Competition for antigen selects T cell responses that undergo memory inflation and maintains clonal dominance during MCMV infection

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Abstract: Cytomegalovirus (CMV) establishes a life-long, persistent/latent infection. Continuous immune surveillance by CMV-specific CD8+ T cells results in their accumulation over time, a process called “memory inflation”. These “inflationary” T cells migrate systemically and comprise the largest T cell populations in humans, making CMV-based vaccines attractive. Why T cells specific for only some CMV-derived epitopes undergo inflation is unclear. We found previously that recombinant murine (M)CMV expressing the SIINFEKL peptide only induced inflation of SIINFEKL-specific T cells, in contrast to wild-type MCMV infection. We show here that:

- Co-infecting mice with viruses expressing and lacking SIINFEKL enabled T cells with multiple specificities to inflate.
- Adoptively transferred OT-I T cells, which have a high affinity for the SIINFEKL peptide, were preferentially enriched shortly after infection.
- Adoptively transferred OT-I T cells inhibited the inflation of host-derived T cells specific for SIINFEKL.
- We observed sporadic, late rejection of OT-Is as a result of minor histocompatibility differences between donor and recipients. Only such rejection enabled host-derived T cells to inflate robustly.

Conclusions:

1. T cell competition for antigen at the level of the APC during MCMV infection dictates which T cell responses inflate.
2. T cell competition for antigen selects T cells with high affinity for MCMV-derived antigens.
3. Relative T cell clonal abundance is actively maintained by competition.

Figure 1

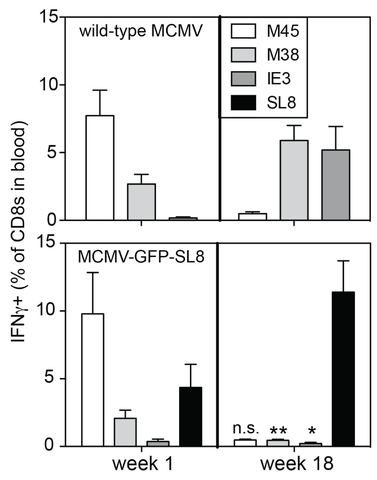


Figure 1: Only SIINFEKL-specific T cells inflate after MCMV-ova infection. B6 mice were infected with MCMV-ova, expressing the SIINFEKL peptide (SL8) from ovalbumin. The frequency of CD8+ T cells in the peripheral blood specific for the indicated antigens was measured after infection by intracellular cytokine staining after peptide stimulation ex vivo.

Figure 2

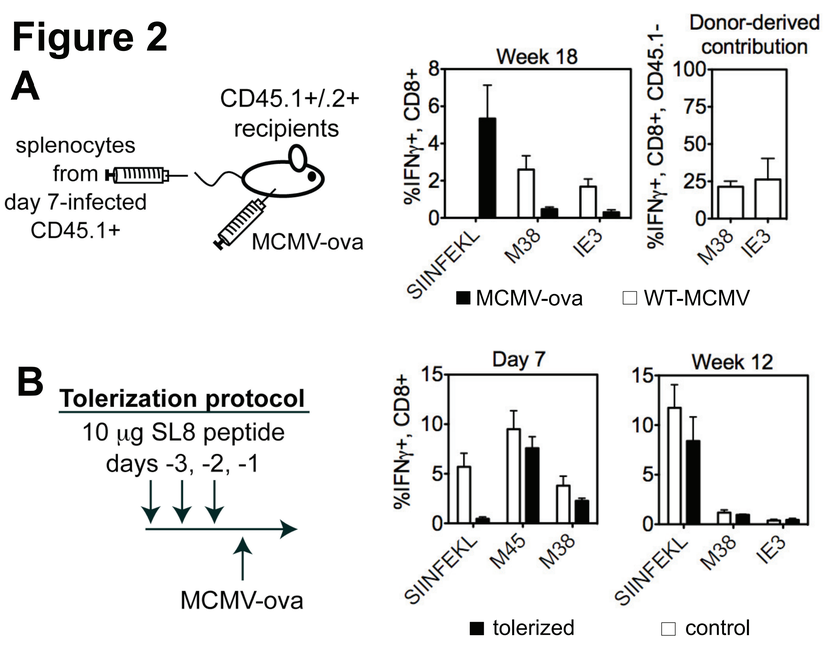
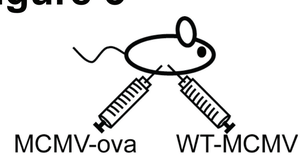


Figure 2: Altering the ratios of functional, epitope-specific cells available to respond to infection does not influence the immunodominance of SIINFEKL-specific T cells. A) To boost the numbers of MCMV-specific T cells, CD45.2+, CD45.1+ naive recipients received splenocytes from CD45.1+ mice infected for 7d with WT MCMV. Recipients were infected with WT MCMV or with MCMV-ova and virus-specific responses were measured in the blood 18 weeks later. T cell responses are shown on the left and percentages of CD45.2− donor cells contributing to either IE3 or M38 responses are shown on the right. **B)** To tolerize SIINFEKL-specific T cells, B6 mice were injected with 10 μg SIINFEKL peptide i.v. on days −3, −2, and −1 prior to infection with MCMV-ova.

Figure 3



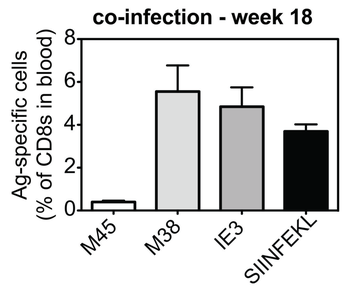


Figure 3. Competition for Ag at the level of the APC shapes immunodominance during chronic MCMV infection. B6 mice were co-infected with MCMV-ova and WT-MCMV lacking SIINFEKL. Virus-specific T cells were measured in the blood 18 weeks after infection using intracellular cytokine staining.

