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Effects of aging on the adaptive immune response to respiratory virus infections

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Abstract

Severe acute respiratory disease caused by respiratory virus infections in individuals aged 65 years and older and in high-risk adults, such as those with chronic cardiopulmonary disorders, is associated with increased hospitalization and mortality rates. Epidemiological studies have identified influenza virus and respiratory syncytial virus as the most frequent causes of virus-induced respiratory disease in elderly and high-risk adults. Studies in both humans and animal models have established fundamental defects in cell-mediated and humoral immune responses in aged individuals. However, it is not well understood how age specifically alters the immune response to respiratory pathogens. In this review, we will focus our discussion on the major causative agents of severe respiratory virus infections in elderly and high-risk adults and the age-associated defects in the immune response that probably contribute to the increased disease severity observed in these populations.

Keywords

aging; B cell; elderly; lung; T cell; virus

Respiratory virus infections are a major cause of morbidity and mortality in elderly and high-risk adults. Deaths associated with respiratory virus infections have increased over the last several decades in the USA, in part reflecting the increased numbers of elderly persons [1]. The number of individuals over the age of 85 years has doubled between 1976 and 1999, and the proportion of adults aged 50–60 years has continued to increase [1]. Owing to increased frailty, senescence of the immune system and high-risk factors, such as chronic cardiopulmonary disorders, adults over the age of 65 years are much more likely to succumb to respiratory virus infections [1–4]. Influenza virus and respiratory syncytial virus (RSV) are the two leading causes of virus-induced severe respiratory disease. Other major causes of virus-

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induced severe respiratory disease include human metapneumovirus (HMPV), human parainfluenza virus (HPIV), rhinovirus and coronavirus [5–7]. Understanding the effects of immune senescence and the contribution of risk factors to increased disease severity in the elderly is paramount for vaccine development and the design of immunotherapeutics.

Senescence of the immune system

Deterioration of the adaptive immune response has been well documented in elderly individuals [4,8–15]. Age-associated defects occur both in hematopoiesis of lymphocytes [13], maintenance of the peripheral lymphocyte pool [4,11,12,16] and during virus-specific responses [10,16,17]. Both cell-mediated and humoral immunity are necessary for virus clearance and protective immunity from reinfection.

Cell-mediated immunity

During a primary virus infection, cell-mediated immunity is primarily responsible for virus clearance [4,18]. It is well established that cell-mediated immunity declines as the immune system ages [4,13,19]. In the thymus, T-cell progenitors develop into mature T cells whereupon they migrate into the periphery. The naive repertoire of T cells is established early in life [20]. Thymic involution begins within 1 year after birth and by the fifth decade of life most of the thymus has been replaced with adipose tissue, although low levels of thymopoiesis continue late into life [13,20,21]. Interestingly, the thymus is capable of regenerating after involution caused by cancer chemotherapy or infection; however, involution caused by aging appears to be irreversible, suggesting senescence-acquired changes in the thymic environment [20]. As thymic output decreases with age, the overall diversity within the repertoire of naive T cells is reduced and existing peripheral T cells age, accruing intrinsic defects that alter the T-cell response to pathogens [16,22–24]. In humans, αβ T-cell receptor diversity does not drastically change until the seventh decade of life, concomitant with significant increases in homeostatic proliferation of peripheral T cells [22]. Homeostatic proliferation increases, perhaps to compensate for decreased thymopoiesis or as a result of accumulating pathogen exposures [13,22]. Over time, there is a skewing in the peripheral T-cell pool from naive T cells to overrepresentation of T cells with a memory phenotype [25,26]. While this phenomenon may in part be from a longer history of antigen exposure, a fraction of $CD4^+$ T-cell recent thymic emigrants (RTEs) in aged mice already have a memory phenotype ($CD44^{hi}CD62L^{lo}$), suggesting that defects are already present in T cells newly generated in aged hosts [27]. As skewing of the T-cell repertoire accumulates, responses to new pathogens may consist of cross-reactive memory T cells or the number of naive T cells required to mount an effective response may fall below a critical threshold [17,19].

