Immune response to COVID-19 in older adults

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The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the third highly pathogenic coronavirus to emerge in the human population in last two decades. SARS-CoV-2 spread from Wuhan, China, across the globe, causing an unprecedented public healthcare crisis. The virus showed remarkable age dependent pathology, with symptoms resembling common cold in most adults and children while causing more severe respiratory distress and significant mortality in older and frail humans. Even before the SARS-CoV-2 outbreak infectious diseases represented one of the major causes of death of older adults. Loss of immune function and reduced protection from infectious agents with age − immunosenescence - is a result of complex mechanisms affecting production and maintenance of immune cells as well as the initiation, maintenance and termination of properly directed immune responses. Here we briefly discuss the current knowledge on how this process affects age-dependent outcomes of SARS-CoV-2 infection.
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SARS-CoV-2 virus and COVID-19 infection

In December 2019, a novel coronavirus was identified as cause of outbreak of severe respiratory illnesses in the city of Wuhan, China.[1] The virus shared 79.6% sequence identity to SARS-CoV, which caused a small global outbreak in 2002, and was thus named SARS-CoV-2. Clinical disease caused by the virus was termed Coronavirus disease-19 (COVID-19). The virus spread globally and in March 2020 World Health Organization declared the outbreak a global pandemic.[2] As of early April, 2021 the virus has infected more than 130 million people globally and caused >3 million deaths.

Of the four families of Coronaviridae (alpha, beta, gamma, and delta), all human corona viruses (CoV) belong to either alpha (229E, NL63) or beta (OC43, HKU1, SARS-CoV,MERS-CoV, and SARS-CoV-2) family,[3] with the latter containing all three CoV highly pathogenic to humans. Four seasonal cold human CoVs (229E, NL63, OC43, and HKU1) account for 10% to 30% of upper respiratory tract infections[4] manifested as common cold, although even these viruses can cause more severe symptoms in frail older subjects.[5]

CoV are single-strand RNA viruses with four structural proteins - S (spike), E (envelope), M (membrane), and N (nucleoprotein) - and multiple ORFs encoding non-structural and accessory proteins.[6] Both SARS-CoV-1 and SARS-CoV-2 enter cells via the interaction between the viral spike protein and the host cell surface enzyme angiotensin-converting enzyme 2 (ACE2),[1,7] although there is evidence for other cell surface molecules such as CD147 (Basignin)[8] and the serine protease TMPRSS2[9] as coreceptors and/or entry co-factors. SARS-CoV-2 binds to ACE2 with 10-20 higher affinity

than SARS-CoV-1, partially explaining the difference in infectivity.[10] ACE2 catalyzes the hydrolysis of angiotensin II and is a critical regulator of renin-angiotensin system and downregulation of ACE2 via virus binding results in disruption of renin−angiotensin system.[11] ACE2 has a protective role in lung,[12] kidney and heart injury.[13] Therefore at least a part of pathology could be a direct consequence of virus binding to the host ACE2 receptor. This is consistent with findings that injection of SARS-CoV-1 spike protein into mice worsens acute lung failure in vivo.[7] ACE2 is expressed in multiple tissues and various epithelial cell types in the respiratory airway, with highest expression in nasal epithelial cells,[14] pointing to these cells as possible loci of original infection. SARS-CoV-2 has a direct cytopathic effect on human airway epithelial cells.[15] Viral shedding primarily occurs from upper respiratory tract but fecal shedding also occurs[16] and is often used for early epidemiological detection.[17]

Person to person transmission is thought to occur mostly by droplets, although evidence supports the possibility of airborne transmission to a lesser extent.[18] Fomite transmission, although possible, presents low risk in real life situations.[19] The possibility of fecal-oral transmission also cannot be excluded.[20,21] Pharyngeal virus shedding is highest before or early after onset of symptoms[16,22] and the majority of transmission is estimated to occur from presymptomatic or asymptomatic subjects,[23] explaining why traditional epidemiological measures were unsuccessful in stopping SARS-CoV-2 spread.

