Respiratory viral infections in the elderly: From the perspective of the aging immune system

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GRAPHICAL ABSTRACT

Defective barrier function
Delayed interferon secretion
Excessive inflammation
Decreased affinity and diversity of antibodies
Impaired cytotoxic T cell responses
Dysregulated antiviral immune responses in the elderly

Assessment of immune status
- Modulating vaccines
- Making use of trained immunity
- Targeting cytokines
- Eliminating senescent cells
- Regulating nutrient sensing pathways

Individualized prevention and treatment strategies

Worse clinical outcomes after respiratory viral infections
Improved prognosis

PUBLIC SUMMARY

■ The elderly have a worse prognosis after respiratory viral infections.
■ Aging immune system with low efficacy leads to ineffective viral clearance.
■ Individualized assessment of immune status is necessary for appropriate treatment.
■ Modulating the aging immune system is a crucial treatment strategy.
Respiratory viral infections in the elderly: From the perspective of the aging immune system

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The susceptibility of the elderly to respiratory viral infections and the challenges posed by an aging population necessitate imperative development of advanced preventive and therapeutic strategies for elderly individuals. The clinical outcome of such infections is intricately determined by the complex interplay among viruses, host tissues, and immune cells. Elderly individuals exhibit a diminished efficacy of their immune system to clear viruses, consequently leading to prolonged viral insults, tissue damage, and an excessive activation of inflammatory cells. These ultimately result in worse clinical outcomes. Targeting the dysregulated antiviral immune responses has emerged as a potential approach to improve the prognosis of geriatric patients. It is noteworthy that the impacts of aging on antiviral immune responses are highly heterogeneous. Thus, individualized patient assessment and management assume paramount importance. This review aims to summarize the current evidence elucidating the effects of aging on immune responses to respiratory viruses, with the ultimate goal of identifying knowledge gaps that can inform future research and enhance the management of elderly individuals.

INTRODUCTION

Lower respiratory infection was the fourth leading cause of both death (2.6 million) and disability-adjusted life-year (105.6 million) worldwide in 2019, as estimated by the World Health Organization.1 Viruses are important pathogens leading to lower respiratory infections, which have been previously overlooked due to diagnostic limitations.2,3 Recent investigations focusing on hospitalized adults with community-acquired pneumonia in the US and in China have demonstrated that respiratory viruses are detected more frequently than bacteria.4 Moreover, the pandemic of Coronavirus disease 2019 (COVID-19), which has led to over 760 million confirmed cases and 6.9 million deaths globally as of June 2023, elicits global attention to respiratory viral infections.5

For a long time, it has been observed that the elderly are more susceptible to respiratory viral infections than young adults. Epidemiological studies across different countries (e.g., China, South Korea, the US, the UK, Spain, Australia, South Africa, etc.) have consistently shown an age-dependent increase in medical visits, hospitalizations, and mortality rates associated with seasonal influenza and respiratory syncytial virus (RSV) infections.6,7 However, the peak of excess mortality occurred in the young adult group rather than the elderly during the 1918 Spanish flu pandemic and the 2009 H1N1 pandemic.8,9 This can be potentially explained by the immunological memory protection resulting from previous exposure to similar strains among the elderly, as well as the effects of immune imprinting and excessive immune responses among young adults.10 These theories suggest that immune memory complicates our understanding of the incidence and progression of infections when aging. Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is a newly emerging pathogen to which the population lack prior immunity, providing valuable insights into how aging influences the severity of respiratory viral infections during primary infection.

According to surveillance data from the US Centers for Disease Control and Prevention (CDC) until June 2023, the rates of COVID-19-related hospitalization and death consistently increase with age. Specifically, compared to individuals aged 18 to 29 years, the rate ratios of hospitalization are 5.0, 9.3, and 15.0 among those aged 65 to 74, 75 to 84, and above 84 years, respectively. Similarly, the rate ratios of death are 60, 140, and 360 in the same three age groups, respectively.10 In a meta-analysis using a Bayesian hierarchical model and data from 36 countries prior to vaccinations, it is estimated that the infection-fatality ratio (IFR) exponentially increases with age in adults. The estimated IFR values are 0.0573% for individuals aged 30 years, 1.0035% for 60 years, and 20.3292% for 90 years.25 Collectively, these findings prompt us to question why the elderly typically experience worse prognosis following respiratory viral infections.

In 2015, approximately 8.5% of the global population (617 million people) was aged 65 years or older. This number is estimated to soar to nearly 20% (1.6 billion people) by 2050,11 corresponding to a substantial vulnerable population at risk of severe outcomes after respiratory viral infections. Thus, it is important to investigate the mechanisms that contribute to the susceptibility of the elderly and explore viable solutions.