Upon infection with a pathogen, naive T cells are activated by antigen-presenting cells, undergo vigorous clonal expansion and develop effector functions to combat the infection [28]. Aged naive T cells exhibit diminished formation of immunological synapses with antigen-presenting cells displaying cognate peptides and poor localization of signaling molecules downstream of the T-cell receptor [29,30]. In addition, activated aged T cells proliferate poorly [31], exhibit decreased trafficking to infected tissues [9], have altered cytokine profiles [26,32,33] and are less efficient at clearing pathogens [34]. Interestingly, defects in $CD4^+$ T cells appear to, in part, depend on the post-thymic age of the naive $CD4^+$ T cell and not the age of the animal [23,35]. Memory $CD4^+$ T cells generated from young naive $CD4^+$ T cells remain functional well into old age [4]. Importantly, not all of the defects observed in naive $CD4^+$ T cells obtained from aged mice can be attributed to the post-thymic age of the cell [27]. $CD4^+$ T-cell RTEs from aged mice exhibit decreased Ca^{2+} flux, proliferation and IL-2 production compared with RTEs from young mice following antigen stimulation or activation via the T-cell receptor. Bone marrow transfer experiments have demonstrated that defects in RTEs from aged mice appear to arise from age-associated changes to both T-cell progenitors and the thymic

environment [27]. Finally, as $CD4^+$ T cells provide help to B cells and cytotoxic $CD8^+$ T cells, there is evidence that aged naive $CD4^+$ T cells provide poor help to B-cell responses [24] and possibly to cytolytic $CD8^+$ T cells [16].

Multiple lines of evidence suggest that age-dependent changes in the overall pool of $CD8^+$ T cells may lessen the efficacy of the response. First, the naive $CD8^+$ T-cell repertoire shrinks with age, reducing the number of naive precursors capable of responding to a particular antigen below a critical threshold. Contraction of the naive T-cell repertoire can greatly alter the magnitude of the response [16]. Second, in addition to increased ratios of memory to naive T cells in aged individuals, memory $CD8^+$ T cells can form clonal expansions that dominate the $CD8^+$ T-cell pool and can possibly compete with naive $CD8^+$ T cells in response to a new or crossreactive pathogen [16,17,19,22,36,37]. Third, trafficking of antigen-experienced $CD8^+$ T cells may be altered with age. For example, following respiratory virus infection, a small pool of virus-specific $CD8^+$ T cells are maintained in the lung airways [9]. As mice age, recruitment of memory T cells into the airways decreases, which may be a result of diminished numbers of effector memory T cells or alterations of memory T-cell trafficking profiles. Decreased recruitment of protective $CD8^+$ T cells to the lung airways could have profound effects on initial control of virus replication in the elderly [9].

Whether $CD8^+$ T cells lose function over time is controversial. Several studies indicate that aged $CD8^+$ T cells retain effector functions following a primary response [11,34,38]. Maintenance of memory $CD8^+$ T cells after acute lymphocytic choriomeningitis virus and Sendai virus infection in young or aged mice was equivalent [39]. Interestingly, $CD8^+$ T-cell memory generated to respiratory viruses in young mice may actually improve over time [40]. Equal numbers of Sendai virus-specific $CD8^+$ T cells isolated from mice infected 1 month or more than 12 months earlier were adoptively transferred into naive congenic hosts that were then infected with Sendai virus. Virus-specific $CD8^+$ T cells generated more than 12 months earlier made up a greater proportion of the secondary response in the lungs and lung airways. Prior to transfer, both populations of memory $CD8^+$ T cells had similar expression patterns of trafficking molecules, suggesting that the observed differences in these two memory T-cell populations were not a result of differential trafficking. Conversely, $CD8^+$ T-cell responses generated in aged mice may be defective and provide poor protection against secondary challenge [40]. When equal numbers of Sendai virus-specific memory $CD8^+$ T cells generated in either young or old mice were adoptively transferred into naive mice that were subsequently challenged with Sendai virus, the memory $CD8^+$ T cells established in aged mice proliferated poorly compared with the memory $CD8^+$ T cells generated in young mice [40]. Although the possibility of poor $CD4^+$ T-cell help cannot be ruled out, this suggests that, similar to $CD4^+$ T cells, naive $CD8^+$ T cells accumulate defects over time.

Humoral immunity

Neutralizing virus-specific antibodies can contribute to virus clearance during primary infection and are important in protecting against reinfection [4,18]. Upon infection with a pathogen, naive B cells that recognize cognate antigen in the presence of $CD4^+$ T-cell help are activated and form germinal centers where hypersomatic mutation, class switching and affinity maturation of the B-cell receptor occurs [28]. Stable memory populations of B cells and antibody-secreting plasma cells are then established to protect against reinfection. Depending on the pathogen, memory B cells and neutralizing antibodies can be maintained for as short as a decade and up to the whole lifetime of the host [41,42].

Much as thymic output decreases over time, aging has deleterious effects on multiple stages of primary B-cell development in the bone marrow [13]. Animal models have demonstrated that there is a significant reduction in pro-B-cell numbers with increased age [43]. B-cell receptor light chain rearrangement is also impaired with age, resulting in decreased numbers

of pro-B cells that successfully transition into pre-B cells [44,45]. After mature B cells exit the bone marrow, they traffic into secondary lymphoid tissues. Trafficking of mature B cells out of the bone marrow into the spleen is diminished in aged animals. As a possible method of compensation, peripheral B cells turnover at a slower rate and have a longer half-life [13,44].

