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Aging and CD8+ T cell immunity to respiratory virus infections

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Abstract

The capacity of the immune system to mediate effective immune responses to pathogens declines with age. In the case of immune responses to newly encountered antigens, several studies have demonstrated that this decline reflects both a loss of naïve T cells and changes in the repertoire and function of these cells over time. However, comparatively little is known about the impact of age on established memory T cells pools. Here we discuss age-related changes in memory $CD8^+$ T cell pools elicited by influenza and parainfluenza viruses and the impact of these changes on immunity in general.

A large number of studies have documented that increasing age is associated with a progressive decline in the capacity to mount effective immune responses (Burns et al., 1993;Cook et al., 1987;Effros, 2003;Grubeck-Loebenstein and Wick, 2002;Haynes et al., 2005;Linton and Dorshkind, 2004;McElhaney, 2003;McElhaney, 2005;Miller, 1991;Miller, 1996;Murasko et al., 2002;Murasko and Jiang, 2005;Nagelkerken et al., 1991;Phair et al., 1978;Wick et al., 2000;Zheng et al., 1997). Aged individuals typically have an impaired capacity to clear infections and also exhibit significant defects in graft rejection, delayed type hypersensitivity, and tumor rejection (Haynes and Eaton, 2005;Miller, 1996;Po et al., 2002). These defects have important clinical consequences for the elderly in terms of susceptibility to infection and poor vaccine efficacy. Recent studies have implicated both the cellular and humoral arms of the immune response in this age-related decline in immune function. For example, studies in animals and humans have shown that aged individuals have a significantly reduced capacity to mount an effective antibody response following infection or vaccination, which is thought to be a consequence of dysfunctional aged $CD4^+$ T cell "help" (High, 2004;Johnson and Cambier, 2004). In addition, involution of the thymus results in a decline in naïve T cell numbers, potentially limiting the breadth and quality of the repertoire of the T cell response (Naylor et al., 2005). However, we still lack a solid understanding of the scope of immune defects or mechanisms underlying the decline in immune efficacy in the elderly.

Whereas most studies addressing the impact of aging on immunity have focused on immune responses to newly encountered antigens, relatively little work has addressed the impact of age on pre-existing memory T cell populations. Studies by Kapasi *et al* using a systemic viral model in mice demonstrated that $CD8^+$ T cell memory remains functional for over a year after its initial generation (Kapasi et al., 2002). This is consistent with human studies suggesting that cellular immunity is relatively long lived (Hammarlund et al., 2003). However, the relative efficacy of the recall of long-term T cell memory (ie. memory that was originally established

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in young individuals) has not been thoroughly studied, especially with respect to pathogens that enter through mucosal sites. We have investigated this issue in the mouse by addressing the impact of aging on the recall of memory $CD8^+$ T cells by respiratory virus infections, such as those mediated by influenza and parainfluenza viruses. This is an important class of pathogens that are a significant clinical problem in the aged. Our data suggest that the recall response is complex and that distinct components of the response are differentially impacted by increasing age.

(i) Basic features of the $CD8^+$ T cell recall response to respiratory virus infections

Memory $CD8^+$ T cells mediate accelerated and enhanced recall responses to secondary virus infections. This is due to both increased numbers of antigen-specific T cells (compared to the naïve T cell pool) and enhanced responsiveness to antigen challenge (reduced co-stimulatory requirements and the higher activation status of the cells). In addition, memory $CD8^+$ T cells can be classified into two major subpopulations (central-memory and effector-memory) based on their migration properties. Central memory T cells express high levels of CD62L and CCR7 and accumulate in secondary lymphoid organs and the bone marrow whereas effector memory T cells express low levels of CD62L and CCR7 and accumulate in the tissues and peripheral organs (Becker et al., 2005;Sallusto et al., 1999). Current data suggest that effector-memory and central-memory $CD8^+$ T cells play distinct roles in the recall response to influenza and parainfluenza virus infection. In general, the response can be divided into three temporally distinct phases (Ely et al., 2003a;Hikono et al., 2006). The initial response to infection (phase 1) involves effector-memory $CD8^+$ T cells that are already present in the lung airways. These are optimally situated to encounter the pathogen as soon as it enters the lung and immediately initiate anti-viral responses. The second phase involves effector-memory $CD8^+$ T cells that are not proliferating and are directly recruited to the lung airways from the circulation (Ely et al., 2003a;Hikono et al., 2006). Finally, the third phase involves both central- and effector-memory $CD8^+$ T cells that proliferate in the draining lymph nodes in response to antigen and are recruited to the lung airways as fully-activated effector T cells (Cauley et al., 2003;Hikono et al., 2006;Sallusto et al., 1999). These three phases operate seamlessly to generate a rapid and sustained recall response to the infection. Importantly, optimal protection is dependent on the strength of the early phases (phase 1 and 2) of the response which occur over the first few days following infection. In support of this, intratracheal transfer studies have shown that memory cells present in the lung airways are able to substantially reduce viral load (Hogan et al., 2001a;Hogan et al., 2001b).

