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Nonmalignant Clonal Expansions of Memory CD8+ T Cells That Arise with Age Vary in Their Capacity To Mount Recall Responses to Infection

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Immune responsiveness declines with age in part due to the development of CD8+ T cell clonal expansions (TCEs) that can dominate the peripheral T cell pool. Although some TCEs arise due to persistent Ag stimulation from chronic infections, others arise in the apparent absence of chronic infection. We have recently shown that this latter class of TCEs can arise over time from the memory CD8+ T cell pool established by an acute viral infection. Unlike TCEs driven by chronic infections, these age-related TCEs do not display the phenotypic and in vitro functional characteristics of exhausted cells. However, the rate at which these age-related TCEs develop from the memory CD8+ T cell pool, as well as their ability to mount a recall response to secondary pathogen challenge in vivo, is not known.

In this study, we analyzed large cohorts of mice over time for the development of TCE following Sendai virus infection and found a progressive increase in the appearance of TCEs, such that most mice showed evidence of TCE within the memory T cell pool by 2 y postinfection. Using a dual adoptive transfer approach to address the recall potential of virus-specific TCEs, we also demonstrate that most TCEs examined are poorly responsive to a secondary infection. Therefore, we provide evidence that the development of TCE is a common occurrence due to the progressive dysregulation of the virus-specific memory T cell pool with age, but many TCEs are profoundly defective in their ability to mediate recall responses.

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Aging is associated with the progressive dysregulation of the immune system and a general decline in immune responsiveness to infection and vaccination (1–6). One feature of this dysregulation is the appearance of CD8+ T cell clonal expansions (TCEs), which are nonmalignant monoclonal populations of CD8+ T cells that appear with increasing frequency as individuals age (7–13). The sizes of these expansions are variable, but they can sometimes represent up to 90% of the entire peripheral T cell repertoire. TCEs in humans are typically observed in individuals that are seropositive for chronic virus infections, such as CMV, suggesting that persistent antigenic stimulation drives these expansions (14, 15). However, TCEs have also been found in the apparent absence of chronic infections, leading to the suggestion that they can be classified into two hypothetical groups that are either dependent or independent of chronic Ag stimulation (16). The sheer size of these expansions has led to speculation that they impact immune responses to both new and recall Ags.

TCEs are frequently observed in the mouse, thus providing a means to study the genesis and consequences of these clonal populations. As in humans, there appear to be two classes of TCEs in mice that are either dependent or independent of chronic Ag stimulation, and the properties of these murine TCEs are essentially identical to those observed in humans. Recently, we analyzed long-term memory in mice that had recovered from an intranasal Sendai or influenza virus infection and conclusively demonstrated that Ag-specific TCE can progressively develop from the memory pool as the mice age (17). These TCEs are indistinguishable from normal memory T cells in terms of phenotype and function, suggesting that they develop stochastically from the existing memory T cell pool.

The impact of TCEs on host immunity is an important issue that is not well understood. It has previously been shown that TCEs found in models of chronic infection are typically nonfunctional (in terms of cytokine production) and presumably reduce overall immune competence (10, 12, 14, 15, 18). For example, in a recent analysis, TCEs that showed no cross-reactivity to HSV-1 nevertheless impaired the de novo response to HSV-1 infection by restricting the naive T cell repertoire (11). Conversely, Ag-specific TCEs that arise in the absence of persistent Ag appear to retain effector function (17). However, it is not clear whether they retain the capacity to mediate protective immunity to secondary challenge since their high frequencies could either benefit or impair a response to the pathogen for which they are specific. To directly address this issue, we have analyzed the development and function of TCEs in mice that have recovered from an acute Sendai virus infection. The data show that memory dysregulation and TCE development occur in most animals over time. Furthermore, we show that memory T cells in many of the mice that exhibit evidence of Ag-specific TCEs are profoundly deficient in their capacity to mediate recall responses to secondary virus challenge. Taken together, these findings demonstrate that T cell memory in aged animals becomes increasingly dysregulated and that this can be associated with a substantially impaired capacity to mount secondary immune responses.

Materials and Methods

Mice, viruses, and infections

C57BL/6, B6.SJL-Ptprca Pep3/BoyJ (CD45.1), and B6.PL-Thy1.1/CyJ (CD90.1) mice were purchased from The Jackson Laboratory (Bar Harbor,
ME) and rederived stocks were maintained at the Trudeau Institute. Sendai virus (Enders strain) was grown, stored, and titered as previously described (19). For intranasal infections, 8- to 12-wk-old mice were anesthetized with 2,2,2-tribromoethanol (200 mg/kg) and administered 250 fifty percent egg infectious doses in a volume of 30 μl. All animal studies were approved by the Trudeau Institute Animal Care and Use Committee.