The humoral response clearly declines with age [12]. This is particularly evident from the decreased efficacy of immunization in the elderly [46–48]. It appears that B-cell memory established in young individuals is maintained for as long as 60 years postimmunization and retains the ability to mount an anamnestic response several decades later [42]. However, compared with young individuals, B-cell responses to pathogens in aged individuals are abbreviated, there is decreased germinal center formation and antibodies are less protective owing to lower titers and affinity [12,13]. There is also an age-dependent increase in levels of low affinity autoantibodies [48]. Since $CD4^+$ T-cell help is necessary for germinal center formation, it is difficult to separate age-related defects intrinsic to B cells or $CD4^+$ T cells or both [12]. Another possible cause of decreased germinal center formation is diminished function of follicular dendritic cells [49].

Similar to age-related changes to the T-cell repertoire previously described, the B-cell repertoire changes with age. A small number of aged adults (5.3 and 7.5% of individuals older than 70 and 85 years old, respectively) develop benign monoclonal gammopathy, defined by a clonal expansion of memory plasma cells that produce a single immunoglobulin [50]. There is also a skewing of the B-cell pool that is mostly comprised of naive B cells in young individuals to predominately a memory-phenotype B-cell pool [48]. This phenomenon requires further investigation as the memory phenotype of B cells is more heterogeneous than previously thought [51]. There is also an age-dependent decrease in B-cell receptor diversity that may further limit the naive B-cell repertoire capable of responding to new pathogens [52].

Influenza virus

Nonpandemic influenza virus is the leading cause of virus-induced severe respiratory disease in the elderly population [1,53]. Approximately 90% of influenza-associated deaths occur in adults over the age of 65 years [1]. Even with a high rate of influenza vaccination, influenza infects 2–5% of healthy elderly patients and 2–7% of high-risk adults [53]. This results in greater than 50,000 deaths and 200,000 hospitalizations annually in the USA [1,54]. The substantial disease burden caused by influenza infection in the elderly emphasizes the need to better understand how aging affects the influenza-specific adaptive immune response.

Adaptive immunity

Cell-mediated and humoral immune responses play important roles in influenza virus clearance and protection from reinfection [10]. Both CD4 and $CD8^+$ T cells are required for clearance of influenza virus during primary infection [10,55]. $CD4^+$ T cells alone can provide partial protection against influenza infection whereas $CD8^+$ T cells are able to resolve the infection [55]. Influenza-specific antibodies protect from reinfection. In addition, there is evidence that the humoral response aids in virus clearance during primary influenza infection [18,55].

Cell-mediated immunity

Various studies have reported no age-dependent changes in the frequency of IFN-γ-producing $CD4^+$ T cells while others show clear decreases [56–58]. These differences may be accounted for by varying histories of natural influenza infection or vaccination in the subjects analyzed or methods of detection. One human study examined the frequencies of effector memory ($CD45RA^-CCR7^-$) and central memory ($CD45RA^-CCR7^+$) influenza-specific $CD4^+$ T cells in young and aged subjects following influenza vaccination [58]. A total of 3 months

postimmunization, aged subjects had significantly lower frequencies of influenza-specific IFN-γ- and TNF-α-producing peripheral blood $CD4^+$ T cells. Whereas young subjects had equivalent frequencies of total effector and central memory $CD4^+$ T cells, aged subjects exhibited an increase in the ratio of central memory to effector memory $CD4^+$ T cells. This same shift was present *ex vivo* in influenza-specific IFN-γ-producing $CD4^+$ T cells. Changes in the balance of effector and central memory $CD4^+$ T cells could alter the ability of memory $CD4^+$ T cells to traffic to the lungs upon reinfection with influenza and could lessen the quality of protection.

Studies in aged mice and humans have demonstrated age-associated declines in $CD8^+$ T-cell responses to influenza [34,59,60]. As outlined previously, the repertoire diversity and size of the $CD8^+$ T-cell response is altered with age in several ways: there is an increase in the ratio of memory to naive T cells with age, there are decreased numbers of naive precursors that can respond to any given epitope, and clonal expansions of memory $CD8^+$ T cells can dominate the $CD8^+$ T-cell pool [16,37,61,62]. First, in aged mice, the magnitude of the $CD8^+$ T-cell response to the immunodominant influenza epitope $NP_{366–374}$ is smaller, owing to a contraction in the number of naive precursors capable of responding [16,34]. In addition to a smaller NP-specific response, the T-cell receptor Vβ profiles are skewed and limited in diversity compared with NP-specific responses in young mice [16]. Importantly, it does not appear that other $CD8^+$ T-cell specificities can compensate for the decreased NP-specific $CD8^+$ T-cell response since the size of the NP-specific response in aged mice directly correlates with virus clearance to heterosubtypic infection [16]. This highlights the importance of examining $CD8^+$ T-cell responses to individual epitopes rather than simply examining the T-cell response as a whole, which may not appear different from young individuals.

