Published in final edited form as:
Ageing Res Rev. 2011 September ; 10(4): 422–427. doi:10.1016/j.arr.2011.02.001.

CD8 T Cell Responses to Influenza Virus Infection in Aged Mice

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Abstract

Influenza is one of the most common infectious diseases afflicting humans, particularly the elderly. The murine model has been widely employed for investigation of immunity to influenza virus infection. In this paper, we review the recent advances in understanding the diminished CD8 T cell immune response to influenza virus infection in aged mice. Possible mechanisms of impaired CD8 T cell responses with aging are addressed, including: 1) the role of dendritic cells (DCs); 2) the effect of CD8 T cell clonal expansion (TCE); and 3) the interactions with CD4 T cells, including T regulatory (Treg) cells and CD4 T helper cells. The aged murine model of the CD8 T cell response to influenza virus is helping to elucidate the mechanisms of immunosenescence which can lead to therapeutic improvements in the primary CD8 T cell response to new infections, as well as the development of new strategies for immunization to prevent influenza in the elderly.

Keywords

CD8 T cell; Aged mice; Influenza virus

1. Introduction

Influenza is a disease with serious consequences including hospitalization and death, especially for elderly individuals over 65 years of age. The influenza pandemic of 1918 killed nearly 50 million people worldwide, with the elderly having the highest mortality rate (Johnson and Mueller, 2002; Ahmed et al., 2007). A strong primary immune response is important for protection against viruses or viral strains previously not encountered, as was the cause of this pandemic. An alteration of the immune system occurs with aging and the primary immune response diminishes, leaving the elderly more susceptible to infectious diseases. Parallel to the aging of the population (CDC, 1998), the incidence of influenza infection has increased dramatically in recent years. Influenza and its complications were responsible for approximately 36,000 deaths annually from 1990-1999 in the U.S. (Thompson et al., 2003), almost double the number between 1976 and 1990. Today, although none of the top five leading causes of death for the overall population is infectious disease (Hoyert et al., 2003), influenza and its associated pneumonia kill a significant proportion of the population over 65 years of age and close to 90 percent of all combined influenza and pneumonia deaths occur in this age group. While the segment of the

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population over the age of 65 has the highest rate of influenza vaccination, these vaccinations offered less protection due to immunosenescence, protecting only 30-34% of recipients age > 65, but up to 90% of those < 65 years (Zheng et al., 2007).

Seventy-five years ago, Andrewers et al. (1934) demonstrated that the virus of human epidemic influenza can infect mice when inoculated intranasally (Andrewers et al., 1934). More recently, it was reported that Swine-origin 2009A (H1N1) influenza virus isolated from humans can infect mice with a high infectivity (Maines et al., 2009). Currently, the murine model is widely employed as a useful model for the investigation of aging, as well as immune response to influenza virus infection (Effros et al., 1983; Po et al., 2002; Asanuma et al., 2007).

In primary virus infections, cell mediated immunity, particularly the $CD8^+$ cytotoxic T lymphocyte (CTL) response, is responsible for virus clearance. The importance of CTLs in primary influenza virus infection has been demonstrated most clearly using athymic, nude mice. When nude mice were infected with influenza virus, these mice were unable to eliminate influenza virus from their lungs. While passive transfer of influenza-specific antibodies could transiently reduce the level of influenza virus in the lungs (Kris et al., 1988), permanent clearance of influenza virus was only achieved by adoptive transfer of influenza-specific CTLs (Lin et al., 1981; Lukacher et al., 1984), which directly implicates CTLs in the antiviral defense mechanism against experimental influenza infection. Therefore, assessment of the differences in CTL responses between young and aged mice has been the focus of most studies investigating the mechanism of the decreased immune response to primary influenza infection in aged mice.