**Tissue harvest**

For serial bleeds, peripheral blood (∼100 μl) was obtained by nicking the tail vein and diluted in PBS containing 10 U/ml heparin. For endpoint assays, cells were isolated from the lung airways by bronchoalveolar lavage (BAL), the lung parenchyma by digestion in collagenase/DNase for 1 h at 37°C followed by Percoll gradient centrifugation, and the mediastinal lymph nodes (MLNs) and spleen by mechanical disruption. Following RBC lysis with ammonium-buffered chloride, live cell numbers were determined by counting and trypan blue exclusion.

**Flow cytometry and cell sorting**

Single-cell suspensions were incubated with Fc block (anti-CD16/32) for 15 min on ice followed by staining with SenNP324–332 Kb tetramer for 1 h at room temperature. Tetramer-labeled cells were incubated with Abs to surface proteins for 30 min on ice. Abs were purchased from BD Biosciences (San Jose, CA; CD8, CD49d, CD127, and Ly6c), eBioscience (San Diego, CA; CD8, CD11a, CD44, CD45.1, CD45.2, CD90.1, and CD90.2), BioLegend (San Diego, CA; CD27 and CD69), and SouthernBiotech (Birmingham, AL; KLRG1). All TCR Vβ Abs were from BD Biosciences. Samples were run on a FACSCanto II flow cytometer (BD Biosciences), and data were analyzed with FlowJo software (Tree Star, Ashland, OR). For cell sorting, total splenocytes were stained with Abs to surface proteins as described above, and bulk memory T cells (CD8+CD44high) were isolated. An aliquot of sorted cells from each donor population was stained with SenNP324–332 Kb tetramer as described above to determine the number of Sendai-specific CD8+ T cells. Sorting was performed on a FACSVantage cell sorter with DIVA enhancement software (BD Biosciences).

**BrdU incorporation and cytokine staining**

For measurement of homeostatic turnover, mice were administered drinking water containing 0.8 mg/ml BrdU (Sigma-Aldrich, St. Louis, MO) every 2 d for 14 d. Following sacrifice, single-cell suspensions were stained with tetramers and Abs to surface proteins as described above, and BrdU incorporation was assessed using a BrdU Flow kit (BD Biosciences). For measurement of cytokine production, single-cell suspensions were incubated with SenNP324–332 Kb or control peptides as previously described (20). Cells were stained for surface markers, fixed, permeabilized (Cytofix/Cytoperm kit, BD Biosciences), and stained for intracellular cytokines with Abs to IFN-γ and TNF-α.

**Dual adoptive transfer**

Dual adoptive transfer experiments were performed as previously described (21). Briefly, sorted total memory CD8+ T cells from the donors were combined such that the number of Sendai NP324–332 Kb-specific T cells in each donor population was equal. The combined cells (1 × 106 Sendai-specific CD8+ T cells from each donor population) were i.v. transferred into naive recipients and then intranasally challenged with 250 fifty percent egg infectious doses of Sendai virus 1 d later. On day 13 postinfection, lymphocytes were isolated from various tissues and host and donor Sendai-specific cells were identified by flow cytometry. The relative response in each tissue was calculated from the numbers of Sendai NP324–332 Kb-specific T cells derived from each donor.

**Statistics**

Statistical analysis was performed with GraphPad Prism software (GraphPad Software, San Diego, CA) using a Pearson correlation. A p value of <0.05 was considered significant.

