induction of genome instability in viral-specific CD8+ memory T cells resulting in senescent or antiapoptotic cells(43). enhancing what has already commenced with aging and reported in association with chronic diseases and lifespan(44).Telomere attrition coupled with

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dysregulated innate and adaptive immune responses to viral infection is another aspect of hallmarks of aging that can explain the severe outcomes in older adults with COVID-19.

Impaired Autophagy

Autophagy is a series of chaperon-dependent and chaperon-independent molecular actions for degrading protein aggregates and maintaining homeostasis of organelles, including mitochondria, peroxisomes and ribosomes, that in addition to protein and energy homeostasis eliminates pathogens such as viral particles. It has been shown that with aging the autophagy process is impaired and contributes to immunosenescence(45, 46).

The autophagy includes several ATG genes and protein complexes that participate in the process. Briefly, autophagy initiation is with creation of a phagophore, with a double membrane structure that originates from the endoplasmic reticulum, Golgi complex, mitochondria, endosomes and/or the plasma membrane, and becomes an autophagosome. mTORC1 and C2 are mammalian targets of rapamycin kinase and nutrient and stress level detectors. In the absence of stress, the mTORC1/ULK1/2 complex inhibits phagophore initiation. While under stress, including viral infections, the energy sensor 5' AMP-activated protein kinase (AMPK) inhibits mTORC1 and in turn activates downstream complexes, such as phosphoinositide3-kinases(PI3)/protein kinase B (AKT1), resulting in the induction of autophagy and virion encapsulation(47). This leads to formation of an autolysosome, and the autophagosomes fuse with lysosomes (phagolysosomes) to degrade the viral contents with lysosomal hydrolases(47).

Additionally, autophagy can augment adaptive immune responses to viral infections. Autophagy increases processing and presentation of viral antigens to the major

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histocompatibility complex (MHC)-I located on antigen presenting cells (APCs), such as B cells, macrophages and dendritic cells. APCs, in turn, present viral antigens to CD4+ T cells to release cytokines and regulate adaptive immune responses. Through phagosome-lysosome fusion, Autophagy interferes with viral replication by inducing release of interferon gamma (IFN- γ) from CD4+ T helper 2 cells, which in turn promotes T cells, NK cells and macrophages(48). Autophagy may also introduce endogenous viral antigens to CD8+ T cells through APCs. It also controls the inflammatory response through degradation of damaged mitochondria, preventing accumulation of ROS and activation of inflammasomes, [nucleotide-binding oligomerization domain-like receptors and pyrin domain-containing protein 3 (NLRP3)]. Moreover, it recognizes targeted pro-interleukin-1β for lysosomal degradation to modulate inflammasome responses(49–51). While some viruses evade direct autophagy, immune-mediated effects of autophagy may help with viral infection control and inflammatory-mediated tissue damage.

Niclosamide and valinomycin, two FDA drugs approved for other purposes, have been shown to enhance autophagy and diminish viral replication(52). While for polyamines, such as spermidine, that were also suggested to bolster autophagy, controversial results have been reported in regards to viral infections(20,53). It has been reported that rapamycin and Vitamin D3, two drugs with suggestive anti-aging effects, have antiviral efficacy by increasing autophagy(49,54). Despite its immunosuppressive effects in transplant patients(55), rapamycin inhibition of mTORC1 improves immune function in older adults(54). Therefore, it may have dual benefits in older adults with COVID-19 infection and presumably would result in improved survival outcomes. Some clinical trials using rapamycin have shown no serious side effects(56,57). Moreover, the antiviral brefeldin A interferes with RNA virus trafficking by inhibiting membrane formation

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from the ER and Golgi system through a noncanonical autophagy pathway(58). Together, impaired autophagy, along with stimulated inflammasome pathways discussed below seen in viral infections(59) may be another hallmark of aging that explains the severity of COVID-19 and poorer outcomes in older adults.

Mitochondrial dysfunction

Mitochondria, a power generator in cells, utilizes oxygen and nutrients to form ATP. ATP is produced by glycolysis in limited amounts and in large amounts by oxidation of Krebs cycle intermediates coupled to electron transport chains. In response to stress and pathogens, mitochondria produce additional amounts of reactive oxygen species (ROS); this has been associated with age-related diseases and decreased life span and is one of the contemporary theories of ageing(60,61). ROS is a normal part of bodily defense mechanisms, but during stress, high amounts are damaging. Detoxifying systems, including catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase with selenium and magnesium as their cofactors(62), vitamin E and C, and coenzyme Q10 (which decreases with aging), help to minimize ROS-induced tissue damage(61,63). Virus infection, including coronaviruses, can induce ROS production and apoptosis(64).