The immune response against respiratory viral infections is a finely modulated process (Figure 1). In clinical practice, both antiviral therapy and host-targeted therapy have shown efficacy in improving the prognosis of elderly patients with such infections. Unfortunately, the benefits of these approaches are limited, because the outcome of respiratory viral infections is determined by the complicated interactions among viruses, host tissues, and immune responses (Figure 2). We propose that the aging process exert a profound impact on the immune system, resulting in delayed clearance of invading viruses, accompanied by overt inflammatory responses and prolonged tissue damage. These factors collectively contribute to the development of more severe disease following respiratory viral infections among the elderly. One piece of evidence supporting our hypothesis is that vaccination, which can augment adaptive immune responses against viral infections, reduces the incidence of severe disease and death in the elderly. Additionally, it is important to note that the elderly are predisposed to concomitant diseases, many of which can influence immune system functions, such as type 2 diabetes,12 chronic kidney disease,13 and chronic obstructive pulmonary disease.14 How these comorbidities affect anti-infection immune responses have been comprehensively reviewed elsewhere. This review will summarize the age-related changes in antiviral immune responses and provide an overview of current management strategies, aiming at figuring out future directions that can improve the prognosis of the elderly affected by respiratory viral infections.

AGING INNATE IMMUNE RESPONSES: WEAKENED VIRAL CLEARANCE, EXAGGERATED INFLAMMATORY RESPONSES, AND COMPROMISED ANTIGEN PRESENTATION

When respiratory viruses invade the lungs, the innate immune response is rapidly activated to control viral replication. Simultaneously, it triggers the...
adaptive immune response to target the viruses more specifically and efficiently. Numerous types of innate immune cells actively engage in this process.

Respiratory epithelium

Respiratory epithelium provides first-line defense against inhaled viruses. Airway epithelial cells secrete mucus to trap inhaled pathogens and their microtubule-based cilia transport mucus through rhythmic beating. Comparisons between nasal epithelial cells isolated from elderly individuals and those from young adults have revealed a higher incidence of ciliary ultrastructural abnormalities, reduced ciliary beat frequency, and impaired particle clearance in the elderly. Impaired clearance capacity has been observed in small airways as well. Additionally, the fluid secreted by airway epithelial cells contains anti-microbial molecules, including surfactant protein A, surfactant protein D, complement protein, and hydrolases. Murine study has demonstrated altered composition of the alveolar lining fluid when aging, including the decreased levels of hydrolases.

Airway epithelial cells are early detectors of viral insults due to their abundant expression of pattern recognition receptors (PRRs) that sense viral pathogen-associated molecular patterns (PAMPs). Upon activation of PRR signaling pathway, these cells produce an array of chemokines, cytokines, and antimicrobial factors to recruit and activate circulating immune cells. Meanwhile, respiratory epithelial cells express major histocompatibility complex (MHC) molecules, enabling them to present antigens to immune cells. When infected with influenza virus A H3N2 in vitro, primary differentiated human nasal epithelial cells (iNBEs) from different age groups showed similar cellular viability and ability to secrete pro-inflammatory cytokines. However, expression levels of molecules critical for antigen processing and presentation were significantly downregulated in the elderly group. This led to a weaker cytotoxic T lymphocyte (CTL) response to clear infected cells, accompanied by reduced production of antimicrobial molecules IFITM1 and MX1. Also, type II alveolar epithelial cells (AECs) in aging mice exhibited an elevated capacity to secrete prostaglandin E2 (PGE2), partially driven by senescence, which in turn impaired the mitochondrial function and restricted proliferation of alveolar macrophages (AMs). Blocking PGE2 signaling improved the survival of aging mice after influenza infection.

Epithelial cells, as the main target of respiratory viruses, are disrupted during infection. Their prompt regeneration to restore tissue integrity is of critical importance for the recovery of infected individuals and the prevention of secondary infections. In influenza-infected mice, aging was associated with a greater loss of both type I and type II AECs. Worse still, there was a delay in the differentiation of club cells into type II AECs. Taken together, these age-related alterations in airway epithelium impose a heavier burden of viral insults on the immune system and result in more extensive tissue damage.

Alveolar macrophages and monocytes

AMs are non-circulating, self-renewal, lung-resident immune cells. They are one of the crucial first-line responders to viral infections with expressions of numerous PRRs and can recruit various immune cells to the lungs. AMs possess strong phagocytic abilities to clear pathogens, infected cells, and apoptotic cells. AMs originate from the fetal liver during embryogenesis, and later from circulating bone marrow-derived monocytes after birth. These two subpopulations display distinct metabolic and cytokine profiles. However, further exploration is required to understand how the proportion of these two subsets affects the outcomes of viral infections.