**Results**

**Progressive development of TCE in the virus-specific memory T cell pool**

To investigate the kinetics with which Ag-specific TCEs develop, we infected a cohort of 100 C57BL/6 mice with Sendai virus and analyzed CD8+ T cell memory to the immunodominant NP324–332 Kb-specific epitope over time. Mice were bled at ~1-mo intervals, starting at 11 mo postinfection, and the samples were analyzed to determine the frequency of NP324–332 Kb-specific T cells. As shown in Fig. 1A (left panel), the frequency of NP324–332 Kb-specific T cells among total CD8+ T cells ranged from 0.5 to 2% in most animals at day 330, although one animal (mouse No. 181, marked in green) had a frequency of ~10%. Over time, the number of animals that expressed elevated frequencies of NP324–332 Kb-specific T cells (≥2% of CD8+) increased. The time at which an expansion could be first identified varied for each animal and ranged from day 320 to 700 in this experiment. For example, an expansion in animal No. 179 was first detected on day 600 (marked in red). However, by day 780, almost all of the mice that were still alive expressed elevated frequencies of NP324–332 Kb-specific T cells. This pattern could not be explained by a change in the ratio of naive and memory cells in the CD8+ T cell pool since almost identical patterns were observed when the data were calculated with respect to the total memory (CD8+CD44high) pool (Fig. 1A, right panel). Analysis of individual mice indicated that the increases in NP324–332 Kb-specific T cell frequencies were generally gradual in nature, and that in some cases the frequencies of these cells actually fell after reaching a maximum. In most mice, the frequency of NP324–332 Kb-specific T cells rose to between 2 and 40% of the CD8+ CD44high pool. However, in one animal, No. 181, the frequency of NP324–332 Kb-specific T cells rose to ~90% of the CD8+ CD44high pool.

The increase in NP324–332 Kb-specific T cell frequencies is characteristic of the development of TCEs in which single or a limited number of clones of Ag-specific cells expand and dominate the memory pool. Since these expansions are monoclonal, or pauciclonal, in nature, they can be followed by analyzing TCR diversity. Given the limited samples available, we followed TCR diversity by analyzing Vβ8 usage (Vβ8.1, Vβ8.2, or Vβ8.3) by NP324–332 Kb-specific T cells. Several distinct patterns of Vβ8 usage were observed, and representative examples are illustrated in Fig. 1B–E. The TCR repertoire of NP324–332 Kb-specific T cells has been previously reported and typically comprises ~20% Vβ8+ T cells (22). This is illustrated by three representative animals in Fig. 1B where the absolute frequency of NP324–332 Kb-specific T cells and Vβ8 usage by those cells remained relatively constant between days 320 and 780 postinfection. In contrast, Vβ8 usage was varied dramatically in animals that exhibited increases in the frequencies of NP324–332 Kb-specific T cells. In some cases, Vβ8 usage decreased dramatically as the frequency of NP324–332 Kb-specific T cells increased, indicating that the expanded cells expressed some other Vβ element (Fig. 1C). In other cases, Vβ8 usage increased dramatically as the frequency of NP324–332 Kb-specific T cells increased, indicating that the expanded cells specifically expressed one of the Vβ8 elements (Fig. 1D). Interestingly, some animals exhibited very high levels of Vβ8 usage at the earliest time point analyzed, day 320, that did not appear to correlate with increasing frequencies of NP324–332 Kb-specific T cells (Fig. 1E). These data suggest that there may be perturbations in the T cell memory pool that do not result in the outgrowth of Ag-specific T cells and further suggest that these perturbations occur earlier than day 320. Taken together, these data indicate that the Ag-independent expansion of virus-specific T cells in these mice are monoclonal or pauciclonal in nature and develop gradually over time in most animals.

**Ag-specific T cell expansions exhibit increased rates of homeostatic proliferation**

We speculated that the gradual development of Ag-specific T cell expansions correlated with an increased rate of proliferation in specific T cells clones in the memory T cell pool. To investigate this possibility we established a second cohort of Sendai virus-infected mice and gave them BrdU in the drinking water on day 600.
postinfection. Fourteen days later animals were sacrificed to determine BrdU incorporation in NP\textsubscript{324-332}/K\textsuperscript{b}–specific T cells. As shown in Fig. 2\textit{A}, analysis of BrdU incorporation in the total memory CD8\textsuperscript{+} T cell pool showed no correlation between the relative size of the bulk memory population and homeostatic turnover. In contrast, focusing the analysis on Ag-specific cells showed a very strong correlation between the frequency of NP\textsubscript{324-332}/K\textsuperscript{b}–specific T cells in the total memory CD8\textsuperscript{+} T cell pool and the level of BrdU incorporation. These data suggest that the Ag-independent development of virus-specific T cell expansions is due to an increased rate of homeostatic proliferation in individual clones.

