Age-related susceptibility to coronavirus infections: role of impaired and dysregulated host immunity

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Human coronaviruses (hCoVs) cause severe respiratory illness in the elderly. Age-related impairments in innate immunity and suboptimal virus-specific T cell and antibody responses are believed to cause severe disease upon respiratory virus infections. This phenomenon has recently received increased attention, as elderly patients are at substantially elevated risk for severe COVID-19 disease and experience increased rates of mortality following SARS-CoV-2 infection compared with younger populations. However, the basis for age-related fatal pneumonia following pathogenic hCoVs is not well understood. In this Review, we provide an overview of our current understanding of hCoV-induced fatal pneumonia in the elderly. We describe host immune response to hCoV infections derived from studies of young and aged animal models and discuss the potential role of age-associated increases in sterile inflammation (inflammaging) and virus-induced dysregulated inflammation in causing age-related severe disease. We also highlight the existing gaps in our knowledge about virus replication and host immune responses to hCoV infection in young and aged individuals.

Introduction

Prior to 2002, human coronaviruses (hCoVs) were best known as causes of the common cold. Two CoVs, HCoV-229E and HCoV-OC43, were identified in the 1960s and caused upper respiratory tract infections that were indistinguishable from those caused by rhinoviruses (1). Other CoVs, HCoV-HKU-1 and HCoV-NL63, which also cause the common cold, were identified in the period after the severe acute respiratory syndrome coronavirus (SARS-CoV) was discovered and after more research efforts were focused on this family of viruses (2, 3).

This characterization of hCoVs as causes of relatively benign infections changed radically with the advent of SARS in 2002 (4). For the first time, an hCoV was shown to cause severe disease. SARS-CoV, the cause of SARS, was shown to originate from bats, with transmission to human populations occurring via intermediary animals such as Himalayan palm civet cats and raccoon dogs in exotic animal live markets in Guangzhou, China (5). SARS caused pneumonia of varying severity, with about 8500 cases and a mortality of approximately 10%. In retrospect, SARS caused a relatively small pandemic because it tended to be transmissible only after an infected person developed symptoms of respiratory disease. Thus, it was easy to identify and quarantine patients, to stop transmission and end the pandemic. In addition, no nonhuman host was involved in SARS-CoV transmission. The last case of SARS was identified in 2004. SARS illustrated two aspects of emerging zoonotic viral infections. It became evident, first, that a zoonotic respiratory pathogen would be readily transmitted under the right circumstances, and second, that humans had no protective immunity to the virus. However, since transmission occurred readily only in hospital and household settings, the pandemic was limited. SARS-CoV caused greater morbidity and mortality in patients with comorbidities such as diabetes and heart disease, which is a common theme of infections caused by pathogenic hCoVs.

In 2012, a second zoonotic CoV, MERS-CoV, causing the Middle East respiratory syndrome (MERS), was identified (6). All cases of MERS have been identified in individuals who lived on the Arabian Peninsula or in travelers from this geographical area (7). Outbreaks initiated by travelers from the Arabian Peninsula were limited in scope, showing that human-to-human transmission of MERS-CoV is uncommon. The one exception was an outbreak in South Korea, in which 186 individuals became infected, with a 20% mortality (8). MERS-CoV also caused pneumonia, with disease primarily confined to the lungs. Unlike SARS, MERS is primarily a disease of camels and continues to enter human populations sporadically from this zoonotic source (9). As of November 2019, MERS-CoV has infected about 2500 people since 2012 with a 35% mortality (10). MERS-CoV, like SARS-CoV, is primarily transmitted only after people are clinically ill. Most reported cases originated in hospitals, after virus was aerosolized and proper precautions to prevent spread were not used (11). More recently, as infection control procedures have been instituted, a majority of cases have been primary, occurring in the community, often in people without any known camel contact (12). Human populations have no preexisting immunity to MERS-CoV, but interhuman transmission remains inefficient. Curiously, while MERS-CoV is detected in camels throughout Africa and Asia (13) and has been in camel populations since at least the early 1980s, clinically evident human disease has never been reported in Africa, and MERS cases were not detected in the Kingdom of Saudi Arabia (KSA), the epicenter of the disease, until 2012. A recent report describes

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MERS-CoV seropositivity in asymptomatic camel abattoir workers in Nigeria (14), suggesting that human infection occurs and is mild. Whether these findings reflect differences in socioeconomic, cultural, or other factors in populations located on the Arabian Peninsula versus in Africa, and whether these factors changed in KSA in 2012, remain to be determined. MERS-CoV, like SARS-CoV, preferentially infected individuals with comorbidities such as diabetes, chronic renal disease, and chronic cardiac disease (15).