(ii) Impact of age on the early phases of the recall response

Effector-memory $CD8^+$ T cells persist in the lung airways of both humans and animals following resolution of a respiratory virus infection (de Bree et al., 2005;Hogan et al., 2001a;Ostler et al., 2001;van Panhuys et al., 2005;Wiley et al., 2001). There is substantial evidence that these cells play a key role in mediating the initial phase (phase 1) of the recall response to secondary infection. However, this population declines rapidly over the first six months post-infection to a minimal, but sustained, number of cells that persists for the life of the animal (Table 1). This progressive loss of memory T cells from the lung airways correlates with a progressive decline in the efficacy of the T cell recall response (Liang et al., 1994). Thus, this important component of the T cell memory pool is not maintained at protective levels as the individual ages.

The early stages of the recall response are also mediated by memory $CD8^+$ T cells that migrate into the lung from the circulation in response to inflammatory signals (phase 2 cells). Despite the fact that these cells are not proliferating and therefore cannot maintain a sustained response,

they nevertheless make a substantial contribution to the overall recall response (Ely et al., 2003a). These cells appears to be derived predominantly from the circulating effector memory T cell pool (Hikono et al., 2006). To determine the effect of age on the contribution of phase 2 cells to the recall response we determined the numbers of antigen-specific (tetramer-positive) memory $CD8^+$ T cells that could be recruited to the lung airways by a heterologous virus infection. As shown in Table 1, the numbers of memory T cells that could be recruited to the lung airways declined dramatically with time. By 20 months post-infection, there was essentially no recruitment of these cells, indicating that phase 2 was completely missing from the recall response at this time. Interestingly, this progressive decline in the capacity to recruit phase 2 effector-memory $CD8^+$ T cells directly correlates with a progressive loss of effector-memory T cells from the splenic memory T cell pool (Roberts et al., 2005). Although the numbers of memory cells in the spleen remains relatively constant, the composition of the population switches from a predominantly effector-memory population (which can be readily recruited to the lung) to a predominantly central-memory population (which cannot be recruited to the lung). The progressive loss of phase 1 and phase 2 memory cells over time corresponds to a decline in the efficacy of cellular immunity to respiratory virus infections.

(iii) Impact of age on the late phases of the recall response

The third phase of the recall response involves memory $CD8^+$ T cells that are stimulated by antigen to proliferate, mature into effector T cells, and then migrate the lungs (Hikono et al., 2006). Both central and effector memory $CD8^+$ T cells have been shown to contribute to this phase of the response (Roberts et al., 2005;Roberts and Woodland, 2004). In light of the changes in the distribution of effector and central memory T cell pools with age, we directly compared the capacity of recent and long-term memory $CD8^+$ T cell pools to proliferate in response to Sendai virus infection *in vivo*. This was done using a dual transfer approach in which the response of two donor T cell populations can be directly compared under identical conditions in the same infected animals (Ely et al., 2003b;Roberts and Woodland, 2004). Surprisingly, the data showed that, on a per cell basis, the 12 month donor memory cells generated a substantially stronger proliferative response than the 1 month donor memory cells (Roberts et al., 2005). This preferential response could not be attributed to clonal $CD8^+$ T cell expansions that have been observed in aging mice, as mice with abnormal memory T cell pools were excluded from the analysis (Callahan et al., 1993;Ku et al., 1997;Messaoudi et al., 2004). Thus, memory $CD8^+$ T cells in the systemic immune system actually increased their capacity to contribute to the recall responses over time. These data suggest that T cell memory in the spleen improves over time on a per cell basis and it appears to take at least a year for stable T cell memory to be fully established (Kaech et al., 2002;Roberts et al., 2005;Wherry et al., 2003).