The carnitine shuttle, a crucial mitochondrial process involved in ATP production from fatty acid beta oxidation (FAO), is required for optimal adaptive T cell mediated immune responses(65). FAO, via depleting fatty acid availability, may reduce viral replication(66).

Accumulation of mutations in mitochondrial DNA (mtDNA), also observed with aging, can also impair mitochondrial function. For example, in response to apoptotic genes and outer membrane permeability, cytochrome c is released to the cytoplasm, where it activates intrinsic and extrinsic

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apoptotic pathways and tissue damage. Moreover, mitochondrial function can influence both innate and adaptive immunity. As a damage-associated molecular pattern (DAMP), mutated mtDNA activates NF-κB and NLRP3 pathways and mediates cytotoxic responses to lung cell stress(60,67). Viral RNA also activates mitochondrial antiviral-signaling proteins (MAVS) by activating RIG-I (a receptor for RNA viruses) which in-turn activates NF-κB and interferon-regulatory factors. Moreover, MAVS activates NLRP3 and release inflammasome factors. Metformin, an anti-diabetic drug recently proposed as an anti-aging modality, has been shown to improve T cell function through increased FAO and AMP-activated kinase (AMPK)(66). Induced hypoxia and shortness of breath, along with ARDS, are the main symptoms of COVID-19. Therefore, mitochondrial dysfunction, a prominent hallmark of aging, would appear to be a most critical system for intervention. Impaired respiration, diminished ATP production, increased ROS, reduced detoxification capacity together with dysregulated immune function, seems likely to play a pivotal role in the development of severe ARDS and mortality from COVID-19. The impact of mitochondrial dysfunction may be especially catastrophic in patients with insulin resistance and other comorbidities(66).

In addition to controlling metabolism and minimizing senescence, other anti-aging modalities such as increasing the expression of the deacetylation enzyme SIRT1 (nicotinamide adenine dinucleotide-dependent acetylase) have been reported to increase the life span and overall health(68). SIRT1 was also reported to have antiviral effects(69), and its interaction with FOXO3 (a transcription factor that is a sensor of the insulin signaling pathway) plays a role in longevity by mediating response to stress(70). However, SIRT1 was also proposed as having proviral activity in MERS-CoV replication(71). The effect of SIRT1 and FOXO3 solely or their interactions with COVID-19 or other lethal RNA viruses require more investigation.

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Thus, there is a nexus of effects between mitochondrial function, immunity, stress and ageing that all come into play upon viral infection.

Innate immunosenescence, inflammation and inflammasomes

The human body uses recognition receptors (PRRs) to identify pathogen-associated molecular patterns (PAMPs) and endogenous danger (or damage)-associated molecular patterns (DAMPs). The most well-known PRRs include the toll-like receptor (TLR), cytoplasmic retinoic acid inducible gene-I (RIG-I) and RIG-I like receptor (RLR), and nucleotide binding oligomerization domain (NOD)-like receptor (NLR). TLRs are induced in response to recognized particles(72). For example, TLR7, which resides on chromosome X, is activated in response to single stranded RNA viruses. TLRs then exert their effects through a set of adaptor proteins such as MyD88 and TRIF, which are responsible for the production of proinflammatory cytokines and IFNs type I and III, respectively(73). IFN-III is released locally when virus invade epithelial barriers, while IFN-I is more potent and systemic. The IFNs limit virus spread, modulate the immune system and promote macrophage, NK, T and B cells activity(74). However, it has also been shown that SARS-CoV can antagonize IFNs and evade the immune system(75).

RIG-I-like receptors are also sensors for RNA viruses and can activate MAVS, as discussed under mitochondrial dysfunction above, which in turn induces NF-κB-related factors, such as IL-6, TNF-α and IRFs, and NLRP3-related factors, such as IL-1β and IL-18, shown to be involved in inflammasome activation(72). Elevated inflammasome pathways in normal aging have also been associated with age-related chronic diseases. RNA viral infections such as coronavirus can result in surging inflammasome levels that can exacerbate the impaired immunological responses in aging(59,76–78).