SARS-CoV-2, the etiological agent of the ongoing COVID-19 pandemic, was first recognized in December 2019 (16). Unlike SARS-CoV and MERS-CoV, SARS-CoV-2 replicates to very high titers in the upper respiratory tract, especially in the presymptomatic phase of the infection (17). Consequently, the virus is readily transmissible from human to human. As of October 10, 2020, there had been 36 million cases and 1,063,429 deaths (3.4% mortality) (18). While SARS-CoV-2 likely originated in bats, a virus identical to SARS-CoV-2 has not been detected in bats. It is probable that the virus crossed species from a not-yet-identified intermediary host. Alternatively, given how well adapted SARS-CoV-2 is to human populations, it is also possible that the virus has been circulating in specific human populations in Southeast Asia for longer than the few months of the pandemic. People with underlying diseases develop more severe COVID-19, as also occurred in SARS and MERS infections (19). Partly as a consequence of the large numbers of COVID-19 infections, several unusual manifestations of the disease have been identified. These include neurological and heart disease, endothelialitis and thrombosis, and a hyperinflammatory syndrome that is especially prominent in children and adolescents (20, 21). The latter has similarities with Kawasaki's disease (KD) and, like KD, can result in permanent damage to the heart (21). While a small proportion of infected children develop this KD-like illness, most remain asymptomatic. However, viral loads are often high in children (22), so these children may serve as the source of the adult infection in some instances. On the other hand, a common presentation of COVID-19 is subclinical disease or asymptomatic disease that is often identified during contact tracing or in routine screening. Some individuals with subclinical or asymptomatic disease may progress to clinical disease, but many do not, and many have high virus loads in their nasopharynx, as measured by quantitative reverse transcriptase PCR (17). Whether people who remain asymptomatic can transmit the virus to susceptible individuals needs to be determined. Development of antiviral therapies and development of vaccines are high priorities as part of the intensive efforts under way to limit the pandemic.

Epidemiology

Common cold CoVs (CCCs) — including HCoV-229E, HCoV-OC43, HCoV-HKU-1, and HCoV-NL63, mentioned above — generally cause mild upper respiratory disease and are especially common in children. However, adults can also be infected and develop disease. CCCs cause colds most often in the winter and fall in temperate areas and account for a variable fraction of upper respiratory tract infections, depending on year and location. In total, about 15% of all colds are caused by CCCs (23). Several volunteer studies have shown that reinfection within one year can occur, although the second infection may not result in clinical disease (24). Shedding may still occur, however, even in the absence of clinical disease. In contrast to the mild disease observed in children, outbreaks of severe respiratory disease caused by HCoV-OC43 and HCoV-229E have been documented in patients with chronic obstructive pulmonary disease (25). These reports demonstrate that severe disease, although uncommon, occurs especially in aged individuals.

In addition to the comorbidities described above, SARS, MERS, and COVID-19 are all more severe in aged patients. SARS rarely occurred in children, with no deaths reported in people under the age of 24 years, compared with a 50% mortality in those over 65 years (4). Similarly, MERS is a disease of aged populations. A few children had severe disease or died from MERS, but these children had underlying diseases that made them more susceptible to the infection (26, 27). COVID-19 is also much more severe in the elderly. While the mortality rate in those 5–19 years of age is estimated to be 9 times lower than that in a comparison group (18–29 years), the rates in those 74–85 and over 85 years are 220 and 630 times higher, respectively (28). Consistent with these data, deaths in nursing homes are believed to account for 41% of all deaths in the United States from COVID-19 (29). Additional studies show that age is an independent risk factor for severe disease. While underlying disease occurs to a greater extent in the elderly, age — independent of underlying disease — is associated with worse outcomes (19). Although the elderly are at increased risk of developing severe disease, individuals of all ages develop a spectrum of clinical illness that includes asymptomatic, mild to moderate, and severe disease. Whether the pathogenic processes that result in severe disease are the same or different in populations of different ages requires further investigation. Interestingly, there are several similarities between the multisystem inflammatory syndrome observed in children and the vasculitis/endothelialitis/thrombosis syndrome observed in adults (20, 21). Notably, as discussed in more detail below, some strains of mice duplicate many of these features of the effects of aging on outcomes. For example, young C57BL/6 mice are resistant to SARS-CoV infection but display an age-dependent increase in clinical disease so that even by 6 months, mice develop respiratory symptoms and may succumb to the infection (30). Similar effects of aging are also observed in macaques infected with SARS-CoV (31). Thus, these animal models will be useful for studies of the role of aging in disease severity.