The mechanisms underlying the increasing proliferative efficacy of memory T cell subpopulations over time are not known. Although it is possible that all of the memory cells (on a per cell basis) gradually improve their capacity to proliferate in response to antigen, it seems more likely that there is simply the progressive accumulation of a specific subpopulation of cells with strong proliferative potential. Since central-memory ($CD62L^{hi}$) $CD8^+$ T cells progressively accumulate in the spleen over time, we speculated that these cells may have a superior proliferative response compared to effector-memory T cells. For example, if the $CD62L^{hi}$ cells had a greater proliferative capacity than $CD62L^{lo}$ cells, their progressive accumulation in the spleen could account for the increase in responsiveness of the total memory pool. However, dual adoptive transfer studies with isolated $CD62L^{hi}$ and $CD62L^{lo}$ populations revealed that both populations increased their capacity to contribute to the recall responses over time. Thus, the increase in recall efficacy of $CD8^+$ T cells over time cannot be entirely attributed to central-, or effector-memory phenotype and must correlate with some other feature of the memory T cell pool. One possibility is that the proliferative capacity correlates with

changes in the TCR repertoire and the overall avidity of the cells for antigen. However, we did not observe differences in the functional avidity of antigen-specific T cells in aged mice (Cole et al., 1994, and data not shown). An alternative possibility is that proliferative capacity correlates with memory $CD8^+$ T cell subsets that differ in activation status. Although memory $CD8^+$ T cell are resting, they express different patterns of activation markers such as CD43 and CD27 and the distribution of these subpopulations changes over time. Thus, memory $CD8^+$ T cells in the spleen progressively change from a more activated ($CD43^{hi}$) phenotype to a less activated ($CD43^{lo}$) phenotype. Preliminary dual-adoptive transfer studies suggest that proliferative efficacy may indeed be linked to low levels of CD43 expression. If this turns out to be correct, the increasing efficacy of the systemic recall response can be attributed to the accumulation of memory cells with the most resting phenotype. Clearly, more needs to be done to understand the differences in recall efficacy of systemic memory $CD8^+$ T cells.

Summary

In summary, increasing age has a significant impact on the memory $CD8^+$ T cell response to respiratory virus infections. There is a significant loss of effector memory cells from peripheral sites over time which may reduce the immediate response of memory T cells to secondary challenge. However, this is counteracted, in part, by the long-term maintenance of large numbers of memory $CD8^+$ T cells in the secondary lymphoid organs and the progressively increasing capacity of these cells to generate proliferative recall responses (Hogan et al., 2001a;Woodland et al., 2001). Overall, it appears that T cell memory is not only maintained for long periods of time, but is also enhanced in the face of an age related decline in the capacity of the immune system to respond to new pathogens (Linton et al., 1997).

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Table 1

The numbers of Sendai virus $NP_{324–332}/K^b$-specific memory cells recruited to the lung airways following heterologous virus infection decline over time[a]

	Sendai $NP_{324–332}/K^b$-specific cells in the lung prior to influenza virus infection		Sendai $NP_{324–332}/K^b$-specific memory cells in the lung on day 4 post influenza virus infection			
			Experiment 1		Experiment 2	
Month post-infection	% Tet[b]	% $CD11a^{high}$	% Tet	Fold increase in cell number[c]	% Tet	Fold increase in cell number
1	80	11	60	9	59	9
4	44	44	32	4	24	5
6	22	48	17	2	16	3
10	5	48	10	2	16	3
20	9	N/D[d]	1	0.4	1	0.4

[a] Cohorts of C57BL/6 mice (6–8 weeks of age, 3 mice per group) were intranasally infected with Sendai virus. At various times post-infection (as indicated in column 1), the mice were intranasally challenged with influenza x31 virus. The percentage of Sendai-$NP_{324–332}/K^b$ specific cells in the lung airways was then determined either one day prior to x31 infection (column 2) and four days post-x31 infection (columns 4 and 6). In addition, the percentage of Sendai-$NP_{324–332}/K^b$ specific cells that were $CD11a^{high}$ was determined for the cells isolated one day prior to x31 infection (column 3). The data are from two independent experiments, as indicated in the table, and used the same cohorts of control animals (the control data are in columns 2 and 3).

[b] % Tet indicates the percentage of Sendai-$NP_{324–332}/K^b$ specific cells among total $CD8^+$ T cells.

[c] Fold increase indicates the fold increase in absolute numbers of Sendai-$NP_{324–332}/K^b$ specific cell in the lung airways at day 4 post-influenza x31 challenge.

[d] N/D, Not Determined. Too few cells were available for an accurate determination.