2. Primary CD8 T cell response

Although aged mice are able to mount a specific CD8 T cell response to influenza virus, the kinetics and magnitude of the response are altered compared to young mice (Effros et al., 1983; Po et al., 2002). After intraperitoneal (i.p.) inoculation with influenza virus, the primary CTL response to influenza reached the peak at Day 5 in young BALB/c mice, while the CTL response was almost completely absent at this time in aged BALB/c mice. The kinetics of the response in aged mice was shifted with maximal CTL activity appearing on Day 7 after infection. In addition, the magnitude of the maximal CTL response generated by aged mice was significantly lower than the maximum generated by young mice (Effros et al., 1983). CTL activity and IFN-γ production by splenic CD8 T cells were also significantly lower in aged than in young BALB/c mice on Days 8, 12, and 17 after infection by small-particle aerosol (Mbawuike et al., 1996). This decreased CTL response continued to be observed when splenocytes from young and aged mice previously immunized i.p. with influenza virus (PR8 strain) were restimulated *in vitro*, and effectors were assayed after 5 days of culture. While 60% (7/12) of aged mice demonstrated responses similar to those of young mice (both 18~45% with means of 29%), the remaining 40% of aged mice demonstrated 0~13% cytotoxicity (Effros et al., 1983).

Recognition of influenza by CD8 T cells occurs through presentation of specific viral peptides on MHC-I molecules. These immuno-reactive peptides, known as CD8 T cell epitopes, are usually 8-10 amino acids in length (Heemels and Ploegh, 1993; Yewdell and Bennink, 1993). Identification of murine influenza epitopes has been useful in laboratory research, as specific clones of CD8 T cells can be stimulated with defined peptides without using the intact virus. Several important epitopes of influenza virus in mice have been identified in the past 20 years, including NP (nucleoprotein)$_{366\text{-}374}$ (Carbone et al., 1988; Cerundolo et al., 1991; Flynn et al., 1998) and PA (acid polymerase)$_{224\text{-}233}$ (Belz et al., 2000) which are restricted to presentation by the $H\text{-}2D^b$ MHC class I allele, and HA

(hemagglutin)$_{518\text{-}526}$ (Kuwano et al., 1991), NP$_{147\text{-}161}$ (Taylor et al., 1987) and PB1$_{703\text{-}711}$ (Meijers et al., 2005), which are H-2K^{d} restricted. Antigen specific CD8 T cells can be detected directly through tetramer staining of the MHC-I/peptide complex (Altman et al., 1996). In addition, detection of specific responses can also be achieved by stimulating cells with the epitope *in vitro* and measuring IFN-γ production by intracellular staining (Appay and Rowland-Jones, 2006; Serbina and Pamer, 2003).

Using the MHC-I tetramer and intracellular IFN-γ assays, Po et al. (Po et al., 2002) analyzed the NP$_{366\text{-}374}$ specific CD8 T cell response in C57BL/6 mice. On Day 10 post-intranasal infection with influenza PR8 strain, significant decreases in both percentage (A vs Y: ~3.1% vs ~9% of total CD8 T cells) and number (A vs Y: ~1×10^5 vs ~5.9×10^5) of NP$_{366\text{-}374}$-specific CD8 T cells were observed in the lungs of infected aged C57BL/6 mice compared to infected young mice. The kinetics of the CD8 T cell response in infected lungs showed peak percentages of NP$_{366\text{-}374}$ $^{+}$CD8 T cells on Day 10 post-infection in young mice, but was delayed to Day 14 in aged mice. Furthermore, the maximal expansion of NP$_{366\text{-}374}$ $^{+}$CD8 T cells achieved in aged mice 14 days post-infection was significantly lower than that of young mice 10 days post-infection. IFN-γ production, maximal cytotoxic activity, and virus clearance paralleled the magnitude and kinetics of the expansion of NP$_{366\text{-}374}$ $^{+}$CD8 T cells in both aged and young mice (Po et al., 2002). Interestingly, on the day of peak response in aged, only 65% of the IFN-γ^{+} CD8 T cells of aged mice bound NP$_{366\text{-}374}$ tetramer, while 90% of the IFN-γ^{+} CD8 T cells of young mice were specific for NP$_{366\text{-}374}$ (Jiang et al., 2009), suggesting that more functional CD8 T cells of aged mice either were reactive to other epitopes of influenza or were non-specific (“bystander activation”).