Clinical features

SARS-CoV, MERS-CoV, and SARS-CoV-2 all have an incubation period of 2–14 days, although the usual period is about 4–7 days (4, 32). A major difference between the three viruses is that COVID-19 may present initially with upper respiratory tract disease, unlike MERS and SARS. While most COVID-19 patients develop mild disease that resolves without any interventions, 10%–20% develop more serious disease, with an overall mortality of 1%–3%. Both the number of total and the number of fatal COVID-19 cases have been underestimated, so that the actual mortality rate is in flux. The primary target organ of infection by SARS-CoV, MERS-CoV, and SARS-CoV-2 is the lung. Severe infection is characterized by acute lung injury and, in more serious cases, acute respiratory distress syndrome. In all three infections, radiographic examination reveals a ground-

glass appearance and airspace opacifications that are especially prominent in the lung periphery (33–35). Pneumonia caused by these three viruses cannot be distinguished, by either clinical or radiographic findings, from severe viral pneumonia due to other causes. As discussed briefly above, severe COVID-19 is characterized by several extrapulmonary manifestations, affecting the heart, brain, and kidney, among other organs (20, 36). Virus has been identified in these organs by electron microscopy (20, 37), but the identity of these presumptive virus particles has been questioned (38). Further, other studies show inflammatory changes without detection of virus (39). Thus, determining the etiology of these changes and identifying host immune factors that contribute to these extrapulmonary manifestations is an important goal. Also, it is not known whether any of these manifestations occur preferentially in aged individuals. A fraction of children develop the KD-like hyperinflammatory syndrome described above (21), but whether the thrombosis and endothelial disease observed in adults have the same pathogenic basis also needs to be determined.

Pathogenesis of hCoV infection in aged animals

Animal models that replicate clinical and immunological features of hCoV-induced pneumonia are critical to understanding disease pathogenesis. Several animal models have been used to (a) examine virus replication in young and aged hosts, (b) study host immune responses and tissue pathology, and (c) test antiviral and vaccine responses to hCoV infections (40–43). A unique feature of all hCoVs, and perhaps of the majority of the RNA viruses causing human respiratory infections, is that they cause more severe pneumonia in aged compared with younger populations (44, 45). Therefore, several small- and large-animal models are used to study age-related changes in disease pathogenesis. In this section, we will discuss animal models used to study hCoV pathogenesis, and their use in the study of age-dependent susceptibility to hCoV infection.

Animal models of seasonal hCoV infections

Studies of seasonal hCoV pathogenesis are limited by poor propagation of these viruses and lack of appropriate animal models. Among the four nonpathogenic seasonal hCoVs, mice expressing human aminopeptidase N (hAPN, the receptor for HCoV-229E), both single transgenic (hAPN/Stat1$^{+/+}$) and double transgenic (immunodeficient mice lacking *Stat1*; hAPN/Stat1$^{-/-}$), support HCoV-229E replication. In vitro adaptation of HCoV-229E in hAPN/Stat1$^{-/-}$ mouse embryonic fibroblasts gave rise to a mouse-adapted strain that replicated in hAPN/Stat1$^{-/-}$ mice and caused mild to moderate disease (46). However, whether HCoV-229E causes age-dependent severe disease in these transgenic mice has not been examined. HCoV-OC43 also infects mice, but tropism is limited to the brain, with limited if any replication in the respiratory tract (47).

Animal models of pathogenic hCoV infections

All three pathogenic hCoVs cause severe disease in the elderly. Therefore, animals that replicate features of age-related severe human respiratory illness are critical to understanding the basis of hCoV pathogenesis.

Animal models of SARS and aging. Several animal models of SARS replicated the age-related severe disease observed in humans. Aged macaques infected with SARS-CoV exhibited increased body temperature, decreased activity, and increased respiratory rates compared with young macaques (31, 48–50). Gross pathological findings showed multifocal consolidations on aged lungs, while innate cell infiltration, edema, and hyaline membrane formation were commonly observed in aged compared with young lungs. Interestingly, there was no difference in lung virus titers between young and aged macaques (31, 48, 50). Standard laboratory rodents are permissive to WT SARS-CoV infection, but fail to develop clinical illness. Consequently, human ACE2–transgenic (hACE2-transgenic) mice were developed to study SARS-CoV-2 pathogenesis, but whether these transgenic mice show age-dependent severe SARS is unknown. While human SARS-CoV (Urbani strain) caused very mild disease in young BALB/c mice, pneumonia was more severe in aged BALB/c mice (41, 51). Serial passage of SARS-CoV-Urbani through the lungs of rats and mice resulted in the outgrowth of rodent-adapted strains that replicated to high titers and caused severe clinical disease (52, 53). One mouse-adapted strain, MA15, is most often used in studies of SARS-CoV in mice. MA15 caused severe disease in both young and aged BALB/c mice when compared with the human isolate. MA15 also caused severe disease with high rates of mortality in aged mice of all strains examined, in comparison with young ones (52, 54). Unlike infection of nonhuman primates, mouse-adapted SARS-CoV replicated to 1 to 2 logs higher titers in the lungs of aged compared with young mice (48, 54).

