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The influence of intrinsic and extrinsic factors on immune system aging

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ABSTRACT

Sex and age-matched wild-type and TCR transgenic mice were infected with cytomegalovirus (CMV) at 6 months of age and followed for 12 additional months to examine aging of the immune system. It was found that viral infection of C57Bl/6 mice resulted in accelerated aging of the immune system as shown by a loss of $CD8^+28^+$ cells and an accumulation of $KLRG1^+$ T cells. CMV infection of OT-1 transgenic mice had no influence on immune aging of these mice which nonetheless demonstrated an accumulation of $CD8^+28^-$ and $KLRG1^+$ T cells with time. $CD4^+$ T cells were unaffected in either strain of mice. Thus, immunological aging was found to be due to both cell-intrinsic and cell-extrinsic factors. Persistent viral infections may accelerate immunological aging but consideration must be given to individual variation in the aging process.

Introduction

Aging of the human immune system is characterized by a gradual decrease in immune function and a skewing of hematopoiesis toward the myeloid lineage, with a reduction in the lymphocytic lineage, and progressive increases in senescent memory T cells at the expense of naïve T cells. Both the innate and the adaptive branches of the immune system are affected, including neutrophils (PMN), macrophages, dendritic cells (DC) and lymphocytes. The hematopoietic stem cell (HSC) population is also detrimentally affected by aging as reflected by its inability to maintain both hematopoiesis and lymphopoiesis (Kovacs et al. 2010; DiCarlo et al. 2009).

Aging of the immune system (and the subsequent loss of function) has been attributed both to "time after birth" (i.e., how old an individual is) and ongoing immune responses to endogenous viral (e.g., herpetic) infections, which focus the immune response on these pathogens at the detriment of other responses (Goronzy et al. 2012; Smithey et al. 2012; Pawelec et al. 2004; Mekker et al. 2012). Which variable (chronological age [intrinsic factor] or persistent viral infection [extrinsic factor]) is more important is not known. However, it is consistently reported that immune aging changes seem to be more pronounced in the cytotoxic T cells ($CD8^+$) subpopulation of lymphocytes, which could reflect the significant impact of persistent viral infections (Smithey et al. 2012; Mekker et al. 2012). Fortuitously, T cell aging can easily be followed phenotypically as demonstrated by the loss of CD28 molecules concomitant with the increased expression of the KLRG1 molecule (Vallejo 2005; Hensen and Akbar 2009; Fagnoni et al. 2000). However, there is controversy as to whether these phenotypic changes occur in both humans and mice (Ohteki and MacDonald 1993; Ortiz-Suarez and Miller 2002; Ku et al. 2001; Connoy et al. 2006; Effros et al. 1994; Boucher et al. 1998; Castle 2000).

To address this question we utilized 6 month old control and virally infected (cytomegalovirus; CMV) "wild-type" C57Bl/6 (B6) and TCR transgenic OT-1 mice. OT-1 mice express a TCR specific for the ovalbumin (OVA) peptide, and thus are unable to "see" and respond to CMV infections (Hogquist et al. 1994). We hypothesized that if immunological aging was due to recognition of persistent endogenous viruses then aging should only be observed in B6 mice. If aging was also due to "time after birth" then immune system aging should be observed in both strains of mice. Infected and control mice were followed until 18 months of age.

We observed that immunological aging was influenced by both cell intrinsic and extrinsic factors, that CMV infection could accelerate this process, but that immunological aging may differ significantly between strains of mice.

Materials and methods

Mice

All (female) mice were obtained from Jackson Laboratories (Bar Harbor, ME) and used according to an IACUC approved protocol.

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Table 1
Expression of MCMV in host tissues.

Strain/animal #	1 month after infection
B6 ($n=4$) control (uninfected)	–
OT1 ($n=5$) control (uninfected)	–
B6-34B	+
B6-34L	+
B6-34N	+
B6-41R	+
B6-43R	+
OT1-41L	+
OT1-46B	+
OT1-46L	+
OT1-46N	+
OT-46R	+

The indicated strains of mice were infected with MCMV as described in Methods. At the indicated times the expression of MCMV was analyzed by PCR. Positive expression is indicated by (+) while lack of expression is indicated by (–).

All care and handling of mice was in accordance with the AAALAC guidelines. The C57Bl/6 and the OT-1 strains of mice were utilized. While the B6 mouse is the "wild type" counterpart, the OT-1 mice contain transgenic inserts for mouse Tcra-V2 and Tcrb-V5 genes, a transgenic T cell receptor (TCR) that is designed to recognize ovalbumin residues 257–264 in the context of $H2K^b$ and used to study the response of $CD8^+$ T cells to antigen (Hogquist et al. 1994). The OT-1 TCR transgenic mice were congenic to the C57Bl/6 background.