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With aging there are innate immunity modifications that diminish normal anti-viral responses, such as attenuated interferon responses to viral infection in monocytes and neutrophils(74,79–81). Also, NK cells shift from less-mature, ready-to-release cytokine states to more mature subsets with decreased function. Migration to the site of infection, response to the PRP ligands, and phagocytosis of apoptotic cells declined in aged macrophages. Moreover, costimulatory signals from the APCs (macrophages, B cells, NK and dendritic cells), that are required to activate T cells, are also decreased(81). These defects in innate immunity with aging, coupled with exposure to coronaviruses with high pathogenicity such as SARS-CoV2, can explain failure of the initial steps to control COVID-19 infections and resulting critical conditions in older adults.

ACE2 is an enzyme converting angiotensin (Ang)-1 to -2 and Ang-(1 to 7). Ang-2 has profibrotic and proinflammatory roles, increases macrophage phagocytosis, reduces hematopoietic progenitor and stem cell proliferation, and increases spleen lymphatic proliferation(82). However, Ang (1-7), and also almandine have anti-inflammatory actions. ACE2 shows a protective impact against lung injury, suggesting a context and tissue dependent effect for ACE2. Diminished ACE2 expression in a rat model of aging also suggest a paradoxical role for ACE2 in older adults. Measuring ACE2 expression and viral loads in older adults with COVID-19 will decipher this enigma(83).

Lung macrophages, including M1 with proinflammatory activity and M2 with regulatory functions, provide localized lung innate immunity. Dysregulated activation of innate immunity plays a primary role in encountering pathogens and in the elevated inflammatory cascade that underlies the pathophysiology of ARDS(84). ARDS is also a systemic inflammatory syndrome with reciprocal involvement of the lungs and other organ systems(85–88). Increased and

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sustained inflammasome-regulated cytokines have been associated with poor outcomes and fibrosis in ARDS patients(89–93).

The SARS-CoV 3a accessory protein activates the NLRP3 pathway. Moreover, in tissue autopsy of patients with SARS-CoV, high levels of both NF-κB- and NLRP3-related cytokines were detected(94,95). Of particular importance was elevated tumor necrosis factor alpha (TNFα) converting enzyme (TACE), a proteolytic enzyme in the processing of TNFα; TNF receptors (TNFRs) and angiotensin-converting enzyme 2 (ACE2). Increased ACE2 and TACE levels have been shown to be associated with poor prognosis in heart failure. In addition to Ang-2 levels, TNFR-1 and TNFR-2 have been strongly associated with kidney failure(96). Thus, the heart and kidney, as well as other organs enriched in ACE2 receptors, may benefit from drugs that influence the renin angiotensin system and that ameliorate inflammation(97).

Another reported complication with COVID-19 is lung fibrosis. Multiple organ fibrosis is also observed with aging, that in part, is believed to be the result of steady low-grade inflammation evoked by senescent cells and increased TGF-beta and IL-13 production(98). In both SARS-CoVs in addition to TGF-beta, overactivation of epidermal growth factor receptor signaling has been shown(15,93). SARS-CoV-2 has also been reported to have a Furin-like cleavage site(99). One premise is that Furin activity is increased after cleavage of SARS-CoV-2 S protein. Furin, a host cell protease, increases both NLRP3 and NF-κB pathways and plays role in various disease and viral infection symptoms(100). Moreover, Furin can cleave the cytokine TGF-beta and increase expression of the serine proteinase inhibitor alpha (1)-antitrypsin Portland(101). TGF-beta can, in turn, increase collagen synthesis and development of fibrosis(102). The role of Furin or other protease activities with SARS-CoV-2 require more elucidation.

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Additionally, with coronavirus infections, the incidence and severity of ARDS is higher in men than women(103,104). Sex-disparity in innate immune response to pathogens has been shown. With SARS-CoV, viral titers and the accumulation of inflammatory monocytes and neutrophils in the lungs was higher among men. Estrogen receptor signaling may play a protective role(105), and low testosterone levels have been implicated in reduced immunity(106). Accordingly, investigation of inflammatory responses, viral loads and immune response patterns across sex and age with RNA viral infections are required. Similar questions may be asked regarding the sex-dependent survival paradox with higher mortality in men and more age-related frailty in women.