Incubation period is 5.7 days on average[24] but SARS-CoV-2 infection remains completely or largely asymptomatic in 20-40% people.[25,26] In symptomatic humans, disease severity ranges from mild flu-like disease to severe respiratory syndrome and death. Clinical picture is nonhomogeneous with most common symptoms being fever and cough.[27] Disease follows a severe course in up to 20% of subjects with acute respiratory distress syndrome (ARDS) as the most frequent complication.[28] Other complications include myocarditis[29] and kidney injury.[30] The vast majority of severe cases, hospitalizations and deaths (8 out of 10) occur in people above 65 years of age,[31] and even people 50 and older exhibit sharp increases in hospitalization (4x) and mortality (10x) relative to those 18-29 years of age. The numbers for those >85 years of age are staggering − 13-fold more hospitalizations, and 630-fold higher likelihood of death than those 18-29 years old (cdc.gov/coronavirus). The elderly often present atypically[31] with lower incidence of fever.[32] COVID-19 shows remarkable age-specific outcomes with log-linear increase in infection fatality rate by age among individuals older than 30 years.[33,34] Other than age, risk factors for severe disease include hypertension, obesity, smoking, type 2 diabetes and male sex.[35] Of interest, age-related frailty assessed by a clinical scale was found to be associated with COVID-19 severity in a prospective cohort study of humans >60 years.[36] Another study showed, disease outcomes were better predicted by frailty than either age or comorbidity.[37] Frailty is a geriatric syndrome characterized by reduced energy

levels, muscle loss and increased vulnerability associated with a hyperinflammable state and elevated levels of proinflammatory cytokines, particularly IL-6.[38,39] This preexisting hyperinflammable state could be contributing to COVID-19 severity which is associated with overactivation of the innate immune system.[40] Prevalence of frailty syndrome is markedly increased in persons above 80 years[41] but physiological and functional changes are distinct from the usual age-related changes.[42]

To effectively protect older adults against SARS-CoV-2, one must dissect potential contributors to the above age-related susceptibility to COVID-19. Age related decreases in respiratory function have the potential to account for high incidence of respiratory symptoms among the elderly.[43] However, chest computer tomography did not show increased lung damage in elderly despite increased disease severity,[44] suggesting that lung aging by itself may not be the determining factor of severe COVID-19. Consistent with that, severity was associated with damage to other organs, mainly heart, liver and kidneys.[35] On the other hand, hypercoagulopathy likely plays a role in organ damage and anticoagulant therapy has been both shown to reduce mortality[45] and is widely used in suspected severe COVID-19 cases. SARS-CoV-2 is able to infect vascular endothelial cells which express high levels of ACE2.[46,47] Resulting endothelial injury and inflammation induces a hypercoagulative state and increased thrombotic events.[48] In addition to direct cytopathic effect of the virus, the downregulation of ACE2 and endothelial damage, there is abundant evidence that immune system dysregulation plays a major role in COVID-19 tissue injury.[49−53] There is evidence that human leukocyte antigen (HLA) class I molecules play a role, as HLA-A*01:01 allele was associated with higher risk of severe COVID-19.[54] A genome-wide association study performed on >2000 intensive care patients found 9 loci associated with severe COVID-19, out of which 5 were genes linked to the immune system, most notably low expression of interferon receptor gene IFNAR2 and high expression of chemotactic receptor CCR2.[55] Immune correlates of disease severity or protection are studied intensively. However, even a year into the pandemic, we do not understand the precise role and importance of the immune aging in the pathogenesis and severity of COVID-19. At least one major obstacle to understanding how aged immune system alterations translate into higher risk of COVID-19 in older adults is lack of adequate (aged) animal models.[56] SARS-Cov-2 virus causes upper respiratory tract infection in Syrian hamsters and ferrets, with mild clinical symptoms and transmission to cage mates.[56,57] However, in both cases there is no resource for aged animals. Only mild clinical disease has been reported in non-human primates[58] but more severe pneumonia and increased viral replication was observed in aged rhesus macaques[59] highlighting the need for aged animals. Viral spike protein of both SARS-CoV-1 and 2 does not bind to mouse ACE2[60] so several transgenic mice expressing human ACE receptor have been developed[61−63] but availability of aged animals is scarce. A different approach is to use mutagenesis to develop mouse adapted viral strains. A

recombinant SARS-Cov-2 virus which can infect BALB/c mice was developed and showed age related pathogenesis.[64] While the immune system is necessary for viral clearance and while severe cases show delayed viral clearance,[65] immune hyperactivation is associated with pathogenesis. Innate immune responses are initiated after viral components, mostly ss and dsRNA, are recognized by pattern recognition receptors (PRR). Their activation induces type I interferon (IFN-I) responses that engender inflammatory cytokine cascades important for limiting viral spread. Initiation of these inflammatory signals leads to recruitment of immune cells to sites of infection starting with neutrophils. Antigen presenting cells, most notably dendritic cells, present viral peptides on MHC molecules to initiate T cell responses, whereas parallel activation of B cells by soluble virus epitopes initiate humoral (antibody) responses. Here, we will briefly outline age-related changes to these processes and how they might directly contribute to poor COVID-19 prognosis.