Aging, MERS, and animal studies. MERS-CoV also causes severe disease in the elderly, especially in individuals with comorbid conditions (55, 56). Efforts to model age-dependent changes have been confined to MERS-CoV–infected mice. Mice are normally resistant to infection with MERS-CoV but can be rendered sensitive to infection by transduction with Ad5 expressing human DPP4 (hDPP4; the MERS-CoV receptor), transgenic expression of hDPP4, or humanization of the mouse DPP4 locus (57–59). The latter results in the most useful model of human MERS. However, age-related disease in these animals has not been well demonstrated, except for increases in proinflammatory responses and prolonged pulmonary inflammation in 25-week-old MERS-CoV–infected hDPP4-transgenic mice compared with 10-week-old mice (60).

Aging and animal models of COVID-19. Several laboratories demonstrated age-related disease severity in SARS-CoV-2–infected animals (60). In SARS-CoV-2–infected macaques, viral titers were higher in nasopharyngeal and anal swabs, and in the lungs, of aged monkeys compared with young ones. SARS-CoV-2–infected monkeys developed interstitial pneumonia characterized by thickened alveolar septa accompanied by inflammation and edema, with diffuse severe interstitial pneumonia observed in aged monkeys (61). Standard laboratory rodents are not permissive to WT SARS-CoV-2 infection. Therefore, several different versions of hACE2-transgenic mice are used to study SARS-CoV-2 pathogenesis, although whether disease severity is age-dependent is unexplored (62, 63). Additionally, SARS-CoV-2 has been genetically manipulated so that it is able to infect standard laboratory mice (64, 65). Notably, middle-aged hamsters, or mice infected with mouse-adapted

SARS-CoV-2, develop more severe respiratory illness and fatal pneumonia compared with young ones. Despite more severe disease in aged rodents, young and aged rodents showed similar virus titers in the lungs, demonstrating that virus-induced inflammatory responses likely drive the severe disease observed in aged hosts (64, 66).

Age-related immune response to hCoV infection and vaccination

With advancing age, there is a gradual decline in the ability of the host immune response to control viral infections. Impaired innate and adaptive immune functions contribute to an age-related increase in susceptibility to virus infections. In this section, we discuss age-associated changes in immune response and its impact on disease outcomes during pathogenic hCoV infections.

Innate immunity in age-related susceptibility to hCoV infections

The innate immune response provides the first line of defense to control initial virus replication and subsequently instructs the development of an effective pathogen-specific adaptive immune response. Detection of pathogen-associated molecular patterns (PAMPs) by innate cell pattern recognition receptors (PRRs) is critical for the induction of early and robust antiviral interferon (IFN) responses to initiate protective immune responses against hCoV infection. Endosomal TLR7 and cytosolic MDA5 are the major sensors that recognize hCoV single-stranded and double-stranded RNA, respectively, to induce antiviral IFNs (67–69). Classical dendritic cells, plasmacytoid DCs (pDCs), and alveolar macrophages (AMs) express increased levels of MDA5 and TLR7 and serve as the major source of IFN-α/β following hCoV infection (69, 70). Aging is associated with a decrease in the total number of DCs, pDCs, and AMs (44, 71), and aged DCs and pDCs express reduced levels of PRRs, including intracellular retinoic acid–inducible gene-I–like receptors (RLRs) and endosomal TLR7 (72, 73), likely contributing to significantly reduced levels of IFN-α/β and leading to increased hCoV replication in the aged lungs. Age-related increased susceptibility to influenza A virus (IAV) and reduced IFN-α production by IAV-infected pDCs from aged donors further support these conclusions (73–75).

Another notable feature of aging is increased myelopoiesis and, as a result, increased numbers of myeloid cells such as neutrophils and monocytes/macrophages in the blood and tissues (76, 77). Despite an increase in myeloid cell numbers, critical functions of neutrophils and macrophages such as phagocytosis, nitric and superoxide production, and migration to infected tissues are defective in aged individuals (reviewed in refs. 78–81). SARS-CoV, MERS-CoV, and SARS-CoV-2 abortively infect myeloid cells. Despite causing an abortive infection, hCoVs induce delayed but robust inflammatory cytokine (IL-6, TNF, and IP-10) and chemokine production by macrophages (82–84). However, it is not known whether aged myeloid cells are more susceptible to hCoV infection compared with those from young individuals. Considering the increased number and elevated basal inflammatory status of neutrophils and monocytes/macrophages in the elderly, it is likely that hCoV infection, albeit abortive, induces robust inflammatory cytokine and chemokine expression leading to exaggerated and dysregulated host inflammatory responses. Additionally, monocyte/macrophage populations isolated from elderly individuals exhibit impaired RLR signaling and thus secrete reduced levels of IFN-α expression following virus infections, despite an unperturbed inflammatory cytokine production (85). The net result is a defective antiviral immune response that is associated with a robust and dysregulated inflammation.