Adaptive immunosenescence

Adaptive immunity includes humoral and cell immunity responses mediated by B cells and CD8+ and CD4+ T cells that identify and respond to specific pathogens. B lymphocytes are triggered to differentiate into immunoglobin (Ig) producing plasma cells by cytokine production from CD4+ cells that recognize viral antigens presented via MHC-II. All immunoglobins, including IgA from respiratory mucosal cells and IgM and IgG, have been reported with SARS-CoV(72). IgM is the first antibody secreted in response to acute viral infection. IgG, produced later and enhanced upon re-infection, presents Fc segments that bind to complement receptors residing on macrophages and dendritic cells, which facilitate the phagocytosis of infected cells. Moreover, the Fc segments participate in antibody-dependent cellular toxicity mediated by both NK and CD8+ cells. With aging, alteration in B cell numbers and decreased IgM and IgD levels (antigen recognition soluble antibodies) shifts the naïve (CD27−) towards memory (CD27+) B cells(107). Moreover, in response to anti-viral vaccines, long-lived plasma cells decrease(108). The key factors mediating the B cell response, including E47 and enzymes responsible for

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hypermutations required for antigen recognition sites, are also affected. Thus, there is diminished effective antibody response to diverse and novel pathogens in older adults.

The communication between antigen recognition receptors (TCR) and co-stimulatory receptors, on T cells and MHC-I on antigen presenting cells (APCs), convert naïve T cells to memory cells which become effector cells to mount a highly specific response. The rest of the memory cells remain in the body to respond to antigen re-exposure(109,110). However, the response is not as vigorous in the aged.

Upon viral infection, activated cytotoxic CD8+ cells release enzymes that lyse infected cells and degrade viral genomes. If CD8+ cells fail to eliminate the virus, cytokines are produced and released from CD4+ T effector cells and induce inflammatory responses that may exacerbate conditions and inflict damage(111).

The dysregulation of both the innate and adaptive immune systems with age has been termed immunosenescence(112), for which accumulated somatic mutations and telomere attrition have been suggested as culprits. Immunosenescence is an emerging concern for the aging population in being able to respond to acute and chronic physical stress. With aging, hematopoietic stem cells, and therefore, progenitors of both B cells and T cell lineages, and the structure of lymph nodes, the secondary immune organ, are affected. Decreased numbers of naïve CD8+ T cells and reduced TCR diversity result in impaired recognition of new antigens and accumulation of dysfunctional memory T cells(113–115). However, there may be a massive increase in memory effector cells in response to a massive pathogen exposure, resulting in augmented inflammatory response. Nevertheless, senescent immune cells were shown to diminish IFN response to viral infection.116 Also, the reciprocal interactions between increased production of inflammation mediators and immunosenescence have been shown(117). Of note, transcription factors FOXO1

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and FOXO3 that are expressed on T cells play an important role in entry to the cell cycle and regulation of T cell function(118,119). Inactivation of FOXO through an autophagy-mediated pathway (PI3/AKT) and their downregulation has been suggested to stimulate T cell proliferation upon activation of TCR in response to PAMP or DAMP. While FOXO1 has been shown to maintain T cell homeostasis, FOXO3 increases polyclonal CD8+ T cell expansion in a tissue-specific manner by reducing apoptosis upon acute viral infection. This suggests a potential use for FOXO3 as an adjuvant in vaccines.

Epigenetic alterations

With aging, cell-specific epigenetic alterations play a pivotal role in distinguishing immune cell phenotypes, especially in T cells(119). Moreover, specific epigenetic modifications, such as chromatin accessibility, via histone acetylation/methylation have been shown with aging(119). Chronic viral infection can accelerate aging as measured through “epigenetic clocks.” Moreover, persons with accelerating “epigenetic clocks” would possibly have more severe outcomes with COVID-19 infections(120,121). Overall, these clocks are highly correlated with chronological age. Moreover, the epigenetic changes in CpG-sites located in sub-teleomeric regions that control innate immunity may mediate inflammatory responses to COVID-19. Changes in chromatin accessibility following such stress are inevitable. However, distinguishing epigenetic patterns based on viral infection from the ones developed based on normal mortality or chronological age may predict the severity of COVID-19-related outcomes. Such epigenetic patterns should be investigated. Moreover, differential methylation rates (DMR) following COVID-19 infection may reveal accelerated aging and subsequent exacerbation of chronic diseases in COVID-19 survivors. In addition, RNA viral infection may play a role in retrotransposon-mediated changes in the genome, which may exert alterations in DMR in

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specific genome regions. Therefore, measuring the epigenetic signatures following COVID-19 in short-term and long-term follow-ups is warranted.