Age related changes of the immune system

Aging results in multiple measurable alterations in the innate and adaptive arms of immunity. Termed immunoscenescence, this process leads to a variable but often marked reduction of immune protection against infections that is deleterious to the health and wellbeing of a substantial fraction of older adults.[66]

Age-related defects in innate immunity can broadly be attributed to decreased phagocytic capacity and impaired/delayed migration, differentiation, and cytokine production by innate immune cells. Neutrophils display reduced cytokine signaling and effector molecule production in older adults. Defects in specific pattern recognition receptor (PRR) expression and signaling have been shown to partially account for the hampered responsiveness of old neutrophils to pathogens. These changes have been correlated with poor prognosis in bacterial infections including sepsis.[67−69] Old macrophages also exhibit reduced migration and phagocytosis, that interestingly leads to reduced removal of dying inflammatory neutrophils in the lung of old mice during influenza infection, suggesting that similar mechanisms could feed into severe COVID-19 pathology.[70,71] Old NK cells exhibit a more mature phenotype and have depressed cytokine secretion and cytotoxic potential. This could be due, in part, to alterations in the expression of activating and inhibitory receptors. In the ectromelia (mouse pox) model, these defects have been shown to explain increased viral susceptibility in old mice.[72,73] Finally, old dendritic cells are less efficient at capturing and processing antigen, which leads to their reduced activation and consequent suboptimal activation of naïve T cells.[74,75] All innate cells subsets exhibit more or less pronounced defects in migration, although it remains to be shown whether these defects occur due to underproduction or dysregulated production of chemokines directing their migration, or to the inability of cells themselves to appropriately respond to chemokine cues.[76] Another

possibility is increased production of negative regulators of chemotaxis such as prostaglandin D2.[77]

There are both quantitative and qualitative changes in B cell function with age. The absolute quantity of both bone-marrow resident B cell progenitors and their naïve daughter cells is reduced with age, leading to underproduction of new naïve B cells. Functionally, the formation and output of germinal center (GC) reactions in primary and secondary responses are both impaired in old age.[78] Defects in the GC reaction is the result of age-related decline in function of both follicular dendritic cells (FDCs) and T cells, along with intrinsic defects within B cells themselves. Decreased FDC functionality is in part due to lower expression of Fc receptors, leading to impaired antigen capture and presentation, while defects in CD4 help may stem from decreased expression of CD40L, an important costimulatory molecule in GC reactions.[79,80]

Antibodies produced in old mice following B cell activation are of lower quality compared to those produced in adult mice. One apparent cause of this defect is the impaired production of the E2A gene-encoded E47 transcription factor.[81] With age, dysregulation of the expression of the mRNA-degradation promoting protein ZFP36 increases with age leads to a higher turnover rate of E47 mRNA in older animals. This instability leads to under-induction of activation-induced cytidine deaminase (AID). Because AID plays a crucial role in both class-switch recombination and somatic hypermutation in activated B-cells, the culmination of these defects is the production of antibodies of inferior avidity and function in old mice.[81,82]

Multiple age-associated defects appear in the T cell compartment in advanced age. The earliest hallmark of T cell aging is thymic involution, marked by degeneration and atrophy of thymic stroma and a concordant and progressive reduction in naïve T cell output.[83] While the homeostatic maintenance of naïve T cells in peripheral lymphoid organs becomes the dominant means of retaining the naïve T cell pool, this process also gradually weakens with aging.[84] Eventually, in the last third of life this leads to reduced diversity of the T cell receptor (TCR) repertoire, and a relative (and in the presence of cytomegalovirus, absolute) accumulation of memory T cells, potentially producing holes in the T cell repertoire mobilized against a given epitope or pathogen.[85,86]

This general collapse of naïve T cell homeostasis is accompanied by decreased primary (new) T cell responses in magnitude and differentiation. This is likely a combination of reduced naïve T cell numbers (and perhaps diversity) and of cell-extrinsic defects in peripheral lymphoid organ structure,[87,88] that fail to orchestrate coordinated and efficient movements and cell-cell communication in the course of primary responses.[89] Studies of human blood have shown that CD8 T cell responses may be either more or differently impacted by aging relative to CD4 T cells.[90,91] However, in both aged mice and humans, it has been demonstrated that naïve T cells are less functional following priming as measured by cytotoxic function, cytokine production, and proliferative capacity.[92,93]