The $CD56^{hi}$ NK cells constitute approximately 10% of peripheral blood NK cells and are highly cytotoxic, as shown by increased production of effector molecules such as perforins and granzymes (86–88). The number and cytotoxic ability of $CD56^{hi}$ NK cells decline with age in humans (88, 89). Impaired migration of NK cells to draining lymph nodes (DLNs) and reduced cytotoxic function contribute to increased susceptibility of aged mice to infection with ectromelia virus, the cause of mousepox (90). Similarly, fewer NK cells and impaired cytotoxic ability correlate with severe IAV-induced pneumonia in murine models (91). SARS-CoV-2 infection causes reduction in peripheral blood NK cell number and impaired cytotoxic function (92–94). However, the role of NK cells in hCoV immunity during aging is yet to be elucidated. In addition to producing antiviral IFNs, DCs play a central role in viral antigen presentation to T and B cells, thus orchestrating effective adaptive immunity. Age-related impaired DC-intrinsic TLR/RLR signaling and thereby reduced IFN-α/β and IL-12 production (reviewed in refs. 71, 95) (as a third signal for T cell activation) may affect T cell priming. Additionally, age-dependent increased prostaglandin D_2 (PGD_2) and increased expression of an upstream phospholipase, PLA_2G2D, in aged hosts dampen migration of classical DCs to DLNs (30). Increased PLA_2G2D expression was postulated to result from the chronic inflammatory response that occurs in the lung during aging.

Adaptive immunity in age-related susceptibility to hCoV infections

Humans are immunologically naive to emerging novel CoVs such as SARS-CoV-2, although cross-reactive T and B cells from CCCs have been detected in several studies. Whether these cells are protective or pathogenic needs to be determined (96–101). Suboptimal neutralizing antibody responses to infection are believed to contribute to severe disease in the elderly (102). However, so far, only a limited number of studies have directly compared antibody responses in young and aged SARS and COVID-19 patients (103, 104). Notably, one study showed a robust neutralizing antibody response in older compared with younger patients with MERS, although the number of patients analyzed was small (105). Similarly, recent COVID-19 studies show high neutralizing and antigen-binding antibody titers in aged SARS-CoV-2–infected individuals in comparison with young ones (103, 104). In correlation with antibody titers, SARS, MERS, and COVID-19 studies show robust plasmablast and antibody responses in individuals with severe respiratory illness compared with those with mild to moderate disease (106, 107). Whether the plasmablast and antibody responses are protective or pathogenic or merely a manifestation of severe disease remains to be determined. Interestingly, a kinetic analysis of serum samples from CCC-, SARS-, MERS-, and COVID-19–recovered individuals demonstrated a rapid decline in neutralizing antibody titers after 3–6 months postinfection (24, 108–111).

Patients with higher antibody titers were less likely to develop clinical disease, although shedding still occurred. Initial studies

showed that SARS-CoV–specific neutralizing antibodies were barely detectable at 3 years post-SARS and were undetectable by 6 years postinfection (109, 112). However, more recent studies showed that anti–SARS-CoV antibodies could be detected for as long as 15 years after infection (112). Whether such low neutralizing antibody titers are protective or pathogenic, possibly contributing to enhanced disease following reinfection, is not known. Limited studies of antibody responses in aged animals experimentally infected with SARS-CoV showed low neutralizing antibody responses and incomplete protection following immunization with an inactivated vaccine (113). However, the basis for reduced antibody titers in aged animals is not well understood. Age-associated reduced numbers of naive B cell precursors, impaired T follicular cell help to B cells, reduced CD40L stimulation, or B cell–intrinsic defects may account for suboptimal B cell response to vaccination in the elderly (reviewed in refs. 114, 115). In addition to studies of circulating B cell and antibody responses, recent reports highlight the role of lung-resident B cells in host protection (116). Elucidating the role of tissue-resident B cells in young and aged hosts will further understanding of the antibody response to hCoV infection and intranasal vaccinations.