Second, clonal expansions of influenza-specific memory $CD8^+$ T cells can develop over time in mice [17]. Although functional and phenotypic profiles of influenza-specific $CD8^+$ T-cell clonal expansions have not been thoroughly studied, clonal expansions to Sendai virus in mice have a similar memory phenotype to a normal $CD8^+$ T-cell response and can produce IFN-γ upon peptide stimulation. Thus, apart from any functional defects acquired with age, alterations in the repertoire of the $CD8^+$ T-cell pool have drastic effects on the overall T-cell response to influenza. Clonal expansions of influenza-specific $CD8^+$ T cells are more difficult to examine in humans owing to the wide heterogeneity in responses but may have an important role in decreasing the quality of the influenza-specific $CD8^+$ T-cell response in the elderly. One human study examined the T-cell repertoire in elderly subjects that failed to produce protective antibodies after influenza vaccination [63]. The majority of these subjects had clonal expansions of $CD45RA^+CD28^-$ $CD8^+$ T cells that dominated in the majority of Vβ families and produced IFN-γ to autoantigens. It was proposed that overproduction of IFN-γ could cause an imbalance between Th1 and Th2 cytokines resulting in deficient humoral responses [33, 63].

Mouse models have also been used to evaluate the effect of aging on $CD8^+$ T-cell effector activity. In aged C57BL/6 mice, there is decreased cytolytic activity by NP-specific $CD8^+$ T cells that strongly correlates with decreased numbers of NP-specific $CD8^+$ T cells as determined by tetramer and IFN-γ production [34,64]. Furthermore, both the $CD8^+$ T-cell response and corresponding peak cytolytic activity are delayed and virus replication is prolonged [34]. Similarly, aged BALB/c mice exhibit decreased T-cell cytolytic activity with peak activity delayed by several days [60]. As expected, decreased cytotoxicity correlated with significantly delayed virus clearance. These results suggest that diminished influenza-specific $CD8^+$ T-cell responses, and not decreased T-cell effector function, cause reduced cytolytic activity and prolonged virus replication in aged mice [34].

In humans, peripheral blood $CD8^+$ T cells in aged adults have decreased influenza-specific cytolytic activity compared with young adults [59]. Several human studies examined cellular responses prior to and after immunization with a trivalent influenza vaccine [57,65]. Relative to young subjects, T cells from aged subjects proliferated less *ex vivo* and produced less IFN-γ. One study compared frequencies of influenza M1-specific $CD8^+$ T cells by monitoring levels of tetramer and IFN-γ [57]. Whereas there was no difference in tetramer frequencies between young and old subjects, in old subjects there were significantly fewer M1-specific $CD8^+$ T cells that produced IFN-γ upon restimulation, suggesting that the $CD8^+$ T cells were functionally impaired. Thus, in contrast to studies performed in mice, human studies have indicated that decreased effector function may also contribute to diminished $CD8^+$ T-cell responses in aged individuals.

Humoral immunity

Humoral immunity wanes with age as illustrated by as much as a 50–75% reduction in vaccine efficacy in individuals aged 70 years and older [4,66]. Whereas the influenza vaccine has a 70–90% efficacy in young adults, there is only a 30–40% efficacy in adults aged older than 65 years [48,66]. Influenza vaccination is particularly ineffective in preventing severe disease in persons with chronic cardiopulmonary conditions [4,64]. The decreased antibody response elicited by the vaccine is probably a combination of deficient B-cell responses and poor help from cognate $CD4^+$ T cells.

Multiple studies have reported no difference in the magnitude of the antibody response elicited by vaccination of young versus aged subjects [67]. By contrast, several studies have reported that 25% of healthy and high-risk elderly adults failed to achieve hemagglutination inhibition (HI) antibody titers greater than 40 following vaccination, which is considered to be the cutoff for protection [68], whereas all young adults that were vaccinated exhibited HI titers greater than 40. Furthermore, the percentage of elderly subjects that achieved a fourfold increase in antibody titers relative to preimmunization titers was appreciably lower [65]. Interestingly, only 10–15% of the elderly compared with 40–50% of young adults exhibited a fourfold or higher increase in antibody titers after booster immunization with the same vaccine components used the previous year. Another group similarly reported that only a fraction of elderly subjects mounted a fourfold or higher increase in antibody titers compared with young subjects after reimmunization [69]. Importantly, one study found that the majority of the immunized elderly individuals that were infected with influenza had HI titers greater than 40 and 31% had titers greater than 640 [70]. This indicates that titers are perhaps not a strong indicator of protection and that the quality of the antibody response may also be important. Alternatively, the level of protection contributed by neutralizing antibodies may be similar in elderly and young adults; however, the second line of defense elicited by memory $CD8^+$ T cells may be deficient in aged individuals.