Proteomics in aging

Recent studies have shown certain protein markers track chronological age and binary outcomes of health versus disease as well as other age-related markers. Notably, growth differentiation factor 15 (GDF15), a member of TGF-beta superfamily, and the most significant marker associated with aging(122) has been associated with viral infection. Overexpression of human GDF15 protein in mice increased inflammation and viral particle release, and in human airway epithelial cells increased inflammation via inhibition of IFN-λ1. Moreover, in patients with ARDS, elevated GDF15 was a significant prognostic marker for poor outcomes(123). Use of GDf15 in COVID-19 patients as a prognosis biomarker should be explored.

Medical Interventions

Targeting the SARS CoV-2 receptor: Among the various targets that impact viral binding and cell entry, targeting the ACE2 receptor as well as the S protein are being considered. A recombinant S protein that binds to ACE2 and thereby outcompetes the SARS-CoV2 for binding and viral entry into Vero cells has been proposed (in review)(124). Up on approval of the surmise of decreased ACE2 expression with aging whether this modality benefit older adults would be a conundrum.

Vaccines: A vaccine (mRNA1273) has been developed based on the SARS CoV-1 epidemic, and testing has just started for COVID-19. The efficacy among older adults remains to be elucidated. It is clear that among the numerous vaccines now being developed, a vaccine, however effective, will not be a universal panacea. The primary problem remains that not all individuals are vaccinated or become immunized and that older adults in particular have

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weakened responses to vaccines(113,125–128). To boost immune response, adding adjuvants to vaccines has been suggested(129). Nevertheless, it is clear that alternate therapeutic strategies, ideally orally active small-molecule agents, in addition to vaccines will be required. Conceptually, and by analogy to HIV-1, it seems likely that a cocktail of agents that interfere in several pathways will be required to deal effectively with the virus.

Minimizing inflammation: One critical phenotype of COVID-19 is ARDS and has been approached by guidelines(5,130). However, to abate cascades of acutely activated proinflammation(131,132), systemic removal of circulatory inflammatory markers and their mediators would be needed. For example, plasma or whole blood exchange with healthy, younger donors may benefit older patients with critical ARDS(133–137) as a crisis modifying modality.

Blocking Viral Fusion and Replication: Recently, theaflavin, a polyphenolic compound in black tea, has been suggested to inhibit viral replication targeting the RdRp enzyme(109–111). Other pre-clinical medications include favipiravir, a more potent antiviral than lopinavir-ritonavir(141,142), combined with tocilizumab, an IL-6 receptor antagonist used in rheumatoid arthritis and other “cytokine storm” conditions(143,144), and remdesivir, an RNA polymerase inhibitor, another potential prophylactic and therapeutic modality candidate against COVID-19(145–147). Moreover, adding hydroxychloroquine to increase pH in endosomes may block virus fusion mediated by the endosomal pathway.148 Although COVID-19 uses host ribosomes as the cellular translation machinery no modality to target ribosome shift has been suggested(149).

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Targeting Aging Related Pathways: Interfering with pathways associated with the hallmarks of aging may improve response to anti-viral drugs(150). Potential drugs or drug targets include rapamycin, and metformin, agents that decrease insulin levels and IGF-1 signaling as well as inhibitors of the mTOR pathway, agents that reduce mitochondrial ROS production and activate AMP-activated kinase (AMPK). The status of metformin as an antiviral remains to be elucidated. On the one hand cellular senescence pathways impact viral replication possibly due to decreases in virus-induced type I IFN expression(116). On the other, senescence may play an anti-viral role as part of the host cell response(151). The role of senolytic drugs improve response to anti-viral modalities and vaccines in older age patients with COVID-19 requires investigation.

At any age, calorie restriction and exercise improve health and immunity. These might help minimize infection with COVID-19 although exercise is not always feasible among the elderly. Although lists of drugs and vitamins have been suggested as candidate modalities, approved anti-COVID-19 medications remain to be recognized(142,152,153). More importantly, at this writing, none of the ongoing clinical trials have considered recruiting older patients. Thus, the impact of all potential therapies and safety in older adults will need to be determined. We suggest that an accelerated understanding of the interactions between COVID-19 and the host hallmarks of aging, as well as targeted therapies, will be more likely to arise from focused understanding of mechanisms in young and particularly old patients.