Generation of strong specific CD8 T cell responses to protective dominant epitopes, such as NP$_{366\text{-}374}$, in lungs after infection or immunization is critical for clearance of influenza. Intranasal (i.n.) infection with influenza virus is a common method of infection employed to study murine influenza since the disease is spread mainly by respiratory droplets, causing pathogenesis and inducing immune responses in the respiratory system. However, the magnitude of the response is related to the amount of virus inoculation. Since aged mice cannot survive when a high dose (i.e. >75 HAU of PR8) of influenza virus is given via i.n. (Po et al., 2002), the response is generally low. Mice, particularly aged mice, are more resistant to a higher dose (i.e. 300 HAU of PR8) of the virus when administered intravenously (i.v.). In order to more carefully examine the differences in response with aging, young and aged C57BL/6 mice were infected i.v. with 300 HAU of PR8, and the specific CD8 T cell response in the lungs was examined using NP$_{366\text{-}374}$ tetramer: Similar to i.n. infection, both a decrease and a delay in response was observed in the lungs of aged mice. On day 7 after infection, 32% and 2.8% NP$_{366\text{-}374}$ $^{+}$CD8 T cells of total CD8 T cells in the lungs were observed in young and aged mice, respectively, while on day 10, they were 25% and 7.1% (Jiang et al., unpublished data). A similar trend of the specific CD8 T cell response in the spleens was observed in young and aged mice, although the magnitude of the response was consistently lower in spleens than in lungs (Jiang et al., 2009). Overall, these results showed that: 1) i.v. infection with flu virus can also generate strong specific CD8 T cell response in the lungs compared with i.n. infection; 2) the peak response in the lungs for both young and aged mice occurs earlier upon i.v. infection (Y vs A: day 7 vs day 10) than i.n. infection (Y vs A: day 10 vs day 14); and 3) the primary immune response following influenza virus infection is diminished in aged mice in both magnitude and rate under various routes of infection.

Since mice are inbred animals, the results obtained from mice may reflect a skewed response due to a specific genetic background. To demonstrate that the response described above is not specific to the C57BL/6 genetic background, BALB/c mice were infected with influenza

virus. Similar results were also obtained when BALB/c mice were i.v. infected with PR8 and the response to their immunodominant CD8 T cell epitope (H-2K^{d}-HA$_{518-526}$) was examined after infection (Jiang et al., 2009). The percentage of HA$_{518-526}$-CD8 T cells detected in spleens was significantly lower in aged than in young mice 7 days after infection. In addition, the peak expansion of the specific CD8 T cells occurred on Day 7 and Day 10 in young and aged mice, respectively. These results verify that this is not a phenomenon unique to or dependent on one particular strain of mice and suggests a similar situation may occur in humans. Further, these results provide explanation why aged mice have a diminished capacity to clear virus, as the decreased expansion of specific CD8 T cells could lead to a decreased influx of CD8 T cell effectors to the primary site of infection, enabling the virus to rapidly disseminate and overwhelm the host response.

3. Memory CD8 T cell response

Specific CD8 T cell recall responses to influenza virus have been well studied in young mice (Flynn et al., 1998; Kedzierska et al., 2004; Crowe et al., 2003), while there have been limited studies in aged mice. It has been shown that the memory CD8 T cell response to NP$_{366-374}$ in aged mice is diminished compared with young mice (Yager et al., 2008; Decman et al., 2010). The number of the NP$_{366-374}$ memory CD8 T cells is significantly reduced in both lungs and spleens of aged mice > day 40 after influenza virus infection. However, the function of the memory CD8 T cells appears to remain intact in aged mice: In the spleens, similar percentages of NP$_{366-374}$ tetramer+ CD8 T cells (~55%) produce IFN-γ in both young and aged mice, and similar percentages of the specific IFN-γ producing cells are able to generate TNF and IL-2, and express CD40L (Decman et al., 2010).