Respiratory syncytial virus

Respiratory syncytial virus is the leading cause of severe lower respiratory virus infections in infants and children under the age of 5 years [71]. More recently, RSV has been recognized as having a significant disease burden in the elderly. RSV is second only to influenza in causing virus-induced severe respiratory disease in adults aged over 65 years [1,53,72]. It has been estimated that 78% of RSV-associated mortalities occur in adults over the age of 65 years [1]. Annually, RSV infects approximately 3–7% of healthy elderly adults with an approximately 8% mortality rate that results in more than 10,000 deaths per year in the USA [53]. This is accompanied by more than 150,000 hospitalizations costing as much as US$1 billion each year [1,53]. The significant disease burden on the elderly population highlights the need for an effective RSV vaccine. In spite of significant pathogenesis in the elderly, little is known about the effects of aging on the immune response to RSV.

Adaptive immunity

It is clear in both humans and mice that both cell-mediated immunity and humoral immunity are important in resolving RSV infection and protecting against reinfection. Children with defective cell-mediated immunity exhibit prolonged virus shedding accompanied with an increase in disease severity and mortality [73–75]. Both CD4 and $CD8^+$ T cells are important in resolving acute RSV infections. In mice, CD4 and $CD8^+$ T-cell depletion prior to primary RSV infection causes prolonged virus replication in the lungs [76,77]. In humans, titers of RSV-specific antibodies correlate with decreased susceptibility to reinfection with RSV; this is substantiated in the BALB/c mouse model whereby antibodies are not important for virus clearance during primary infection but protect against reinfection [78–80]. However, unlike mice, in humans RSV-specific antibodies may not provide sterilizing immunity since reinfection commonly occurs throughout life. Thus, understanding the effects of aging on the adaptive immune response is critical to understanding increased disease caused by RSV infection in the elderly.

Cell-mediated immunity

Several studies have directly examined the immune response to RSV infection in humans and in aged animal models [81–83]. Similar to influenza infection in aged animal models, aged mice and cotton rats demonstrate a decreased cell-mediated immune response accompanied by increased morbidity during RSV infection. Aged animals exhibit delayed virus clearance, a decrease or delay in inflammatory cytokine levels such as IFN-γ, and an increase in the Th2 cytokine IL-4 [81–83]. Supportive of a degenerative T-cell response, RSV-infected senescence-accelerated mouse strain P1 (SAM-P1) mice and aged BALB/c mice exhibit decreased *ex vivo* cytolytic activity by $CD8^+$ T cells and NK cells [81,82]. In addition, SAM-P1 and aged BALB/c mice have decreased numbers of pulmonary CD4 and $CD8^+$ T cells compared with young mice [81,82]. Owing to either increased virus burden or immunopathology, disease severity in animals increases with age. SAM-P1 mice exhibit increased weight loss and increased inflammation in the lungs following acute RSV infection [81].

There are few human studies examining the effects of aging on RSV-specific T cells. It has been reported that the frequency of RSV-specific $tetramer^+$ $CD8^+$ T cells slowly decreases with age [84]. All of the subjects with chronic obstructive pulmonary disease (median age: 69 years) and several of the healthy subjects older than 65 years did not have detectable frequencies of RSV $tetramer^+$ $CD8^+$ T cells [84]. In agreement with tetramer frequencies, another study demonstrated that elderly adults have significantly fewer RSV-specific IFN-γ-producing peripheral blood T cells compared with young adults as determined by enzyme-linked immunosorbent spot (ELISPOT) assay [85]. By contrast, another group did not observe significant decreases in the frequency of RSV-specific IFN-γ-producing CD4 and $CD8^+$ T cells between young and elderly adults [56]. They did see an age-dependent decrease in the ratio of IL-10 to IFN-γ-secreting $CD4^+$ T cells, suggesting that the balance between anti- and pro-inflammatory $CD4^+$ T cells may shift with age. However, further human studies are needed to determine whether the magnitude and the cytokine profiles of the RSV-specific T-cell response changes with age.

