CD27\textsuperscript{high}/KLRG1\textsuperscript{low} CD8+ T cells that persist throughout MCMV infection are highly expansive and have the ability to reestablish MCMV immunity

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ABSTRACT:
Cytomegalovirus (CMV) is a herpesvirus that establishes lifelong latency in 60-80% of Americans. Constant immune surveillance is necessary to prevent viral reactivation from latency and results in the accumulation of functional CMV-specific CD8+ T cells (CD8s) over time, a process termed memory inflation. As such, CMV reactivations remain a clinical concern for immunosuppressed patients and reconstituting CMV immunity is critical for the long-term prevention of CMV disease. Understanding the maintenance of memory inflation may reveal novel approaches to restore CMV immunity.

Previous work has shown that the majority of inflationary CD8s express a terminally-differentiated “effector” (T\textsubscript{EFF}) phenotype, have a short half-life and appear unable to sustain long-term CMV immunity. Interestingly, inflationary populations also include a minor subset of CD8s that express a “memory” phenotype (T\textsubscript{M}).

Conclusions:
Utilizing the murine model, MCMV, our data showed that:
1. The minor T\textsubscript{M} population was responsible for the majority of the inflationary population’s expansive capacity following transfer into naive mice and MCMV challenge.
2. Transferred T\textsubscript{M} cells produced both T\textsubscript{M} and T\textsubscript{EFF} progeny, which inflated and persisted in the recipients.
3. When T\textsubscript{M} cells were transferred into chronically-infected mice, they survived and produced T\textsubscript{EFF} CD8s if host immunity was lost.
4. T\textsubscript{M} CD8s appear to be a crucial component for the establishment and maintenance of CMV immunity.

Figure 1: CD8+ T cells for certain MCMV antigens inflate during chronic MCMV infection.
The frequency and number of MCMV-specific CD8s in the blood and spleen was measured by tetramer staining and flow cytometry. T\textsubscript{M} (CD27\textsuperscript{high}/KLRG1\textsuperscript{low}); T\textsubscript{EFF} (CD27\textsuperscript{low}/KLRG1\textsuperscript{high}).

Figure 2: Proposed Model.
A T\textsubscript{M} population persists throughout MCMV infection that divides and differentiates asymmetrically to produce both T\textsubscript{M} and T\textsubscript{EFF} populations.

Figure 3: Adaptive transfer of T\textsubscript{M} or T\textsubscript{EFF} sorted CD8+ T cells. Splenocytes from MCMV chronically-infected mice were CD8-enriched, sorted, transferred into naive C57BL/6 and challenged with MCMV. Purity of the T\textsubscript{M} or T\textsubscript{EFF} populations was ~90% (data not shown).

Figure 4: MCMV-specific T\textsubscript{M} cells expand and produce phenotypically diverse progeny.
50,000 T\textsubscript{M} or T\textsubscript{EFF} CD8 T cells were transferred and challenged as described in Figure 3. Donor tetramer+ CD8 frequency (A) and phenotypic fold change (B) was measured 7dpi in blood and spleen. Number of transferred tetramer+ CD8s was estimated by flow cytometry. A) T\textsubscript{M} donors expand more robustly than T\textsubscript{EFF} cells. B) M38-specific T\textsubscript{M} donors, in contrast to T\textsubscript{EFF} donors, produce phenotypically diverse progeny. Colored boxes indicated the initial phenotype of transferred cells.

Figure 5: MCMV-specific T\textsubscript{M} cells recapitulate memory inflation and reestablish a T\textsubscript{M} population that can inflate again when restimulated.
50,000 T\textsubscript{M}, CD8 T cells were transferred and challenged as described in Figure 3. Donor tetramer+ CD8 frequency (A) and phenotype (B) was measured in the blood at indicated time points. A) M45- and M38-specific T\textsubscript{M} donors follow their non-inflationary and inflationary kinetics, respectively (refer to Figure 1) B) M38-specific T\textsubscript{M} donors produce effector progeny that inflate, but also establish a stable T\textsubscript{M} population. C) Naïve or T\textsubscript{M} OT-1 CD8 T cells were transferred into naïve C57BL/6 mice and rechallenged with MCMV-Ova. Frequency and phenotype of OT-1s was measured in the blood. Even after multiple stimulations, T\textsubscript{M} OT-1s can reproduce inflation and generate a new T\textsubscript{M} population (data not shown).

Figure 7: T\textsubscript{M} and T\textsubscript{EFF} cells are not detectable when transferred into chronically-infected mice.
50,000 T\textsubscript{M} or T\textsubscript{EFF} CD8+ T cells from chronically-infected mice were transferred into chronically-infected mice as described in “A”. Number of transferred tetramer+ CD8s was estimated by flow cytometry. B) Frequency of donor CD8s was measured in the blood of recipients 9-12 weeks post-transfer by flow cytometry (note: the most dominant response was only 0.5% of total CD8s). Data from two independent experiments.

Figure 8: T\textsubscript{M} cells, but not T\textsubscript{EFF} Cells, can expand and differentiate in chronically-infected mice.
A) Host immunity of the mice described in Figure 7 was depleted via intraperitoneal injections of anti-Thy1.1 (Clone HISS1), CD4 (Clone GK1.5) and NK1.1 (Clone PK136). B) Following the depletion schedule, the frequency of tetramer+ donor CD8s in the blood was measured by flow cytometry. T\textsubscript{M} donors did not expand in the absence of antigen. C) T\textsubscript{M} donors made diverse tetramer+ responses in 6/6 recipients. In contrast, T\textsubscript{EFF} donors made a detectable donor response in only 2/6 recipients, and these responses were specific for only one tetramer. (Check marks indicate a tetramer+ donor population of >10 events collected by flow cytometry). D) The phenotype of tetramer+ donor CD8s was determined by flow cytometry. T\textsubscript{M} donors in 6/6 recipients produced phenotypically diverse progeny (representative FACS plots from a single mouse shown). Frequency displayed is the average of the six mice +/- the standard deviation. Data from two independent experiments.

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