Compared with young mice, protective immunity generated after primary influenza infection in aged mice is decreased upon secondary influenza infection (Yager et al., 2008; Decman et al., 2010). In these studies, young and aged C57BL/6 mice were infected i.n. with PR8 (H1N1), and then challenged i.n. with a different subtype strain, x31 strain (H3N2). This strategy avoids the complication of cross-neutralizing antibodies and allows T cell immunity to be examined directly since anti-H1 and -N1 antibodies generated during the first infection cannot neutralize the x31 strain, allowing the x31 strain to replicate and to re-stimulate NP$_{366-374}$-specific CD8 T cells and induce a CD8 T cell recall response (Decman et al., 2010; Liang et al., 1994). With this experimental system, Yager et al. (2008) found that NP$_{366-374}$-specific CD8 T cell frequencies were strongly correlated to clearance of influenza virus in lungs on Day 7 post challenge. All young mice had undetectable viral levels and had an average of 15% NP$_{366-374}$-specific CD8 T cells. However, only 1/3 of aged mice cleared virus by Day 7, and the aged mice had lower levels of NP$_{366-374}$-specific CD8 T cell expansion. The aged mice which cleared the virus had 5-15% NP$_{366-374}$-specific CD8 T cells, while those with detectable viral loads had less than 5% specific CD8 T cells (Yager et al., 2008). These data demonstrate that the magnitude of CD8 T cell recall response to the dominant CD8 T cell epitope of influenza virus is correlated with viral clearance and is impaired in aged compared with young mice.

Decman et al. (Decman et al., 2010) found that morbidity and mortality is increased in aged mice upon secondary infection. Weight loss was more severe in aged mice and 30% of the aged mice died upon re-challenge, while all of young mice survived. In addition, virus was cleared by Day 5 in the lungs of all young mice, but was still present at high levels (~10^4 TCID$_{50}$/g of lung) in the majority of aged mice. Interestingly, there was a distinct difference in the numbers of memory CD8 T cells produced, with a marked decrease in immunodominant epitope NP$_{366-374}$ specific CD8 T cells in aged mice, while responses to subdominant epitopes appeared less impaired by age (Decman et al., 2010). In aged mice, impaired response to a CD8 T cell immunodominant epitope, e.g., NP$_{366-374}$, correlates with

a decreased ability to eliminate virus after secondary challenge with an influenza strain that contains the immunodominant CD8 T cell epitope, but a different antibody reactive epitope (primary vs secondary infection: H1N1 vs H3N2) (Yager et al., 2008). The loss of this dominant CD8 reactivity may be the cause of decreased protection that has been shown to occur with aging (Yager et al., 2008).

When equal numbers of $NP_{366-374}$-specific memory CD8 T cells from both young and aged mice were transferred into congenic young mice prior to infection with recombinant vaccinia virus expressing $NP_{366-374}$ peptide, the $NP_{366-374}$-specific memory CD8 T cells from aged mice did not expand as much as those from young mice. In the blood, 3 to10 fold lower frequencies of $NP_{366-374}$-specific CD8 T cells derived from aged donors were observed at days 7, 14, and 21 post-challenge compared with their young counterparts, demonstrating that intrinsic defects in memory CD8 T cells of aged mice may, at least partially, impair their ability to mount vigorous recall responses during secondary influenza infection (Decman et al., 2010).