Collectively, these data suggest an alteration/attrition of the T-cell response in the elderly. If the aged T-cell response is indeed diminished, it is not clear whether RSV-specific T-cell responses are decreased owing to a contraction in the naive repertoire or competition with memory T-cell clonal expansions, whether T cells have intrinsic defects, whether the T-cell response is not efficiently primed by antigen-presenting cells, or whether it is any combination of these possibilities. Furthermore, since human studies are limited to analyzing T cells from circulating peripheral blood, these data may not reflect differences in numbers or the quality

of RSV-specific lung-resident T cells; in humans and animals, virus-specific memory $CD8^+$ T cells are preferentially maintained in the lungs long after resolution of the infection [9]. Since these are the cells poised to respond immediately to respiratory virus infection, it is important to determine what age-associated changes occur in these cell populations.

Humoral immunity

Respiratory syncytial virus neutralizing antibodies reduce susceptibility to reinfection with RSV in both young and aged adults [3,79,80,86–88]. In humans, mucosal RSV-specific IgA antibodies correlate more closely with protection than serum-neutralizing antibodies [86]. These observations are validated in the BALB/c mouse model in which antibodies protect against RSV infection [78]. Reinfection with RSV is common despite the presence of RSV-specific antibodies and memory T cells, suggesting that naturally acquired immunity to RSV either rapidly wanes or is never fully established [71,89]. Consistent with this observation, there is a fairly rapid decay in serum antibody titers after natural RSV infection; following RSV infection, neutralizing antibody titers decrease more than fourfold over 1 year in the majority of subjects [88]. After an initial decrease in titers, decay rates probably level off since subjects not reinfected with RSV maintain stable antibody levels with a low decay rate [88]. It does not appear that the decay rate differs with age, although this has yet to be carefully assessed. It is unknown if the same serum decay rates apply to mucosal antibody titers.

It is unclear whether the humoral response is intact in the elderly. SAM-P1 mice infected with RSV had decreased mucosal RSV-specific IgA antibodies that correlated with a decreased $CD4^+$ T-cell response [81]. Young and aged SAM-P1 mice did not have differences in mucosal IgG2a and IgG1 antibodies. In humans, multiple groups have observed similar baseline RSV antibody titers between young and aged subjects [90,91]. One prospective study actually found that the elderly cohort had a more vigorous antibody response than young adults; following natural RSV infection, the mean increase in neutralizing antibodies in aged adults was approximately eightfold compared with an approximately fourfold increase in young adults [90]. This study did not examine individual antibody isotypes or mucosal antibody titers that might have revealed age-associated differences. A recent study in Italy found that while nearly all elderly (>80 years of age) and young (20–60 years age) subjects had RSV-specific antibodies, only 36% of aged individuals had neutralizing antibodies compared with 92.5% of young individuals [92]. Compared with the young cohort, seropositive subjects in the aged cohort also had significantly lower mean neutralizing titers, perhaps reflecting the decay of RSV-specific antibodies.

Further investigation is required to more closely examine the diversity and affinity of RSV-specific neutralizing antibodies in the elderly. It may be that *in vitro* RSV neutralization assays are not sensitive enough to detect age-related differences, and the affinity and/or diversity of antibody specificities may be decreased enough in the elderly to diminish protection from reinfection. It would also be informative to assess the RSV-specific $CD4^+$ T-cell and B-cell responses in parallel with antibody titers in order to link age-associated decreases in humoral immunity with upstream events. It is currently unknown whether age-related decreases in the B-cell repertoire or poor germinal center formation limit the humoral response to RSV. In addition to any defects that aged B cells may accrue, $CD4^+$ T-cell help to B-cell responses may be poor in the elderly. Aged mouse models will be useful in analyzing the effects on aging on the magnitude and quality of the $CD4^+$ T-cell and B-cell response and how this affects antibody production.

Human metapneumovirus

Human metapneumovirus was identified in The Netherlands in 2001 and has since been identified as a major cause of respiratory tract disease in infants and young children worldwide

[93–97]. HMPV causes approximately 5–15% of severe respiratory infections in infants with clinical symptoms similar to RSV [5]. The majority of children are infected by the age of 5 years and almost all adults are seropositive to HMPV [98]. Reinfection with HMPV suggests that, similar to RSV, protective immunity is not established following natural infection. It is now appreciated that HMPV infects people of all ages, including the elderly [98–101]. While HMPV infection rates are high in young adults, probably owing to increased exposure to children, disease severity is greatest in high-risk elderly adults with underlying chronic heart and lung conditions [100]. This emphasizes the combined contribution of cardiopulmonary disorders, frailty and immune senescence to increased severity of respiratory virus infections in the elderly population.