SARS, MERS, and COVID-19 studies comparing virus-specific T cell responses in young and aged individuals are lacking. The majority of human studies assess hCoV-specific T cell response in individuals with severe versus nonsevere disease or symptomatic versus asymptomatic individuals. Evidence from clinical hCoV studies indicates that patients with severe disease exhibit lymphopenia and reduced virus-specific T cell immunity. In contrast, individuals with subclinical, mild, or moderate symptoms show high lymphocyte numbers and robust T cell immunity (16, 117–119). Aging is associated with reduced naive antigen-specific T cell precursors due to thymic involution (120), and reduced T cell function due to age-related changes in cell-intrinsic and -extrinsic factors that dampen T cell immunity (121). Considering that elderly individuals exhibit severe disease, it is likely that the elderly mount much weaker virus-specific T cell responses compared with young individuals. However, a study of a small cohort of MERS-CoV–infected young and aged individuals showed comparable MERS-CoV–specific T cell responses (105). Therefore, large-cohort studies are required to assess whether different numbers of hCoV-specific T cells in young and aged individuals account for severe disease in the elderly. Antigen-experienced memory T cells are central to providing protective immunity upon pathogen rechallenge. Virus-specific memory T cells are detected in the peripheral blood of SARS-recovered individuals for up to 17 years following SARS-CoV infection (122, 123) and may provide long-term immunity to hCoV infection in both young and aged individuals. A protective role for hCoV-specific primary and memory T cell responses is well documented in young and aged mouse models of SARS and MERS (30, 69, 124–126). These studies show age-related declines in numbers of virus-specific T cells, which is, in part, due to impaired migration of antigen-coated DCs to the DLN. As discussed above, mice express increased levels of lung PGD_2 levels during aging, and these levels are increased upon SARS-CoV infection (30). PGD_2 suppresses DC migration from lungs to DLNs. Blocking PGD_2/PLA_2G2D activity in aged mice resulted in increased antigen-loaded DC migration to DLNs and, as a result, improved virus-specific T cell responses and mouse survival (30). In addition to these defects, loss of virus-specific precursor cells due to thymic involution (127), expansion of clones of T cells resulting from chronic stimulation by specific viral antigens (e.g., CMV and EBV) (128, 129), increased expression of checkpoint inhibitors such as PD-1, LAG3, etc. (130, 131), and loss of lymphoid tissue integrity (132) may contribute to age-related suboptimal virus-specific T cell immunity and impaired hCoV clearance from the lungs.

Despite a decline in the number and function of peripheral blood virus-specific T cells, tissue-resident memory (TRM) T cells increase with advancing age in humans and mice (133). TRM T cells are a subset of memory T cells that reside within the tissue parenchyma and elicit rapid and robust antiviral immunity at the site of infection, such as airways and lungs (134, 135). hCoVs induce protective lung- and airway-resident $CD69^+$ TRM T cells in young mice (124, 125). Similarly, IAV-specific TRM $CD8^+$ T cells are protective in young hosts (135), but these cells accumulate in aged lungs and elicit inflammatory and fibrosis-associated lung pathology (136). Thus, the role of hCoV-specific TRM T cells in aging needs further investigation. It is important to note that the above conclusions are derived from studies of specific pathogen–free (SPF) mice in controlled laboratory environments. Recent studies suggest that the immune response of SPF mice resembles that of human neonates, while that of barrier-free "dirty," or pet store, mice is similar to that of adult humans (137, 138). Therefore, the use of "dirty" pet store mice in hCoV research might help to increase understanding of human immune response to these viruses.

A summary of immune responses to hCoV infection in young and aged hosts is shown in Figure 1.

Potential role of inflammaging in hCoV pathogenesis

Inflammaging is an age-associated progressive increase in baseline sterile inflammation and is characterized by elevated levels of serum inflammatory mediators such as IL-6, TNF, IL-8, and C-reactive protein, as well as PLA_2G2D as described above (139–141). Inflammaging is considered to be a significant risk factor for various age-related immuno-inflammatory conditions, such as Alzheimer's disease, cardiovascular disease, diabetes, cancer, and autoimmune conditions (142, 143). Inflammaging has a multifactorial origin including age-related chronic activation of immune cells by persistent viruses such as CMV, cellular immunosenescence, impaired clearance of dead or dying cells, obesity, age-related increases in leakage of intestinal microbiota, DNA damage, and excessive release of mitochondrial DNA (139, 140). With advancing age, hematopoietic and nonhematopoietic senescent cells secrete high amounts of inflammatory cytokines (TNF, IL-6, and IL-1β) and chemokines (IL-8 and CCL2) (139, 144, 145). Elevated levels of these inflammatory mediators have a negative impact on the activation and function of both innate and adaptive immune cells. For instance, inflammaging-associated increases in TNF levels correlate with reduced numbers and function of innate cells such as myeloid DCs and pDCs (146). Similarly, increased TNF activity reduces T cell number and function, and impairs B cell class switch recombination and antibody responses (143, 146,