4. Mechanisms of impaired CD8 T cell response to influenza virus in aged mice

The mechanisms of impaired CD8 T cell response in the aged are complex and still remain largely unknown, although it has been shown that both extrinsic and intrinsic factors may contribute to this impairment (Linton et al., 2005; Jiang et al., 2007; Jiang et al., 2003). Investigation of specific T cell immune responses has recently been made easier by the development of CD8 TCR transgenic (Tg) mice in which the majority of CD8 T cells have receptors that recognize one specific epitope. The oldest reference was that of Katz et al. (Katz et al., 2004) who stated that adoptively transferred NP-specific TCR Tg T cells from young mice displayed extensive expansion in response to the NP epitope when the recipients were young mice, but underwent more limited expansion in aged recipients after influenza virus infection. Conversely, transferred Tg T cells of aged mice also proliferated better in response to antigen in young than in aged recipients (Katz et al., 2004). However, this review article did not provide specific details regarding the magnitude of expansion in either young or aged mice. In our recent studies (Jiang et al., 2009), we found less expansion of transferred CD8 T cell from young Clone-4 mice (BALB/c Tg mice in which CD8 T cells recognize $HA_{518-526}/K^d$ of influenza virus) in aged than in young BALB/c mice (A vs Y: 11% vs 23% of total CD8 T cells). These results indicate that environmental factors extrinsic to the CD8 T cells are partially responsible for the defects in CD8 T cell expansion that occur with aging. Several possible extrinsic parameters that can influence the altered CD8 T cell response to influenza virus infection with aging include: 1) the role of dendritic cells (DCs); 2) the effect of CD8 T cell clonal expansion (TCE); and 3) the interactions of CD8 T cells with CD4 T cells, e.g., T regulatory (Treg) cells and CD4 T helper cells.

4.1. The role of DCs in aged mice

DCs play an important role in generating both innate and adaptive immune responses (Lanzavecchia and Sallusto, 2001; Sallusta and Lanzavecchia, 2002; Iwasaki, 2007), but information regarding alterations in DCs with aging during influenza virus infection is very limited (Linton et al., 2005). It has been reported that the number of DCs recovered from spleens of aged mice is decreased to approximately 50–70% of that of young mice (Linton et al., 2005). While our recent data show that both aged BALB/c and C57BL/6 mice demonstrate a substantially decreased number of DCs in spleens, a comparable or even increased percentage of DCs in the total splenocytes was found in the aged mice (Jiang et al., 2009). Interestingly, no significant differences in the representation of lymphoid,

myeloid, and plasmacytoid DC subsets in the spleens of young and aged mice have been reported (Linton et al., 2005).

After immunization with influenza virus, dramatic decreases in the expression of the DC antigen-recognition receptor, DEC-205 (Jiang et al., 1995), and in the number of $CD11c^+$ DCs in both the spleen and lymph nodes were found in aged compared to young mice (Linton et al., 2005). In addition, the recruitment of CFSE-labeled airway DCs to the draining lymph nodes was significantly decreased in aged mice after i.n. immunization with influenza virus (Linton et al., 2005). These findings suggest changes in maturation and/or recruitment of DCs in aged mice.

To examine whether the function of DCs in priming CD8 T cells is altered with aging, we incubated DCs from young and aged BALB/c mice with CFSE-labeled Clone-4 TCR Tg CD8 T cells and $HA_{518\text{-}526}$ peptide *in vitro*. The proliferation and expansion of the Clone-4 cells was comparable after culture with DCs of young and aged mice at 48h and 72 h post stimulation, with similar results being observed in the LCMV Tg C57BL/6 mice system (P14)(Jiang et al., 2009). These data suggest that DCs of aged mice demonstrate no decrease, at least *in vitro*, of antigen presenting function.

However, we have recently demonstrated that an alteration in DCs is one of the factors in the aged environment that contributes to the impaired specific CD8 T cell response to influenza virus infection *in vivo*. Having observed that aged mice have a decreased number of DCs, but that they appear to have comparable subsets and comparable function on a per cell basis as determined *in vitro* (Jiang et al., 2009; Linton et al., 2005), we postulated that increasing the number of DCs might enhance the CD8 T cell response of aged mice. To examine this hypothesis, we co-transferred purified Clone-4 Tg CD8 T cells from young mice with enriched $CD11c^+$ DCs of young BALB/c mice into young or aged BALB/c mice. The recipients were then infected with influenza virus. Transfer of TCR Tg CD8 T cells alone resulted in limited expansion in aged compared to young mice on Day 3 after infection. However, when the DCs were co-transferred with young TCR Tg CD8 T cells, the expansion of the Tg cells significantly increased in aged, but not young, mice. Along with the expansion of TCR Tg CD8 T cells, the number of Tg cells producing IFN-γ as demonstrated by intracellular staining similarly increased in aged mice when DCs from young mice were co-transferred (Jiang et al., 2010). These results demonstrate that DCs from young mice can enhance the specific CD8 T cell response to virus infection in aged mice, indicating that either the number of DCs is insufficient or their function may be impaired in aged mice.