Aged BALB/c mice exhibit delayed weight recovery, increased clinical illness, mortality and increased airway obstruction compared with young mice following acute HMPV infection [102]. Peak virus titers in the lungs are higher in aged mice but virus clearance is similar to young mice. This suggests that the early innate immune response to HMPV declines with age. Similar to influenza and RSV infections, the cell-mediated immune response is important in controlling HMPV replication [103,104]. Aged BALB/c mice infected with HMPV have similar numbers of $CD8^+$ T cells in the lung airways but significantly more $CD4^+$ T cells accompanied with increased numbers of IL-4 and IFN-γ-producing $CD4^+$ T cells 6 days postinfection [102]. Aged mice exhibit decreased IFN-γ levels and increased IL-4 and IL-6 levels 6 days postinfection as detected by ELISA from bronchoalveolar lavage fluid. While it is not clear what accounts for the overall decrease in IFN-γ levels, these data suggest that there is a shift from a T-helper cell (Th)-1 to a Th2-type $CD4^+$ T-cell response, which may contribute to exacerbated disease in aged mice.

Human metapneumovirus-specific antibodies are protective against reinfection [105,106]. In BALB/c mice, infection of aged mice with HMPV induces significantly lower levels of IgG; IgG1, a Th2 isotype; and IgG2a, a Th1 isotype, than in young mice 25 days postinfection [102]. Levels of IgG2a are significantly lower 14 days postinfection, again suggesting an age-related shift from a Th1 to a Th2-type $CD4^+$ T-cell response. Neutralizing antibody titers are approximately threefold lower in aged mice compared with young mice [102]. While changes in the type of $CD4^+$ T-cell response may cause decreased antibody titers, it is also possible that age-associated deficiencies in $CD4^+$ T-cell help contribute to a poor humoral response. Since HMPV is such a newly described respiratory pathogen, there have been few human studies comparing the humoral and cell-mediated immune response in elderly and young adults.

Other respiratory viruses

The disease burden of respiratory virus infections in elderly and high-risk adults by viruses other than influenza virus, RSV and HMPV has received little attention. Other respiratory viruses that contribute to disease burden in the elderly and high-risk adults include HPIV, rhinovirus and coronavirus [6,7,71,107–111]. The disease burden by these viruses has probably been overshadowed by the predominance of influenza and RSV infection during the winter months, overlapping clinical symptoms between viruses, and owing to insensitive diagnostic tests [71,107,112]. The relative contribution of HPIV, rhinovirus and coronavirus to severe respiratory disease in the elderly will probably increase as rapid real-time PCR tests become more common. In addition, studies limited to the winter months during peak RSV and influenza infections may not accurately assess severe respiratory disease caused by viruses such as rhinovirus and HPIV, which can occur throughout the year and with various periodicities [71,112,113].

Several studies have tested patients admitted to the hospital with acute respiratory conditions for a wide variety of common respiratory viruses. While these studies were biased toward high-

risk patients, one study that examined elderly adults hospitalized for severe lower respiratory tract infections found that HPIV was associated with 2.5–3.1% of the infections [107]. Another study found that HPIV infections accounted for similar frequencies of respiratory virus infections as RSV in hospitalized patients older than 65 years of age with chronic pulmonary disorders (11.5% with HPIV compared with 10.4% with RSV) [6]. A prospective study in a nursing home found that HPIV infections accounted for 4–14% of respiratory illnesses [112]. The differences in infection rates reported by these studies may be attributed to methods of identification, periodicity of HPIV infections and environmental risk factors. Together, these studies demonstrate that HPIV significantly contributes to the disease burden in elderly and high-risk adults. Additional prospective investigations identifying the natural rate of HPIV infection and mortality in healthy young and elderly adults are required to more accurately assess the relative disease burden in the elderly population. It is currently not clear how age-related changes to the immune system contribute to disease severity of HPIV infections.

Rhinovirus and coronavirus infections in healthy adults are generally mild with the majority of infections being asymptomatic or not requiring hospitalization [108,114,115]. However, there is accumulating evidence that both viruses significantly contribute to the disease burden in aged adults, especially those with chronic cardiopulmonary conditions [109,112–114,116–120]. A prospective 2-year study in England that examined community-dwelling adults over the age of 60 years reported that rhinoviruses and coronaviruses were identified in 26 and 9.5% of reported respiratory infections, respectively, compared with 9.5% by influenza and 3% by RSV [7]. Another study in the USA identified rhinovirus and coronavirus infections in 12% of elderly patients admitted to the hospital with respiratory conditions, all of whom had underlying cardiopulmonary disease or cancer [109]. There have also been reports of severe rhinovirus outbreaks in nursing homes [114,116]. Rhinovirus infections are commonly identified in dual respiratory virus infections, which could contribute to increased disease severity [113]. As with HPIV, age-related changes to the adaptive immune responses to rhinovirus and coronavirus are not well studied. In conclusion, while influenza, RSV and HMPV infections probably account for the majority of severe acute respiratory virus infections, it is important to recognize that other virus infections are a significant cause of morbidity in elderly and high-risk adults.