In respiratory viral infections, circulating monocytes are recruited to respiratory tracts in response to chemokines, where they can secrete type I IFNs
to clear pathogens and release chemokines to attract additional immune cells. Meanwhile, monocytes can differentiate into dendritic cells (DCs) and macrophages to serve as antigen-presenting cells. The expression levels of multiple PRRs on monocytes remain unaltered in the elderly compared to the young. However, the secretion of cytokines (e.g., IFN-α, IFN-γ, and IL-1β) and chemokines (e.g., CCL20 and CCL8) in response to PRR agonists was reduced and delayed from cells of the elderly. Specifically, following A/PR8 influenza infections in vitro, circulating monocytes from geriatric donors produced notably less type I IFN. This process was regulated by both primary and secondary retinoic acid-inducible gene I (RIG-I) signaling through the adaptor protein tumor necrosis factor receptor-associated factor 3 (TRAF3) and the transcription factor interferon regulatory factor 8 (IRF8), respectively.

**Neutrophils**

Neutrophils have a dual role in viral infections. They are important in clearing viruses through direct phagocytosis, secretion of antimicrobial agents, formation of neutrophil extracellular traps (NETs), and deposition of trails to guide virus-specific CD8+ T cell migration. However, their overt and prolonged activation can cause immunopathology through excessive production of gelatinases and collagenases, oxidative burst, and NETs formation. In mice, aging was associated with excessive neutrophil infiltration in the lungs during influenza strain PR8 infection, primarily attributed to upregulated secretion of CXCL1 and CXCL2 from local senescent AECs. Another contributing factor was the impaired clearance of neutrophils by AMs, as the AMs have a dampened phagocytic ability and a lower expression level of the scavenging receptor CD204. Depleting neutrophils prior to PR8 infection numerically increased mortality rates of older mice while depleting at day 6 post-infection significantly reduced lung damage, local inflammatory cytokine levels, and mortality rates without affecting viral elimination. However, in young mice, the pre-infection depletion markedly increased mortality rates, whereas the depletion at day 6 only numerically reduced mortality rates. These findings indicate that aging leads to defective antimicrobial function of neutrophils during the early stage of influenza infection, accompanied by a prominent immunopathological effect during the late phase.

**Natural killer cells**

Natural killer (NK) cells can be categorized into three distinct subsets, namely CD11b+CD56dim cytotoxic NK cells, CD27+CD56high regulatory NK cells, and CD27-CD11b+CD56bright tolerant NK cells. The precise role of NK cells in respiratory viral infections has yet to be fully elucidated. On the one hand, recruited NK cells have been proven to eliminate viruses by the interaction with hemagglutinin (HA) molecules through natural cytotoxicity receptors Nkp44 and Nkp46, leading to lytic effects on infected cells. On the other hand, the accumulation of activated NK cells in the lungs may cause immunopathology, as evidenced by the beneficial effects of NK cell depletion on the mortality of influenza-infected mice, particularly when high-dose influenza was utilized. In aging mice, NK cells exhibited a reduced number in the lungs, less mature phenotypes, curtailed cytotoxicity, and decreased IFN-γ production compared to young mice after intranasal influenza infection. These alterations were associated with delayed viral clearance, greater weight loss, and decreased survival.

**Dendritic cells**

DCs from three distinct developmental lineages, including conventional dendritic cells (cDCs), plasmacytoid dendritic cells (pDCs), and monocyte-
derived DCs (moDCs), collectively constitute the dendritic cell population in the lungs. cDCs are key antigen-presenting cells bridging innate and adaptive immune responses. Both of the two important pulmonary cDC subsets, CD103+ DCs and CD11b+ DCs, can migrate to draining lymph nodes to activate antigen-specific T cells once they capture viruses in the lungs. moDCs also possess antigen presentation capabilities and have been shown to adopt a pro-inflammatory phenotype during influenza infection to mediate tissue injury in mice. Aging was correlated with a delayed cDC expansion in the lungs following influenza strain PR8 infection in mice; worse still, these cDCs exhibited poor activation status. Moreover, during SARS-CoV and influenza A virus infection, the augmented prostaglandin D2 (PGD2) expression in the lungs resulted in decreased DC migration to draining lymph nodes in aging mice, subsequently priming fewer antigen-specific T cells. Treatment with PGD2 antagonists reversed this phenomenon and improved the survival rates of mice. In vitro studies using human peripheral blood mononuclear cells (PBMCs) to generate moDCs have demonstrated that influenza virus H3N2 induced poor maturation of moDCs from older individuals in an autocrine TNF-a-dependent manner, and the antigen-specific CD8+ T cell responses were then attenuated. Consequently, elderly individuals exhibited impaired elicitation of adaptive immune responses, which are crucial in controlling viral infections. pDCs can secrete type I IFNs to combat viruses. Circulating pDCs isolated from aged individuals secreted lower levels of type I and III IFNs in response to stimulation with inactivated influenza virus A/PR/8/34 in vitro compared to young individuals, potentially mediated by decreased phosphorylation of IRF-7. Besides, moDCs from the elderly have also exhibited deficient production of type I and III IFNs.