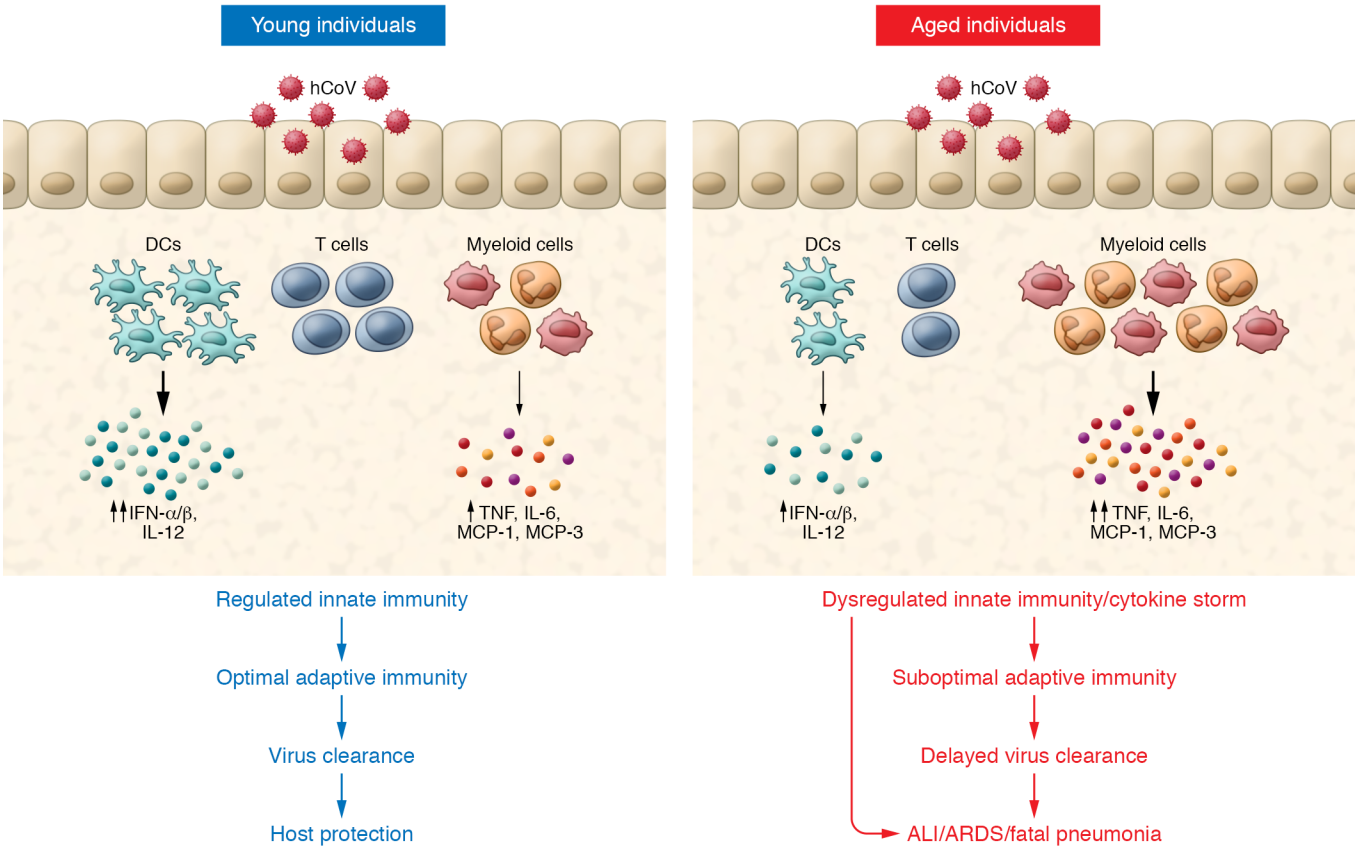

Figure 1. Immune response to respiratory hCoV infection in young and aged individuals. In young hosts, hCoV infection induces an early elevation of IFN-I and IL-12 responses and controlled proinflammatory cytokine/chemokine production, leading to effective adaptive immunity and enhanced virus clearance. In contrast, aged hosts mount reduced and delayed IFN-I and IL-12 responses and excessive proinflammatory cytokine/chemokine responses, leading to excessive inflammation, impaired adaptive immune response, delayed virus clearance, and fatal pneumonia.

147). Although the impact of inflammaging on host immunity to hCoV infections is not well defined, high baseline inflammatory mediators and their potential to dampen hCoV-specific antiviral response may dysregulate host immunity and contribute to severe SARS and COVID-19 pneumonia in the elderly.