Immunological features of COVID-19 in aged subjects

SARS-CoV-2 virus control seems to be directly related to COVID-19 severity, as virus load in severe cases was found to be higher and clearance delayed compared to mild cases.[65] Even after adjusting for age and comorbidities higher viral load was predictive of mortality.[94] Peak viral load was increased in aged humans suggesting that immune system in elderly is less able to counteract viral replication and spread.[95] The initial step in counteracting viral spread is induction of anti-viral defenses in different cells by type I interferons. SARS-Cov-2 evades IFN-I response more efficiently than MERS and SARS-CoV-1 via its nsp1 and nsp6 proteins which suppress IFN-I signaling.[96] Impaired IFN-I responses in white blood cells of severe COVID-19 patients were found to be associated with a persistent viremia and exacerbated inflammatory response.[97] In contrast, bronchoalveolar lavage fluid of severe COVID-19 patients showed increased expression of IFN stimulated genes.[98] Contradictory findings regarding IFN-I responses and COVID-19 severity might be explained by differences in sampling time and tissue sampled, and resolving them would be highly significant as recombinant IFN-I is currently investigated as potential COVID-19 treatment.[99] Progression to severe clinical picture is associated with hyperactivation of the immune system manifested by increased levels of inflammatory cytokines in circulation, also called "cytokine storm".[40] Levels of C-reactive protein (CRP) and interleukin-6 (IL-6) were particularly strongly associated with increased mortality.[100] Other cytokines elevated in severe cases included IL2, IL7, IL10, GSCF, IP10, MCP1, MIP1A, and TNFα.[101] Basal IL-6 and TNF-α levels in circulation increase with age, a condition sometimes termed inflammaging,[102] and increased IL-6 is in particular associated with age-related frailty and all cause mortality.[38,103] Given that IL-6 levels predicted COVID-19 mortality, blocking IL-6 signaling was considered a promising therapeutic target; however, clinical trials of tocilizumab and sarilumab, monoclonal antibody directed against IL-6 receptor, have yielded mixed results on survival in hospitalized COVID-19 patients[104,105] for reasons not understood at this time.

In addition to increased cytokine levels, multiple cellular immunity defects are associated with COVID-19 severity. Severe cases show decreased lymphocyte blood counts and increased neutrophil counts, such that the neutrophil to lymphocyte ratio emerged as an independent predictor of mortality.[106] Autopsy samples from the lungs displayed neutrophil infiltration in pulmonary capillaries,[107] raising the possibility that reduced removal of neutrophils by alveolar macrophages with aging, which contributes to severity of influenza in old mice, could be at play here too. Microvascular thrombi, containing neutrophil extracellular traps associated with platelets and fibrin were found in the lung, kidney, and heart post mortem.[108] Activated neutrophils are known to contribute to large-vessel thrombosis[109] and excessive reactive oxygen species production is also suspected to contribute to tissue damage in severe COVID-

19.[50] Previous research in rodent viral[71] and bacterial[110] models showed that aged animals displayed increased infiltration of neutrophils in lungs which contributed to pneumonia severity. This excessive neutrophil infiltration was associated with increased chemokine production by senescent epithelial cells[71] and impaired toll like receptor activation.[110] Lymphopenia in severe cases affected primarily T lymphocytes, particularly CD4+ and CD8+ T cells.[111] Multiple reports showed a decrease in naïve CD4+ T cells in bloodstream of severe cases and increased expression of activation markers such as CD38 and HLA-DR.[111–113] Although decreased naïve T cells in the blood were associated with severity most of these studies lacked older participants with moderate disease so some of the observed phenotypes might be features of aging by itself. Moderate cases were characterized by the presence of highly clonally expanded CD8+ in bronchoalveolar lavage fluid suggesting a strong T cell response is protective.[114] Antigen specific CD4+ T cell responses correlated with SARS-CoV-2 specific IgG and IgA antibody titers.[115] However, SARS-Cov-2 specific T cells responses have been detected in up to 40% healthy controls[116–118] leading to hypotheses that cross reactive memory T cells from previous common cold CoV infection might be protective.[119] Ex vivo peptide stimulation revealed a range of preexisting memory T cells that are cross-reactive between SARS-CoV-2 and the common cold coronaviruses. Cross-reactivity was associated with epitopes derived from SARS-CoV-2 spike, N, nsp8, nsp12, and nsp13 proteins.[120] At the moment, it is unclear whether and how the presence and the exact specificity of these cross-reactive T cells affects disease severity.