AGING ADAPTIVE IMMUNE RESPONSES: IMPAIRED EFFICIENCY TO CONTROL INFECTION

It is the efficient and specific adaptive immunity that lies at the core of complete viral elimination. A clinical study revealed some patients whose B cells were depleted with anti-CD20 therapy could survive viral infection, but the viral clearance was much delayed. This demonstrates that the two arms of the adaptive immunity have some redundancy in viral control, but their coordination is essential for achieving high-efficiency viral clearance. Given that the adaptive immunity is highly dependent on complex cell-cell interactions, it is more vulnerable to immune defects accumulated during aging. Therefore, we propose that the dysregulation of the adaptive immune responses could be a major cause of delayed viral clearance and severe clinical outcomes observed in the aged patients.

Limited plasticity of aging lymphocytes

When exposed to a pathogen for the first time, naïve lymphocytes are activated and differentiated. The shrinkage of naïve lymphocytes in the elderly has been extensively studied. This can be attributed, in part, to the accumulation of oxidative DNA damage in hematopoietic stem cells (HSCs) as they age, which impairs their self-renewal capacity. Meanwhile, HSCs tend to favor myeloid lineages over lymphoid lineages, especially lymphoid B lineage. Age-related changes also occur in the microenvironment of lymphoid organs such as the bone marrow, thymus, and lymph nodes, which are critical for lymphocyte generation and maintenance. Bone marrow stroma would be gradually occupied by fat when aging, providing poor support for HSCs. In the thymus, the thymic epithelial space shrinks with age, and the cytokine expression profiles resemble those of adipose tissue, leading to restricted generation and maintenance of naïve T lymphocytes.

T-cell receptor (TCR) and B-cell receptor (BCR) are surface molecules of lymphocytes that recognize antigen fragments presented by MHC molecules. Numerous studies have demonstrated that the diversity of TCR or BCR repertoire contracts with selective clonal expansions when aging. Specifically, one study tracked the evolution of TCR repertoire longitudinally over 2 years, presenting a progressive reduction in repertoire diversity accompanied by expanded clonality of CD8+ T cells. Not only does the overall repertoire narrow, but the repertoire specific to a particular pathogen also becomes limited. Studies have found that TCRs recognizing antigens from influenza virus or SARS-CoV-2 had restricted diversity and enhanced cross-reactivity in older individuals. The diminished number of naïve cells and the narrowed repertoire of BCR/TCR partially result in a relatively poor adaptive response to novel pathogens, which in turn correlates with increased severity following respiratory viral infections.

B lymphocytes

Activated naïve B lymphocytes can differentiate into plasma cells to produce antibodies and clear viruses in an antigen-specific manner. T follicular helper (Tfh) cells, located in secondary lymphoid organs, are responsible for generating antigen-specific B cells. Studies in aged mice have revealed a reduction in fully differentiated NP-specific Tfh cells in germinal centers (GCs) following intranasal influenza PR8 infection, which was correlated to compromised GC development and antibody generation. Furthermore, on day 14 after infection, aged mice exhibited more NP-specific T follicular regulatory (TfR) cells, suppressing the responses of both Tfh and B cells in an antigen-specific manner. In one study, the general levels of elicited SARS-CoV-2-specific antibodies were shown to be similar across different age groups, but IgG tended to bind non-structural/accessory proteins in patients older than 70 years with severe COVID-19. Similarly, another study showed that elderly individuals generated antibodies against shared antigens of human coronaviruses (HCoVs), such as nucleoprotein (NP) and S2, after SARS-CoV-2 infection. In contrast, children were able to produce more targeted antibodies against SARS-CoV-2 owing to fewer prior HCoV infections. The failure to effectively generate antibodies with high neutralization potency is associated with increased disease severity following infections.

T lymphocytes

During respiratory viral infections, CD8+ T cells are activated to exert a direct cytotoxic effect on infected cells. In aged mice, the activation of NP-specific CD8+ T cells in the lungs was delayed with a decreased peak after intranasal influenza strain PR8 infection. This phenomenon may be attributed to the expansion of regulatory T (Treg) cells, which are known to impair antiviral CD8+ T cell responses. Moreover, Treg cells from the aging subjects expressed higher levels of activation markers. A similar phenomenon has been observed in SARS-CoV-2 infection. When stimulated with peptides derived from SARS-CoV-2, CD8+ T cells from older donors could not differentiate into IFN-γ+, TNF+, or CD107+ T cells as efficiently as those from younger donors. Additionally, the cytotoxicity of CD8+ T cells was compromised in COVID-19 patients over 80 years old. T cells are involved not only in the clearance of respiratory viruses, but also in immunomodulatory processes during post-infection recovery, such as CD8+ tissue-resident memory T (TRM) cells and Treg cells. In aging mice, elevated TGF-β signaling in the lungs led to the accumulation of TRM cells during the repairment stage. Depleting these lung-resident CD8+ T cells, but not those in lymphoid organs or peripheral blood, could reduce infiltration of monocytes and neutrophils, decrease local expression of multiple pro-inflammatory cytokines and chemokines, and ultimately alleviate tissue inflammation and chronic fibrosis. This suggests the detrimental role of TRM cells in lung sequelae after respiratory viral infections when aging. As for Treg cells, RNA sequencing and DNA methylation analysis of lung-resident Treg cells revealed a deficient reparative response but a profound pro-inflammatory phenotype in aged mice, which was regulated by DNA methylation. Transfer of splenic Treg cells from young mice to aged mice 24 hours after infection significantly improved survival.