Ag-specific TCEs vary in their capacity to mediate recall responses

The development of Ag-specific TCE clearly disrupts the normal composition of the Ag-specific memory T cell pool. However, the impact of this dysregulation on the capacity of the memory T cell pool to mediate recall responses is not known. To address this question we focused on four animals (Nos. 179, 181, 292, and 281) that exhibited substantial TCEs (TCE Nos. 292 and 281 were selected-based on screening several additional cohorts of Sendai memory mice; see Supplemental Fig. 1). The animals were sacrificed and splenocytes were analyzed using a panel of V\textbeta–specific Abs to identify V\textbeta usage. As shown in Fig. 3, animal No. 179 exhibited a major expansion of NP\textsubscript{324-332}/K\textsuperscript{b}–specific T cells that were V\textbeta\textsuperscript{8.3}+ (comprising 81% of the total NP\textsubscript{324-332}/K\textsuperscript{b}–specific T cell pool). Animals No. 181 and No. 292 both exhibited expansions that were primarily V\textbeta\textsuperscript{5}+ (comprising 96 and 80%, respectively, of the total NP\textsubscript{324-332}/K\textsuperscript{b}–specific T cell pool). Interestingly, the expanded NP\textsubscript{324-332}/K\textsuperscript{b}–specific T cells present in animal No. 181 expressed reduced TCR levels. Finally, animal No. 281 exhibited a major expansion of NP\textsubscript{324-332}/K\textsuperscript{b}–specific T cells inasmuch as there was a profound loss of V\textbeta\textsuperscript{8.1/8.2} usage. However, V\textbeta usage could not be assigned with the panel of Abs available.

To determine the ability of these virus-specific TCEs to respond to a secondary infection, we used a dual adoptive transfer approach in which the responses of two separate populations of memory T cells could be compared in the same animal and under the same infection conditions (21, 23). Spleen cells from animals Nos. 179, 181, 292, and 281 were enriched for CD8\textsuperscript{+} cells on a negative selection column and then flow cytometrically sorted to isolate CD44\textsuperscript{hi} memory cells. A corresponding population of memory CD8\textsuperscript{+} T cells was isolated from young B6.CD90.1 mice that had recovered from an intranasal Sendai virus infection (45 d postinfection). The
memory T cells from mice exhibiting T cell expansions were then mixed together with young memory T cells such that the ratio of NP324–332/Kb-specific T cells was 1:1 and i.v. injected into B6.CD45.1 recipient mice, as depicted in Fig. 4A. One day later, recipient mice were infected with Sendai virus, and NP324–332/Kb-specific T cells were analyzed 13 d later in the lung airways (BAL), lung parenchyma, MLNs, and spleen. Cells derived from each donor and from the host were distinguished on the basis of CD90 and CD45 expression, and the ratio of the response of the two donors was determined (Fig. 4A) (24). As shown in Fig. 4B, young memory NP324–332/Kb-specific T cells were substantially more efficient at mediating the recall response to Sendai virus infection than were memory cells from three of the mice exhibiting TCE (animals Nos. 179, 181, and 281). In each of these cases, the young memory T cells mounted responses that were ~10-fold greater than those from mice with TCE. Moreover, the reduced responsiveness of these TCE memory cells was exhibited in all tissues tested, suggesting a defect in the capacity of the Ag-specific memory T cells to expand in vivo rather than a defect in migration to the site of infection. There was some minor skewing of the young memory T cell/TCE ratio among different tissues within the same experiment, suggesting there may be some small differences in trafficking to or survival in infected tissues. In contrast, memory NP324–332/Kb-specific T cells from one animal that exhibited a TCE (No. 292) mounted a response that was equivalent to that of young memory T cells. These data demonstrate that Ag-specific memory T cells from mice that exhibit dysregulated memory T cell pools vary in their capacity to mount recall responses.

We next analyzed NP324–332/Kb-specific T cells from two of the animals that expressed TCEs (Nos. 179 and 292) in more detail. Phenotypic analyses (Fig. 5A) revealed that the cells were essentially indistinguishable from memory NP324–332/Kb-specific T cells from young mice that did not exhibit expansions. Both of the TCEs identified in these mice expressed CD44, an activation marker that is upregulated on effector and memory T cells, and a variety of other activation markers. The only minor difference was that the TCEs in these two animals expressed slightly reduced levels of CD49d, suggesting that these TCEs were not functionally exhausted (25).

To confirm that these TCEs retained their functional capacity, we examined cytokine production following stimulation with the NP324–332 peptide. As shown in Fig. 5B, both TCEs were able to produce IFN-γ and TNF-α at levels comparable to those observed in young memory T cells. The production of cytokine was dependent on Ag, since irrelevant Kb-binding peptides did not elicit cytokine production from either TCE or young memory T cells.