Virus infections

Mouse cytomegalovirus (MCMV, strain smith MSGV) was purchased from American Type Culture Collection (ATTC, VR-1399). Mice were inoculated by the intraperitoneal route with 1×10^4 plaque forming units (pfu) of MCMV. Both B6 and OT-1 mice were infected at 6 months of age, and followed over the next 12 months. MCMV was measured in peripheral blood and occasionally in tissues by real time PCR (Vliegen et al. 2004; Gold et al. 2004). Briefly genomic DNA was extracted from blood followed by outer PCR amplification using primers, primer A 5′TTCGTTCGGACCATGGCCG (+) and primer B 5′TCGCCGTTCGTGCAGTCCAA (–) followed by inner PCR amplification using primers, primer C 5′TCGCCCATCGTTTCGAGA (+) and primer D 5′TCTCGTAGGTCCACTGACGGA (–). The outer PCR was performed at 95 °C (30 s), 55 °C (30 s) and 72 °C (2 min) for 35 cycles and inner PCR at 95 °C (30 s), 60 °C (30 s) and 72 °C (2 min) for 35 cycles. The inner PCR yielded a 105 bp band visualized on 2% agarose gel.

Fluorescence activated cell sorter (FACS) analyses

Approximately 20 μl of peripheral blood was collected from the cheek pouch and lysed in ACK lysis buffer for 5 min at room temperature to remove red blood cells. Cells were washed once and resuspended in 100 μl of PBS–2% FBS. All Fc receptors were blocked using 10 μl (0.01 mg/ml) of mouse IgG for 5 min to reduce spurious antibody binding. Cell suspensions were then stained using the following antibody-fluorochrome conjugates: CD3-Brilliant Violet 421, CD4-PE, CD8-FITC, CD28-PE/Cy7, B220-PerCP/Cy5.5, and KLRG1-APC. Cells were stained with all antibodies simultaneously. After staining cells were fixed in 1% paraformaldehyde. Cells were analyzed on an LSRII flow cytometer using FacsDiva software.

Results

6-Month old B6 and OT-1 mice were infected with murine CMV and followed over the next 12 months. As shown in Table 1 both strains of mice displayed persistent viral infections as MCMV could be detected at multiple time points for up to 1 year post-infection. At no time did the animals become overtly viremic or unhealthy.

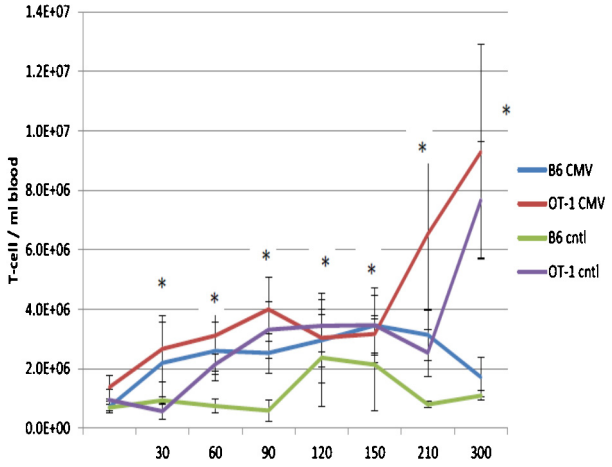

Fig. 1. Effects of age and viral infection on total T cells. B6 and OT-1 mice were infected with MCMV at 6 months of age as described and followed over the next 12 months. At the indicated times the numbers of total T cells in the peripheral blood was determined. Data are presented as the mean ± standard deviation for six mice for each time point and condition. * Significant difference at $p < 0.05$ by Student's t-test for the B6 mice only.

Mice were analyzed monthly for almost 1 year after infection for any phenotypic changes associated with persistent viral infection. As shown in Fig. 1, there was a small but significant change in total T cell numbers between control and MCMV infected B6 mice. However, there was no significant different in total T cell numbers in OT-1 mice as a result of MCMV infection.

When $CD8^+$ cytotoxic T cells were analyzed there was a significant increase over time in $CD8^+KLRG1^+$ T cells in the MCMV-infected B6 mice only. The levels of such cells in OT-1 mice did not change (Fig. 2).

Concurrently, as shown in Fig. 3, there was a loss of $CD8^+CD28^+$ T cells over time in these MCMV-infected B6 mice that was significantly different that the uninfected control B6 mice. Unexpectedly, OT-1 mice also displayed a loss of $CD8^+CD28^+$ T cells with increasing age which was observed in both control and virus-infected animals. Only at very late time points did viral infection accelerate the loss these cells in B6 mice. These observations were in contrast to some reports in the literature for CD28 expression in aging mice (Weng et al. 2009; Ortiz-Suarez and Miller 2002).

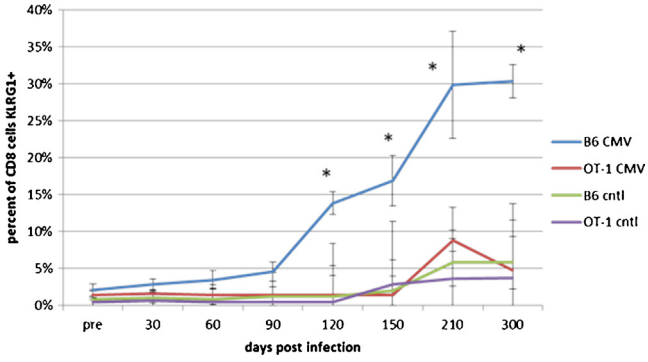

Fig. 2. Effects of age and viral infection on KLRG1 expression by cytotoxic T cells. B6 and OT-1 mice were infected with MCMV at 6 months of age as described and followed over the next 12 months. At the indicated times the percentage of $KLRG1^+CD8^+$ T cells in the peripheral blood was determined. Data are presented as the mean ± standard deviation for six mice for each time point and condition. * Significant difference at $p < 0.05$ by Student's t-test for the B6 mice only.