Memory T lymphocytes play a crucial role in mounting a robust and efficient immune response upon reinfection with a previously encountered pathogen. However, the functionality of these cells has been found to vary with age, although the findings have been inconclusive. One study demonstrated that when stimulated by peptides from the seasonal coronavirus OC43, the magnitude of OC43-specific memory CD4+ T cell response diminished with age, characterized by reduced expression of IFN-γ, IL-2, and TNF. Conversely, another study observed higher percentages of H7N9-specific IFN-γ+, TNF+ and CD107+ T cells in older individuals approximately 12 to 15 months after infection. Interestingly, when monitoring the phenotypes of T cells longitudinally, the levels of IFN-γ and TNF-α+ T cells remained relatively stable in young adults, but tended to increase over time in the elderly during the first 15 months post-infection. Such a difference might partially stem from different disease severity during the acute phase of infection across different age groups.
INDIVIDUALIZED EVALUATION OF THE IMMUNE STATUS IN THE ELDERLY

Though aging has been proven to be an independent risk factor for mortality after respiratory viral infections,\textsuperscript{92} clinical outcomes are still highly heterogeneous in patients of the same age. Given that the outcome of infections is determined by the interaction among viruses, host tissues, and immune responses, a comprehensive assessment from these three perspectives is imperative to tailor individualized treatment strategies. Current clinical practices mainly focus on evaluating viruses and tissue damage,\textsuperscript{93-96} but often overlook the assessment of immune status,\textsuperscript{97} which can differ widely among individuals.\textsuperscript{98,99}

Traditional assessment of antibody responses, such as the hemagglutination inhibition test for influenza infection and the measurement of IgG, IgM, and IgA antibodies to receptor binding domain (RBD) and spike protein for SARS-CoV-2 infection, lack quantification. Though neutralization assay is more quantitative, it is time-consuming. Regarding T lymphocyte responses, subset analysis based on flow cytometry often neglects the specificity to certain antigens. Moreover, while several clinical studies have identified associations between levels of specific cytokines in peripheral blood and disease severity/mortality in patients with respiratory viral infections, these findings have not yet been translated into practical predictive models with broad applicability.\textsuperscript{100}

Novel advancements in technology have opened up innovative avenues for assessing immune status in the context of respiratory viral infections. One example is mapping the BCR/TCR repertoire that has been discussed above, the diversification of which can serve as a predictive indicator of viral clearance efficacy.\textsuperscript{78,80} Meanwhile, single-cell sequencing can provide a comprehensive and unbiased understanding of the immune status of an individual.\textsuperscript{78,101} Current studies primarily focus on transcriptomics. With the growing knowledge of epigenomic changes in the aging immune system,\textsuperscript{102} future research could incorporate single-cell epigenomics. It is important to note that these cutting-edge technologies currently come with a high cost, highlighting the need to fully exploit their significance in assessing immune status before their wide implementation in clinical practice.

STRATEGIES TO MODULATE THE ANTIVIRAL IMMUNE RESPONSES

Consistent with our hypothesis that the reduced efficacy of the aging immune system to eliminate viruses results in more severe outcomes following respiratory viral infections, clinical evidence suggests that modulation of the immune responses under some circumstances may benefit the geriatric population.

Targeting cytokines

IFNs are vital antiviral cytokines, the secretion of which from various types of cells is downregulated during the early stage of infection in the elderly as introduced above.\textsuperscript{93,94,95} Randomized controlled trials (RCTs) have illustrated that the early treatment with pegylated IFN-λ could benefit high-risk outpatients with COVID-19,\textsuperscript{103} whereas IFN-β1a did not improve the clinical outcomes of hospitalized patients with COVID-19.\textsuperscript{104} Such inconsistent results underscore the need to consider factors such as the type of IFN molecules, the target population, and the timing of treatment.

As viruses persist, overt inflammatory responses occur, leading to tissue damage. This process is accompanied by elevated levels of multiple pro-inflammatory cytokines, including IL-6, IL-8, IL-1β, which have been associated with unfavorable clinical prognosis in patients with respiratory viral infections.\textsuperscript{93,94} Efficacy has been demonstrated in several studies for IL-6 receptor antagonists and Janus kinase (JAK) inhibitors in hospitalized patients with COVID-19.\textsuperscript{104,105} These medications act by blocking downstream effects of pro-inflammatory cytokines. However, whether they can be applied to other respiratory viral infections needs further investigation.