Conclusion

The COVID-19 outbreak is a worldwide public health problem whose consequences are likely to persist for several years. Older adults and patients with comorbidities are at higher risk and develop more severe and critical complications. Both the incidence and severity of disease appear to be more prominent in men than women and more epidemiological reports from

different geographical regions across age and sex are required. Coronaviruses use host factors for replication that are impacted by age. Innate immunity neutralizing antibodies, cellular immunity responses such as CD4+ and CD8+ T cells, B cells, natural killer cells, macrophages, inflammatory host responses, along with specific virus antigen epitopes, play interactive roles in disease development. The imposition of acute stress in older adults, where diminished reserves and stress response capacity and reduced immune response to vaccines, place them at higher risk for critical health complications and mortality. Together with anti-viral interventions, key hallmarks of aging may offer insights for identifying and treating patients most at risk as well as those with no or minimal risk. The study of patients with COVID-19 from time of infection to long-term follow-up across the age spectrum in men and women will shed more light on the basis for age and sex differences in response to such stressors.

Conflict of interest

Non.

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Table 1. COVID-19 Structural proteins, non-structural proteins, and accessory proteins

Replication phase	Host factor	Virus factor	Function
Binding and entry	ACE2	Spike glycol protein (S)	Cellular receptor
Attachment and entry		S glycoprotein	
Viral transcription/replication, ribosome frameshift		Replicase polyprotein 1a (R1a)	
Viral transcription/replication, ribosome frameshift		replicase polyprotein 1ab (R1ab)	
Protein 3a		Independent budding	
Envelope small membrane protein (E)		Independent budding	
Membrane protein (M)		Virion morphogenesis	
Non-structural protein 6 (NS6)			Unknown
Non-structural protein (Ns) 7a (Ns7a)			Unknown
Protein 7b (Ns7B)			Unknown
Ns8a			Unknown
Nucleoprotein (N)		Viral genome packaging	
Ns14			
Ns 9b			Unknown
Ns10			Unknown
	IFITM (interferon-induced transmembrane)		Inhibit cell entry
	TMPRSS2 (Transmembrane Protease Serine2)		Cleave and activate S protein
	Furin		Cleave and activate S protein
Genome replication and transcription	GSK3 Glycogen synthase kinase 3		Phosphorylate N protein and facilitate viral replication
Translation of structural proteins	N-linked glycosylation enzymes in Golgi		Modify S and M protein; N-linked glycosylation of the S protein facilitates lectin-mediated virion attachment and

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			constitutes some neutralizing epitopes
	Endoplasmic reticulum chaperones		Proper folding and maturation of protein

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Figure1: Schematic of severe acute respiratory syndrome coronavirus-2(SARS-CoV2) and Biological features clockwise: Telomere length, genome instability, mitochondria, stem cell, extracellular matrix, Golgi, endoplasmic reticulum, cell membranes, epigenetics, senescent cells, inflammation, autophagy.

Figure2.a: COVID-19 genome and proteins.

Figure2b: Coronavirus structure, cell entry and replication.

ACE2: Angiotensin-converting enzyme2, ERGIC: Endoplasmic reticulum-Golgi intermediate compartment, ER: endoplasmic reticulum

Figure3. Mitochondria, outer membrane permeability and apoptosis pathways:

Apoptosis induced by coronavirus infection including intrinsic and extrinsic apoptosis. **Ligands:** FasL, Fas ligand**,** TNF-α, tumor necrosis factor alpha**, anti-apoptotic factors:** Bcl2-associated X; Bcl-xL, Bcl-2-like protein 1; Bcl2, B cell lymphoma 2, Mcl1, myeloid cell leukemia 1; **proapoptotic factors:** PUMA, p53-upregulated modulator of apoptosis; BAD, Bcl2-associated agonist of cell death; BAX; BID, BH3-interacting domain death agonist; BIM, Bcl2-interacting mediator of cell death; APAF1, apoptotic peptidase-activating factor 1; Casp: caspase; FADD: Fas associated via death domain; AKT: RAC-alpha serine/threonineprotein kinase; SARS, severe acute respiratory syndrome(64).