Age-related dysregulated inflammation in hCoV pathogenesis

Protective host immunity against an acute virus infection involves successful coordination between innate and adaptive responses that effect pathogen elimination with minimal damage to the host. A regulated protective immunity is characterized by the recognition of viral PAMPs leading to early IFN and inflammatory cytokine (e.g., IL-12) induction that nonspecifically limits virus replication and spread and successfully facilitates the development of effective virus-specific T and B cell responses to clear virus or virus-infected cells, with minimal tissue damage. In response, hCoVs encode numerous proteins that inhibit IFN induction and IFN-stimulated gene expression (reviewed in refs. 148, 149), while promoting excessive inflammation, leading to suboptimal T and antibody cell responses and delayed or impaired virus clearance. This is evidenced by studies in humans and mouse models of severe SARS, which showed that robust and protracted IFN-γ and IFN-α/β responses were associated with failure to elicit a virus-specific antibody response (69, 70, 150). These results highlight the importance of controlled innate immunity in successful transition to an effective adaptive immunity. In agreement with these studies, severe SARS in aged macaques correlated with robust inflammation characterized by increased NF-κB and reduced IFN signaling, without a change in lung virus titers (31). Interestingly, early recombinant IFN treatment reduced SARS severity in mice and macaques with marginal changes in SARS-CoV load in the lungs (31, 70), while IFN treatment at or after the peak of virus infection resulted in increased morbidity and mortality (69, 70). These results suggest a critical role for early IFN response in host protection during aging. Similarly, studies in aged mice showed that disease severity correlated with prolonged inflammatory gene expression and enhanced magnitude and kinetics of a disproportionately strong host innate immune response (48).

Analyses of peripheral blood cells from naive young and aged individuals using multiple omics technologies demonstrated (a) polarization of immune cells toward an inflammatory phenotype, (b) reduced T and B cell receptor diversity and increased clonal expansion, and (c) accumulation of myeloid cell populations with advancing age (151, 152). Using a similar methodology, results from a small cohort of COVID-19 patients and recovered individuals demonstrated an increase in monocytes/macrophages and a decrease in T cell populations in aged patients (152). Further, a

recent study suggests that the SARS-CoV-2–specific T cell and antibody responses are poorly coordinated in aged individuals, contributing to poor outcomes (153). Notably, recent studies showed equivalent nasopharyngeal SARS-CoV-2 RNA levels in young, adult, and aged COVID-19 patients (22, 154–156), suggesting a role for host immune response in facilitating severe disease in the elderly. Although the basis for severe COVID-19 is yet to be established, published COVID-19 reports suggest at least two possible explanations for fatal disease in humans. One school of thought is that SARS-CoV-2 is highly efficient in suppressing the induction of protective antiviral responses, as evidenced by low serum IFN-α/β levels and loss of pDCs (a major source of IFN upon CoV infection) in the peripheral blood of patients with severe COVID-19 compared with those with mild to moderate disease (157, 158). The second possibility is that a delayed but ultimately excessive IFN response facilitates an exaggerated inflammatory response with increased mortality (159–161). As a consequence of either of these initial immune responses, COVID-19 is more severe and is characterized by elevated levels of several serum inflammatory markers (IL-6, TNF, C-reactive protein, GM-CSF, D-dimer, ferritin, and MCP-3) and significantly reduced lymphocyte numbers in the peripheral blood (158–162). SARS studies in aged animals showed low IFN but prolonged inflammatory gene expression compared with younger animals. Based on these results, one can speculate that an initial low IFN response associated with delayed and robust NF-κB–mediated inflammatory cytokine/chemokine responses contributes to severe COVID-19 in the elderly.

Concluding remarks

Although it is well established that SARS, MERS, and COVID-19 are more severe in the elderly, the basis for this increased severity needs further investigation. It is unclear whether the severe disease in aged individuals is caused by impaired virus clearance due to ineffective innate and adaptive immune responses or age-related excessive/dysregulated inflammation, or a combination of the two. Studies of experimentally infected aged nonhuman primates revealed the presence of severe SARS without any change in lung virus burden (31, 50). Similarly, COVID-19 studies show severe disease in the elderly without a change in SARS-CoV-2 load in the upper airways (22, 154, 155). These results suggest a role for robust inflammation in disease pathogenesis. In contrast, severe pneumonia in SARS-CoV-2–infected aged macaques correlates with high virus titers and delayed virus clearance, indicating suboptimal T and B cell responses (61). Additionally, SARS-CoV– and SARS-CoV-2–infected aged mice show high virus titers and/or delayed virus clearance (48, 65), suggesting impaired innate and adaptive immune responses. A thorough examination of virus load in the upper and lower respiratory tract and host immune responses in young and aged individuals is critical to distinguish whether severe hCoV disease in aged individuals is caused by impaired and/or dysregulated host immunity. Nonetheless, therapies directed at suppressing virus replication, controlling excessive inflammation, and improving lymphocyte responses will likely improve disease outcomes in aged individuals.

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