Targeting innate immune cells

Trained immunity describes the phenomenon that transient exogenous or endogenous stimulation can induce functional reprogramming of innate immune cells, leading to a non-specific augmented response upon rechallenge. Epigenetic modifications and metabolic reprogramming primarily underlie the molecular mechanisms of this phenomenon.\textsuperscript{106-108} Stimulation of trained immunity through specific vaccines or molecules may confer nonspecific protective effects against respiratory viral infections in elderly individuals. According to one RCT, the elderly who have received BCG vaccination have a markedly decreased incidence of respiratory tract infections of probable viral origins compared to the placebo vaccination group. Serological analyses revealed epigenetic modification in monocytes and altered cytokine secretion profiles of PBMCs upon stimulation with non-mycobacterial ligands, suggesting the involvement of trained immunity.\textsuperscript{109} In another RCT which recruited individuals aging 50 years or older, BCG vaccination reduced the incidence of SARS-CoV-2 infection within six months after vaccination.\textsuperscript{110} A similar decrease was exhibited in an observational study involving hospital employees in the Netherlands who received quadrivalent inactivated influenza vaccine.\textsuperscript{111} However, a separate RCT recruiting 3988 participants reported that BCG vaccination did not decrease the risk of symptomatic COVID-19 or severe COVID-19 among healthcare workers, and the results of subgroup analyses among individuals aging at least 60 years old were similar.\textsuperscript{112} Consequently, the existing evidence remains controversial, and the efficacy of trained immunity in protecting older individuals from respiratory viral infections requires further confirmation in large-scale clinical trials and real-world studies.

Targeting adaptive immune cells

Adaptive immune memory can provide rapid and targeted protection upon exposure to a previously encountered antigen. The use of vaccination, which leverages adaptive immune memory, is a widely accepted and acknowledged approach to prevent respiratory viral infections. However, it is important to note that due to changes in the immune system with aging, the elderly get less effective protection compared to younger population.\textsuperscript{113} In the elderly, delayed and attenuated antibody and T-cell responses have been observed following vaccination against influenza or SARS-CoV-2.\textsuperscript{113-116} Though limited studies provided direct evidence of vaccine efficacy or effectiveness in different age groups, there are data verifying the correlation between serological immune responses and clinical protection provided by vaccines.\textsuperscript{116,117} Therefore, appropriate strategies are necessary to enhance vaccine efficacy in the elderly.\textsuperscript{118} High-dose vaccine may be a potential solution, as a pooled analysis of two RCTs proved that the elderly receiving high-dose vaccine had a remarkably lower incidence of laboratory-confirmed influenza infection (n=41141, risk ratio=0.76, 95% confidence interval 0.65-0.90).\textsuperscript{119} The incorporation of adjuvants including AS03 and MF59 has shown promise in augmenting vaccine efficacy in the elderly as well.\textsuperscript{120,121} Other strategies, such as incorporating PAMPs to induce innate immunity, mapping epitopes to generate stronger T cell responses, and mitigating baseline inflammation, are also under investigation now.\textsuperscript{122}

Targeting senescent cells and dysregulated cellular metabolism

The aging of the immune system takes place along with the aging of the whole body. Therefore, targeting the hallmarks of aging may ameliorate the dysregulated antiviral immune responses observed in the elderly.\textsuperscript{123} Mounting evidence suggests the potential benefits of interfering with cellular senescence and dysregulated nutrient sensing. Some other dimensions of aging, such as telomere attrition and epigenetic alterations, have been associated with impaired antiviral immune responses, but their potential as therapeutic targets remains unknown.\textsuperscript{124} As for the rest, including genomic instability, loss of proteostasis, etc., knowledge of their role in immune responses is still limited.

Cellular senescence. Cellular senescence is defined as the permanent arrest of the cell cycle, and the accumulation of senescent cells promotes organismal aging.\textsuperscript{125} Senescent cells undergo many phenotypic alterations, which can serve as biomarkers for detection or targets for elimination. These alterations include but are not limited to the accumulation of lysosomal enzyme senescence-associated-β-galactosidase (SA-β-gal),\textsuperscript{126} cyclin-dependent kinase inhibitors p21 and p16, and senescence-associated heterochromatin foci (SAHF).\textsuperscript{127} Then, senescent cells can release a range of molecules known as the senescence-associated secretory phenotype (SASP), comprising many pro-inflammatory cytokines, chemokines, and extracellular matrix remodeling protease.\textsuperscript{128} While SASP may confer benefits in tissue repair, senescence clearance, and tumor suppression, it may also
Table 1. The changed antiviral functions of immune cells in the elderly and the related modulating strategies.