Although recall responses from three of four animals that exhibited NP324–332/Kb-specific TCEs were impaired, they were not completely negative. We speculated that residual, nonexpanded memory in these mice may be mediating this recall response. To investigate this idea, we compared the TCR Vβ usage of the NP324–332/Kb-specific T cells prior to adoptive transfer and following recall to infection in a host animal. We focused on animals Nos. 179 and 292, since these memory T cell pools differed in their capacity to mediate recall responses. As shown in Fig. 6, and as discussed earlier, NP324–332/Kb-specific memory T cells from animal No. 179 were primarily Vβ8.3+ prior to adoptive transfer into the host animal. This is consistent with the presence of a monoclonal or pauciclonal expansion of part of the virus-specific memory T cell pool in this animal. Thirteen days following a secondary Sendai virus infection, the responding donor population became predominantly Vβ5.1/5.2+, suggesting that a minor subpopulation of T cells in the memory T cell pool had dominated the response. Importantly, it was apparent that the previously dominant Vβ8+ cells, which contained a monoclonal or pauciclonal expansion, were selectively decreased in the recall response. Importantly, the recall of young memory T cells in the same host showed no skewing of the Vβ repertoire, demonstrating that there is no intrinsic bias toward a Vβ5.1/5.2+ response during a secondary infection. In contrast to mouse No. 179, analysis of mouse No. 292 revealed a different pattern. In this case, NP324–332/Kb-specific memory T cells were primarily Vβ5.1/5.2+ prior to adoptive transfer into the host animal and this did not change in the recall response to Sendai virus infection. These data suggest that the expanded population of NP324–332/Kb-specific T cells in this animal responded well to secondary infection in vivo and that the presence of one or more expansions did not impact the strength of the response.
Discussion

There is increasing evidence that the immune system becomes progressively compromised with age, resulting in poor immune responses to newly encountered Ags. In the case of T cell memory to pathogens that elicit acute responses, such as influenza and paramyxoviruses, we have recently demonstrated in a mouse model that there is a progressive dysregulation of Ag-specific memory CD8+ T cells characterized by the outgrowth of Ag-specific T cell clones. This was the first time that pathogen-specific TCE had been linked to the memory T cell pool in the absence of any form of chronic or persistent infection. In this study, we have extended this finding to show that Ag-specific TCEs begin to appear ∼12 mo postinfection and that they are present in most animals by 24 mo postinfection. Additionally, we have shown that in many instances, these expansions are not able to participate in recall responses to secondary infection and consequently impair the response of the total memory T cell pool.

An interesting feature of the data is that dysregulation of the memory T cell pool was readily observed in most of the surviving animals by day 780 postinfection. This suggests that the outgrowth of specific clones occurs much more frequently than originally anticipated. Furthermore, it is likely that the frequency with which Ag-specific TCE occur may be underestimated in these studies. First, it is possible that the identification of TCE on the basis of elevated frequencies may lead to their underestimation. In this study, we identified expansions as populations of NP324–332/Kb-specific memory T cells that were present at frequencies >2% of the total CD8+ T cell pool (2% was selected on the basis that it is three standard deviations above the frequency of recently generated NP324–332/Kb-specific memory T cells in young mice) (17). However, it is apparent from the data that memory T cell populations that fall below this threshold may be dysregulated in terms of their repertoire diversity. For example, at least two of the animals illustrated in Fig. 1B exhibit frequencies of NP324–332/Kb-specific memory T cells <2% of the total CD8+ T cell pool (2% was selected on the basis that it is three standard deviations above the frequency of recently generated NP324–332/Kb-specific memory T cells in young mice) (17). However, it is apparent from the data that memory T cell populations that fall below this threshold may be dysregulated in terms of their repertoire diversity. For example, at least two of the animals illustrated in Fig. 1B exhibit frequencies of NP324–332/Kb-specific memory T cells <2% threshold, suggesting that the T cell memory pool is normal. However, Vβ8+ T cells are overrepresented in this population (60% versus the more normal 20%), indicating substantial dysregulation of the memory pool. Second, the use of Vβ8 expression alone to identify perturbations in the repertoire may
miss cases where more than one TCE has developed if two distinct clones happen to both use a Vβ8 element. Based on these two possibilities, we think that many TCEs may be overlooked and that dysregulation of the memory T cell pool may be even more dramatic than observed in this study. In this regard, the large TCE outgrowths simply represent the extremes of a more general pattern of dysregulation in the memory T cell pool. We think that future studies should be directed at better understanding the true kinetics and extent of memory T cell dysregulation.