While lymphopenia affected B cells to a lesser extent,[121] there were pronounced oligoclonal expansions of plasmablasts in severe cases.[122] Overall antibody titers were increased in severe cases and were not affected by age.[123,124] However, in elderly subjects neutralizing antibody titers were less correlated with antigen specific CD4+ and CD8+ T cell responses, suggesting that potential lack of coordination in adaptive immune responses may contributes to disease severity.[125]

The protective ability of early adaptive immune responses is highlighted by development of multiple successful SARS-CoV-2 vaccines (Figure 1). While several vaccine candidates are still in clinical trials, two mRNA vaccines have been approved and are in mass use in the US as of December 2020.[126] Both of these vaccines were shown to induce neutralizing antibodies and Th1 cell response[127,128] and reduce the incidence of symptomatic and severe COVID-19 with high efficacy even in participants 65 years of age or older.[129] It remains to be seen how broadly protective and durable these responses will be in older adults.

In lieu of a conclusion...

We have learned an extraordinary amount of information about the virology, pathogenesis and immunology of SARS-COV-2 over the past year, with >100,000 papers containing COVID-19 and/or SARS-CoV-2 listed in
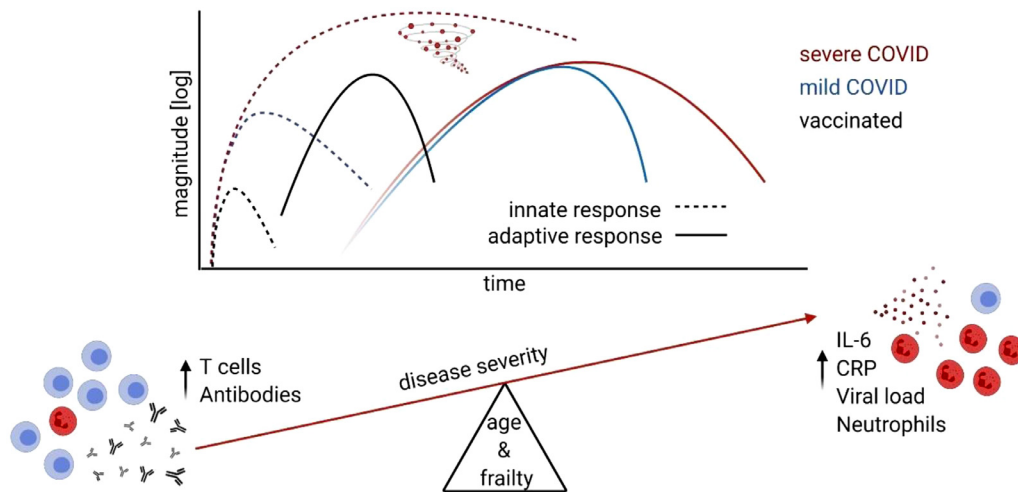
Figure 1 Severe cases of COVID-19 are characterized by prolonged hyperactivation of innate immunity manifested by increased levels of inflammatory cytokines in circulation, also called "cytokine storm" as well as increased neutrophil count. This primarily occurs in aged and frail subjects. Development of successful vaccine shows that early adaptive immune response from T cells and neutralizing antibodies is protective and prevents the severe course of COVID-19.

PubMed and preprint servers in 2020 alone. However, we still very much lack a comprehensive picture of the disease and of virus pathogenesis, as well as of the interactions of SARS-CoV-2 with its (human) host. It is evident that COVID-19 severity is associated with delayed viral clearance, hyperactivtion of the innate immune system, increased antibody titers and T cell lymphopenia (Figure 1). At the moment, it is unclear how frailty and aging predispose for these phenotypes and increased severity. Below, we outline some of the most burning immunological questions, and hope that these and related questions will be answered with utmost urgency:

- ☐ Are innate sensors specifically disabled in older adults to make them more vulnerable to COVID-19?
- ☐ Are there aging-related cytokine dysfunctions similar to recently discovered type I IFN genetic defects that underlie severe COVID-19 in older adults? Do they kick in only when the older adaptive immune system cannot terminate infection on time?
- ☐ Do low numbers of naïve T and B cells with aging predispose towards poor immunity and poor outcomes?
- ☐ Is the reduced diversity of the T and B cell response with age linked to impaired immune responses?
- ☐ Can the older immune system target all the key antigens of the virus, or is the virus more likely to slip by it?
- ☐ Do the remaining CD4 and CD8 cells respond with correct and strong effector function?
- ☐ Is the sum of immune defects sufficient to permit variant selection in in older adults?
- ☐ Do prior coronavirus infections differentially shape the ability of the older immune system to respond to SARS-CoV-2?
- ☐ Do older adults generate long-lived and protective memory responses?

- ☐ Do T memory responses in older population target the same array of virus epitopes as in adults?
- ☐ Are virus escape variants more likely to slip by older T memory (Tm) responses?
- ☐ How well will older T cells respond to SARS-CoV-2 vaccination?

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