<table>
<thead>
<tr>
<th>Cell types</th>
<th>Changes in antiviral functions with age</th>
<th>Modulating strategies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory epithelium</td>
<td>- Decreased ciliary beat frequency, particle clearance ability, and secreted hydrolase.</td>
<td>- Targeting cytokines: administering IFNs and blocking the signal of pro-inflammatory cytokines.</td>
</tr>
<tr>
<td></td>
<td>- Decreased levels of antigen-presenting-related molecules.</td>
<td>- BCG vaccination to induce trained immunity.</td>
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<td></td>
<td>- Secrete more PGE$_2$ to impair the proliferation of alveolar macrophages.</td>
<td>- Senolytics (e.g., fisetin, navitoclax, quercetin plus dasatinib, etc.) to eliminate senescent cells.</td>
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<tr>
<td></td>
<td>- Delayed regeneration after infection.</td>
<td></td>
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<tr>
<td>Alveolar macrophages</td>
<td>- Decreased and delayed secretion of cytokines (e.g., IFN-α, IFN-γ, and IL-1β) and chemokines (e.g., CCL20 and CCL8) after stimulation.</td>
<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td>- Excessive infiltration after influenza infection.</td>
<td></td>
</tr>
<tr>
<td>Natural killer cells</td>
<td>- Decreased cytotoxicity and IFN-γ production after influenza infection.</td>
<td></td>
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<tr>
<td>Conventional dendritic cells</td>
<td>- Delayed expansion, poor activation, and decreased migration to lymph nodes after influenza infection.</td>
<td></td>
</tr>
<tr>
<td>Plasmacytoid dendritic cells</td>
<td>- Decreased secretion of type I and III IFNs after influenza infection.</td>
<td></td>
</tr>
<tr>
<td>B lymphocytes</td>
<td>- Decreased number of naïve B lymphocytes and decreased diversity of BCR repertoire.</td>
<td>- Vaccination with appropriate modifying strategies to induce high-quality pathogen-specific immune memory.</td>
</tr>
<tr>
<td></td>
<td>- Compromised germinal center development with fewer Tfh cells and more Tfr cells.</td>
<td></td>
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<tr>
<td>T lymphocytes</td>
<td>- Decreasde number of naïve T lymphocytes and decreased diversity of TCR repertoire.</td>
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<tr>
<td></td>
<td>- Decreased CD8$^+$ T cell activation and cytotoxicity.</td>
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<td></td>
<td>- Expanded Treg cells.</td>
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<td></td>
<td>- Accumulated CD8$^+$ TRM cells to promote tissue inflammation and chronic fibrosis.</td>
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<td>- Altered memory T lymphocyte reaction.</td>
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<tr>
<td>Natural killer cells</td>
<td>- Decreased cytotoxicity and IFN-γ production after influenza infection.</td>
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<tr>
<td>Conventional dendritic cells</td>
<td>- Delayed expansion, poor activation, and decreased migration to lymph nodes after influenza infection.</td>
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Abbreviations: BCR, B-cell receptor; IFN, interferon; PGE$_2$, prostaglandin E$_2$; TCR, T-cell receptor; Tfh, T follicular helper cells; Tfr, T follicular regulatory cells; Treg, regulatory T cells; TRM, tissue-resident memory T cells.

Promote aging-related dysfunction and chronic inflammatory. One study demonstrated that the elevated levels of circulating SASP were associated with an increased risk of adverse postoperative outcomes, suggesting their potential as indicators of disease status. Senolytics are drugs selectively eliminating senescent cells, and senormorphics are compounds targeting SASP signaling. These two have emerged as potential therapeutics for age-associated health issues, which have been extensively reviewed elsewhere.

The relationship between cellular senescence and respiratory viral infections has been explored in several studies. For instance, one study illustrated that the accumulation of senescent AECs in mice led to excessive neutrophil infiltration and subsequent immunopathology following influenza infection. However, the overall impacts of an increased burden of senescent cells in respiratory viral infections remains to be elucidated. Among specific-pathogen-free (SPF) mice exposed to pet store mice or their bedding, the older population had a markedly elevated mortality rate. Both prophylactic and therapeutic usage of the senolytic fisetin could elevate the survival rate of old mice. Interestingly, cellular senescence accompanied by SASP could be induced by viral infections and was associated with disease pathophysiology, as evidenced by recent findings in patients with COVID-19. Animal studies showed that senolytic drugs, such as navitoclax, quercetin plus dasatinib, and fisetin, could effectively eliminate senescent cells and improve survival rates in animals developing COVID-19-like pneumonia. Furthermore, two RCTs with small sample size have suggested that quercetin administration in COVID-19 outpatients had the potential to shorten the time to viral clearance, ameliorate symptom severity, and prevent progression to severe disease or death. These studies are fundamental for the subsequent clinical work assessing the efficacy of senolytics in preventing and treating respiratory viral infections.