Conclusion & future perspective

Aging affects cell-mediated and humoral responses during T- and B-cell development, the diversity and size of the peripheral naive T- and B-cell pool, the primary adaptive immune response to respiratory viruses, the maintenance of immunological memory and the ability to mount secondary responses. Owing to either increased virus replication or inappropriate immune responses, senescence of the immune system may be a major contributor to increased disease severity of respiratory virus infections in the elderly. However, high-risk factors, such as chronic cardiopulmonary conditions, undoubtedly contribute to increases in morbidity and mortality. High-risk and functionally disabled adults are more likely to require hospitalization for severe respiratory virus infections and have a higher mortality rate [3].

Most of our understanding of age-associated defects in adaptive immune responses to respiratory virus infections comes from influenza and RSV studies. In general, cytolytic $CD8^+$ T-cell responses have decreased magnitudes and are delayed, resulting in prolonged virus replication. Diminution of virus-specific $CD8^+$ T-cell responses probably occurs in part from decreased numbers of naive $CD8^+$ T cells able to respond to viral antigens, competition of new $CD8^+$ T-cell responses with clonal expansions of memory T cells and poor proliferative capacity. The effect of age-associated defects in $CD4^+$ T-cell help to $CD8^+$ T-cell responses has received little attention. $CD4^+$ T-cell help is important in maintaining memory $CD8^+$ T cells and in generating robust secondary responses [121,122]. Defects in $CD4^+$ T cells have

primarily been studied in aged animals using model antigens or T-cell receptor transgenic $CD4^+$ T cells. Future studies need to address age-related defects in $CD4^+$ T-cell responses to respiratory viruses and the effects of poor $CD4^+$ T-cell help on $CD8^+$ T cells and B cells.

The decline of humoral responses to respiratory virus infections is best exemplified by decreased influenza vaccine efficacy. There remains disagreement in the field regarding whether antibody responses diminishes with age. Age-related defects in antibody-mediated protection are probably a result of reductions in both quantity of neutralizing antibodies and quality (e.g., decreased affinity and diversity of antibodies). Since B-cell responses require $CD4^+$ T-cell help, it is important to establish whether defective $CD4^+$ T-cell responses contribute to attenuated antibody protection. The effects of aging on $CD4^+$ T-cell help and humoral responses to respiratory virus infections have not been directly explored. In addition, future studies need to establish whether contraction of the B-cell repertoire or decreases in antibody quality are linked with poor humoral responses in the elderly.

As we gain a better understanding of the basic cellular changes that occur during senescence of the immune system, we will be able to design vaccines and immunotherapies that circumvent defects or boost the immune response. Since vaccines are the most effective method of preventing illness, priority should be given to designing vaccines that protect elderly and high-risk adults. Vaccines to respiratory viruses will probably need to be able to induce both humoral and cell-mediated immunity to provide multiple lines of defense against infection.

Executive summary

Background

- Influenza virus and respiratory syncytial virus (RSV) are the leading causes of virus-induced severe respiratory disease in elderly and high-risk adults.
- Human metapneumovirus, human parainfluenza virus, rhinovirus and coronavirus also contribute to the respiratory disease burden in the elderly and high-risk adults.
- Immune senescence in addition to increased frailty and high-risk conditions, such as chronic cardiopulmonary disorders, lead to increased hospitalization and mortality rates in the elderly.

Age-related defects in cell-mediated immunity

- The generation of cell-mediated responses decreases with age owing to changes in the peripheral T-cell pool and the accumulation of T-cell intrinsic defects.
- Aged individuals exhibit decreased virus-specific $CD8^+$ T-cell responses and delayed peak cytolytic activity concomitant with prolonged virus replication.

Age-related defects in humoral immunity

- Humoral immune responses decrease with age. This is illustrated by an approximately 50% reduction in influenza vaccine efficacy in elderly individuals.
- In general, virus-specific antibody titers are lower in elderly adults compared with young adults.
- After influenza vaccination, aged individuals with antibody titers that would normally be protective in young adults exhibit a higher risk for infection compared with young vaccinated individuals, suggesting that antibodies in the elderly are of decreased quality.

Conclusion & future perspective

- Defects in both cell-mediated and humoral immunity contribute to increased infection rates and disease severity in elderly adults.
- It is not clear whether decreases in humoral immunity are due to B cell-intrinsic defects or deficient $CD4^+$ T-cell help. Future studies need to directly examine if age-related changes to $CD4^+$ T cells alter the humoral response to respiratory viruses.
- Designing vaccines that induce strong humoral and cell-mediated immunity in the elderly is a priority. The use of adjuvants or cytokines with vaccines may be particularly useful in eliciting protective immunity.

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