An important observation is that memory T cell pools exhibiting substantial dysregulation often mediate a reduced capacity to participate in recall response, despite retaining the capacity to secrete inflammatory cytokines ex vivo (26, 27). Note that the dual adoptive transfer approach used for these studies effectively integrates all of the factors involving the accumulation of activated effector T cells at the site of infection (such as their capacity to migrate to lymph nodes prior to infection, the overall proliferative and death rates of the responding cells, and the capacity of the cells to migrate to inflammatory sites) (28). Furthermore, the response of the dysregulated memory T cell population is measured in direct comparison to the responding cells, and the capacity of the cells to migrate to inflammatory sites (such as their capacity to migrate to lymph nodes prior to infection, the overall proliferative and death rates of the responding cells, and the capacity of the cells to migrate to inflammatory sites) (28). Therefore, the dysregulated memory T cell population is measured in direct comparison to recently generated memory in the same host and under identical conditions of infection and inflammation. Thus, these data suggest that TCEs are generally detrimental to a secondary immune response. However, note that in many cases, there are hugely increased numbers of memory cells and that this increased number may compensate for the overall decrease in proliferative potential in individual animals. A key question in this regard is the extent to which TCEs displace normal memory T cells. Also note that not all examples of dysregulated T cell memory correlate with impaired recall responses. One of the four animals that we tested, No. 292, elicited a response that was clearly equivalent to recently elicited T cell memory. More studies are needed to determine the true frequencies of responsive and nonresponsive TCEs and the underlying mechanisms associated with nonresponsiveness.

The mechanisms that control the development of TCEs are currently undefined. In the case of chronic infections, it has been speculated that persistent stimulation by Ag results in the outgrowth of specific clones of T cells (29). However, in this study the agent that elicited the memory was cleared by the immune response and did not establish a chronic or persistent infection. Moreover, we have shown that Ag-specific TCEs can be elicited by both Sendai and influenza virus infections, ruling out any specific biological characteristics of a particular pathogen (17).

Based on these observations, we hypothesize that Ag-specific TCEs are a natural outcome of the long-term homeostatic proliferation of the memory T cell pool. It is interesting that TCEs display enhanced homeostatic proliferation despite showing severely impaired proliferation to antigenic stimulation, but this may be due to signaling defects after TCR triggering, or to defects in cytokine rearrangements, which are found in aged T cells (30, 31). Since homeostatic T cell proliferation is asynchronous and individual clones may have slightly different proliferation rates, it might be expected that some clones would tend to overgrow the pool over time. In fact, one could argue that it would be unexpected for the immune system to maintain normal TCR diversity within any given memory CD8+ T cell pool given the relatively rapid rate of cell turnover. The large TCEs that develop in some individuals and mice may represent one extreme of a spectrum of gradual degradation of the memory T cell repertoire. In these cases, the system has selected for a clone that exhibits a slightly higher rate of homeostatic proliferation compared with the average. Our data support this view by showing that there is a higher rate of homeostatic proliferation in mice that exhibit large TCEs. Furthermore, the phenotype of large Ag-independent TCEs is consistent with normal memory cells, indicating that they are not substantially different from other memory T cells. A central facet of this hypothesis is that homeostatic cytokines should play a key role in TCE development, and, consistent with this, TCEs that arise from the normal memory T cell pool typically express receptors for cytokines that are involved in CD8+ T cell homeostasis, namely CD122 (IL-2Rβ/IL-15Rβ) and CD127 (IL-7Rα) (16). Additionally, TCEs isolated from mice can be maintained in the absence of Ag by transfer into β2-microglobulin-deficient mice (10). Although the TCEs that develop in Sendai-immune mice are clearly specific for viral Ag, it seems likely that expansions of unknown specificity in specific pathogen-free mice also develop from the memory T cell pools that are invariably present. Although the idea that TCEs represent homeostatic outgrowths of otherwise normal memory T cells is attractive, further investigation is needed to explain why some TCEs exhibit an impaired capacity to mount recall responses.

In conclusion, the data we present in this study demonstrate that T cell memory in aged animals becomes increasingly dysregulated and that this can lead to clonal expansions with a substantially impaired capacity to mount secondary immune responses.
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Disclosures

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