4.2. The effect of TCE on CD8 T cell response

The ability to generate effective CD8 T cell responses to newly encountered infections and to respond to immunization requires the maintenance of a diverse repertoire of T cell specificities (Messaoudi et al., 2002; Yewdell and Haeryfar, 2005; Kedzierska et al., 2005). It has been reported that the functional diversity of the αβ TCR repertoire in the spleens of young mice is about 2×10^6 different clones of about 10 cells in each clone (Casrouge et al., 2000). However, TCE, i.e., clonal expansion of T cells expressing a specific TCR, has been shown to occur in both aged humans (Posnett et al., 1994; Hadrup et al., 2006; Ouyang et al., 2003) and animals (Callahan et al., 1993; Posnett et al., 2003; LeMaoult et al., 2000; Messaoudi et al., 2004; Ely et al., 2007). TCE can comprise 70-80% or more of the total CD8 T cell compartment in some aged mice (Posnett et al., 1994; Callahan et al., 1993), filling up space that could be used for other specificities of CD8 T cells. Thus, it has been speculated that TCE and the resulting loss of diversity associated with aging is a contributing factor to the impaired immune response to infections and vaccines.

CDR3 regions are the most polymorphic parts of the TCR and their diversity is representative of T cell diversity in a given T cell population. Using CDR3 length spectratype analysis of the naive CD8 T cell repertoire in young and aged mice, Ahmed et al. (Ahmed et al., 2009) found that naive T cells from aged mice have altered spectratypes compared with the characteristic bell-shaped "Gaussian" spectratype profiles of naive CD8 T cells from young mice. DNA sequence analysis confirmed a loss of diversity related to these altered spectratypes. Recently, Yager et al. (Yager et al., 2008) studied the effect of aging on the diversity of the CD8 T cell response to influenza virus infection. They observed a selective decline or even a lack of the $NP_{366-374}$-specific T cell responses in aged mice infected with influenza virus. The $NP_{366-374}$-specific CD8 T cells express $V\beta8.3^+$ TCRs (Deckhut et al., 1993), and the repertoire of CD8 TCRs responding to NP366-374 in C57BL/6 mice after primary infection with influenza virus has been well characterized (Townsend et al., 1986; Zhong and Reinherz, 2004). The decreased $NP_{366-374}$-specific CD8 T cell responses were associated with altered spectratypes of the $V\beta8.3^+$ TCRs of $NP_{366-374}$-CD8 T cells, and correlated with impaired viral clearance after re-challenge. These findings suggest that the impact of aging on the CD8 T cell response to influenza virus is caused, at least partially, by limited T cell repertoires and particularly by the selective loss of the repertoire for a typically immunodominant epitope, which in the case of influenza, is $NP_{366-374}$. This age-associated decline in T cell repertoire diversity may impact the immune response to new or emerging viruses by limiting the range of epitopes to which T cells can react.

4.3. CD4 T cells: Treg cells and CD4 T helper cells

Two subsets of CD4 T cells, Treg cells and CD4 T helper cells, interact with CD8 T cells and thus alteration in these cell populations due to aging may influence the function of CD8 T cells during influenza virus infection. Very little is known regarding the relationship between Treg cells and decreased immune responses to virus infection in aged mice. However, it has been demonstrated that the number and percentage of Treg cells in secondary lymphoid organs (spleen and lymph nodes) of aged mice are significantly higher than that of young mice (Sharma et al., 2006; Williams-Bey et al., 2010; Chiu et al., 2007; Zhao et al., 2007; Lages et al., 2008). Treg cells in aged mice also showed modified Vβ family distribution, which suggests the presence of selective clonal expansion (Chiu et al., 2007). Despite these changes, Treg cells of aged mice still retain their inhibitory function. Recently, we found that splenic Treg cells of both young and aged mice can significantly and comparablely inhibit expansion of the Clone-4 Tg CD8 T cells after stimulation *in vitro* with $HA_{516-524}$ peptide of influenza virus (Williams-Bey et al., 2010). Interestingly, depletion of Treg cells from splenocytes of aged mice can significantly enhance the proliferation of the Tg CD8 T cells, but there is no effect when Treg cells are depleted from splenocytes of young mice (Jiang et al, unpublished data). Upon influenza infection, aged, but not young, mice have a significant expansion of Treg cells, which may contribute to the impaired CD8 T cell response to primary infection (Williams-Bey et al., 2010). In this study, the maximal expansion of Treg cells in aged mice occurred on Day 7 returning to pre-infection levels on Day 10, the time of peak CD8 T cell response in aged mice. Treg cells may affect CD8 T cell response via intermediaries. For example, when Treg cells were inactivated by anti-CD25 antibody, the low expression of co-stimulatory molecules CD40 and CD86 on DCs of aged mice were restored to the levels of young mice (Chiu et al., 2007).