**Dysregulated nutrient sensing.** Immune cell aging is associated with dysregulation of the mTOR signaling pathway, an important nutrient-sensing pathway, the inhibition of which has been shown to enhance longevity. Specifically, hyperactivation of the mTOR signaling pathway has been linked to T-cell aging, offering a theoretical basis for using mTOR inhibitors as a therapeutic approach. One RCT demonstrated that mTOR inhibitor RAD001 could significantly increase hemagglutination inhibition titers as well as seroconversion rates following influenza vaccination in elderly participants aging ≥ 65 years, although data on clinical efficacy or effectiveness were not available. Then, in another RCT, 6-week co-administration of two mTOR inhibitors, BEZ235 and RAD001, resulted in approximately 38% reduction in the rate of overall infections, particularly respiratory infections, during the year after drug initiation in elderly volunteers. Mechanistic analyses revealed that the co-treatment upregulated IFN signaling pathways. These findings highlight the potential of mTOR inhibitors as a preventive strategy in the elderly against respiratory viral infections.

**CONCLUSIONS AND PERSPECTIVES**

Advanced age poses a heightened risk of adverse clinical outcomes in the context of respiratory viral infections. The curtailed ability of the aging immune system to efficiently eliminate viruses leads to persistent viral insults and tissue damage, concomitant with overt activation of deleterious inflammatory immune cells, which subsequently result in the unfavorable prognosis. Extensive research has substantiated that epithelial cells, various innate...
immune cells, and T and B lymphocytes experience quantitative, phenotypic, and functional alterations throughout the aging process, together contributing to the enhanced susceptibility of the elderly (Table 1). Therefore, we propose that targeting the aging immune system represents a crucial avenue for the management of respiratory viral infections in the elderly, with the identification of potential targets as the initial step in this direction.

Aging is a highly heterogeneous process. A longitudinal study indicates that the status of immune aging served as a stronger predictor of cardiovascular disease outcomes than well-established risk factors. Then, a recent study demonstrated that clustering COVID-19 patients based on their immune response profiles during infection could help predict treatment response and clinical prognosis. These findings underline the immense potential of mapping immune profiles prior to or during the early stages of respiratory viral infections to enable the stratification and then individualize treatment approaches. Assessing cytokine levels, TCR/BCR repertoire diversity, transcriptionomics and epigenomics of immune cells, and hallmarks of aging (i.e., burden of cellular senescence, nutrient sensing dysregulation, telomere attrition, and epigenetic alterations, etc.), all offer valuable insights from diverse perspectives. Such studies can provide potential markers for assessing immune aging, and the combination of these markers help comprehensively portray the types of immune defects present in each subject, ultimately facilitating personalized treatment strategies. Equally crucial is considering how to translate this knowledge into point-of-care tests.

Even though numerous alterations have been observed, whether they are casual factors for the impaired antiviral responses needs to be carefully confirmed, and finding or establishing appropriate research subjects/models is essential to accomplish this. Human-based studies typically use immune cells derived from peripheral blood ex vivo, lacking intercellular communications and the impacts of microenvironment. The widely-employed murine models could avoid such concerns, but introduce other challenges simultaneously. First comes the disparity in immune systems across species, as well as the modifications of viruses for animal studies. Then, the most suitable mouse strains, virus strains, and inoculum conditions for immune-aging-related studies should be identified, the combination of which would significantly influence the study results. Finally, unlike humans, SPF animals used in experiments rarely have prior infections, making the immune system less experienced. Aforementioned factors necessitate consideration before translating discoveries in murine models into clinical applications. Nonetheless, the rapidly developing technologies of organoids and lung-on-a-chip could avoid such concerns, but introduce other challenges simultaneously. We hope that all progress achieved in mechanism studies could be translated into clinical practice to improve the prognosis of the elderly afflicted by respiratory viral infections. Firstly, making use of immune memory can prevent infections, but certain strategies should be adopted to improve their efficacy, which has been discussed in detail above. Then, accurate diagnosis of respiratory viral infections and differentiation from other diseases at an early stage is critical for timely treatment and intervention. In addition to pathogen-based diagnosis, blood-based host gene expression test now provides new options for diagnosis.

As for the treatment strategies, virus-targeted therapy and immune-targeted therapy are both crucial. Unfortunately, unlike antibiotics, current clinical practice has a very limited availability of antiviral drugs, with the absence of broad-spectrum antivirals. To target host immune system, efforts can be directed towards strengthening antiviral immune responses or mitigating the damaging effects of overt inflammatory responses, which may dominate at different stages of infection, respectively. However, many of these treatments have only been studied in small-scale clinical studies or been validated solely in patients with COVID-19, not to mention a variety of drugs still in their pre-clinical stages. Given our expanding knowledge of the significant burden respiratory viral infections have posed to the health of elderly individuals, we urge for increased attention to be devoted to resolving the aforementioned issues. Ultimately, this will better equip the elderly population and improve their prognosis in the future.

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AUTHOR CONTRIBUTIONS

Bin Cao, Baidong Hou, and Xulong Zhang supervised and revised the manuscript. Xueyang Zhang wrote and edited the manuscript. Jiuyang Xu, Yeming Wang, Hui Li, Jiabei Yu, Xiao Shang, and Lianhan Shang edited the manuscript. All authors contributed to the article and approved the submitted version.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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