In addition to CD4 Treg cells, CD4 T helper cells may contribute to the diminished CD8 T cell response with aging. As described earlier, the magnitude of the CD8 T cell recall response to the dominant CD8 T cell epitope of influenza virus has been shown to be decreased in aged mice (Yager et al., 2008; Decman et al., 2010; Sambhara et al., 2001);

however, the reasons for this effect still remain to be elucidated. It has been demonstrated CD4 T cell help is required during the priming phase for the generation of long-term memory CD8 T cells in young mice (Janssen et al., 2003; Shedlock and Shen., 2003; Sun and Bevan, 2003). In addition, it had been repeatedly reported that naïve CD4 T cell function including IL-2 production and helper function is impaired in aged mice (Linton et al, 1996; Haynes et al., 2000; Haynes et al., 1999; Haynes et al., 2003). Therefore, the decreased CD8 T cell recall response to influenza virus infection may partially result from the deficiency in CD4 T cell help with aging. While the decreased helper function of CD4 T cells of aged mice is established regarding B cell response, and adjuvant or an adjuvant containing proinflammatory cytokines can enhance the helper function resulting in improved expansion and differentiation of B cells (Maue et al., 2009; Maue and Haynes, 2009), no similar studies have been reported for CD8 T cell function in influenza. More studies need to be done to determine the role of CD4 T cell help in the diminished memory CD8 T cell response to influenza virus infection in aged mice, as this could present a target mechanism for the improvement of vaccinations in the elderly population.

5. Conclusions

Aging affects the CD8 T cell response to influenza virus infection, causing a diminished ability of the immune system to mount both primary and secondary immune responses. The impaired primary response increases the concern of exposure to new or evolving influenza strains, while simultaneously, the decreased secondary response inhibits the effectiveness of vaccination in the elderly. Using the aged murine model to study the CD8 T cell response to the virus has facilitated understanding of the altered immune response with aging. The intrinsic alterations of CD8 T cells of aged mice during both primary and secondary infection need to be further elucidated; aged-related changes occur in proliferation, cytokine production, and repertoire diversity, but the contribution of each of these changes to the decreased response is yet unknown and needs to be investigated. It is also known that environmental changes extrinsic to the CD8 T cells can impact the CD8 T cell immunity to influenza in aging. Thus, future studies of strategies to enhance the CD8 T cell response to virus infections in aged mice should include how to improve the function of DCs and CD4 T helper cells and to decrease the suppressive function of Treg cells. Discovering the mechanisms of these alterations with aging, as well as the interaction between the intrinsic and extrinsic changes, will have important implications for developing new strategies to decrease morbidity and mortality of influenza in the elderly.

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Research Hightlights

- In this paper, we review the recent advances in understanding the diminished CD8 T cell immune response to influenza virus infection in aged mice.
- Possible mechanisms of impaired CD8 T cell responses with aging are addressed, including: the role of dendritic cells; the effect of CD8 T cell clonal expansion; and the interactions with CD4 T cells, including Treg cells and CD4 T helper cells.
- The aged murine model of the CD8 T cell response to influenza virus is helping to elucidate the mechanisms of immunosenescence.