Figure 4

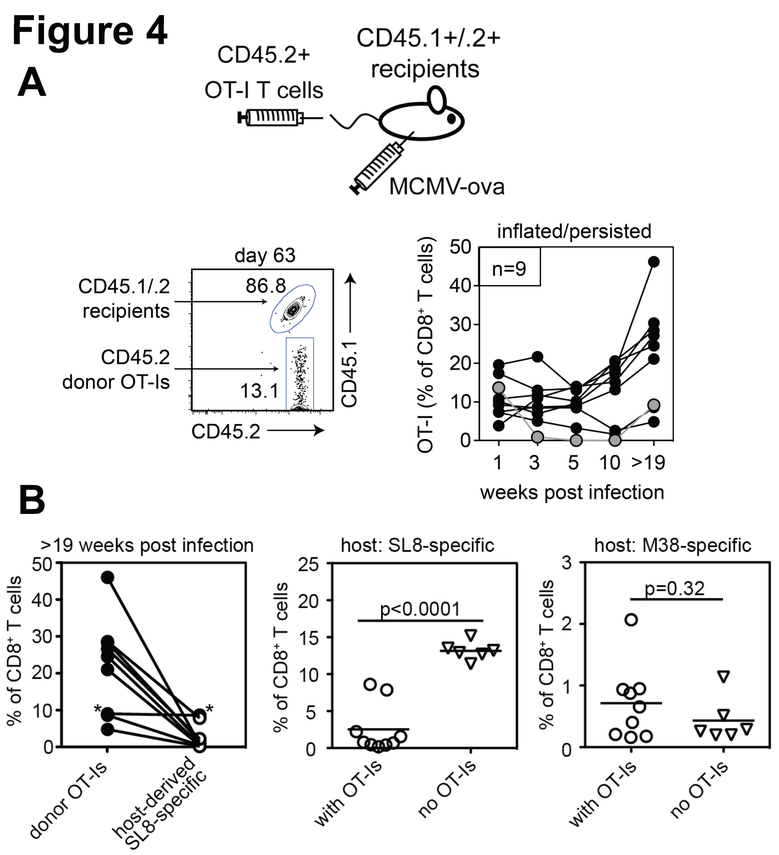


Figure 4: OT-I T cells undergo inflation after transfer and dominate SIINFEKL-specific T cells. A) Schematic of adoptive and a representative FACS plot of donor OT-I and host CD8+ cells is shown. The frequency of OT-I T cells was analyzed in the peripheral blood over time. In one mouse, the transferred OT-I T-cell population fell below the limit of detection for several weeks and then reappeared at a chronic time point (gray line). **B)** The frequency of CD8+ T cells that are SIINFEKL-specific (pentamer-binding) and either donor- or host-derived was determined (left). The lines connect the donor and host populations from the same animals. Asterisks refer to the mouse in which OT-I T cells were temporarily undetectable (gray line in A). The frequency of host-derived SIINFEKL-specific (middle) or M38-specific T cells (left) in mice that did or did not receive OT-Is.

Figure 5

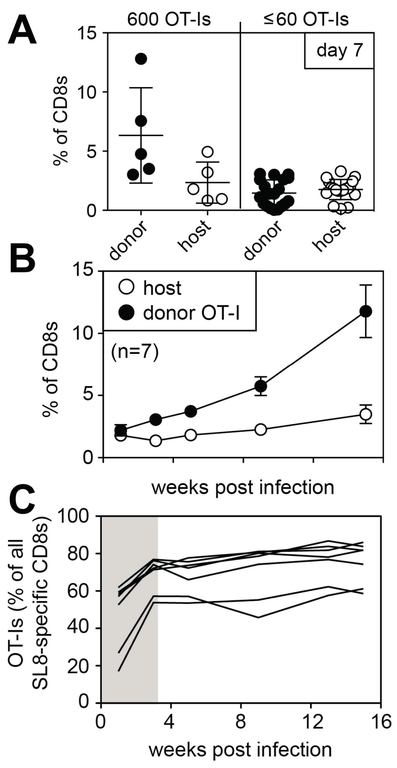


Figure 5: OT-Is are selected to inflate early in infection. Mice received the indicated number of OT-Is and the frequency of donor and host SIINFEKL-specific T cells were assessed **)** by intracellular cytokine staining 7 days later **(A)** or over time for mice that received 60 or fewer OT-Is **(B)**. **C)** The proportion of total SIINFEKL-specific T cells that are donor-derived (from B) is shown. Each line represents one animal. The grey box indicates a window of OT-I enrichment.

Figure 6

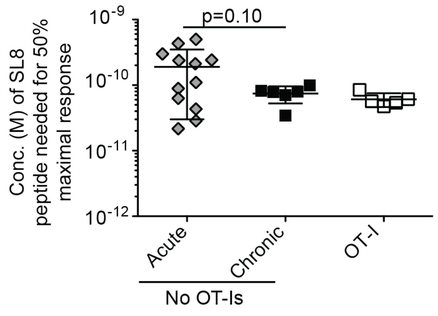


Figure 6: The avidity of SIINFEKL-specific T cells narrows and increases over time. Functional avidity of SIINFEKL-specific splenocytes was measured after 7 days (acute), or more than 3 months (chronic) in mice that did not receive OT-Is. The avidity of OT-I T cells was measured after they had undergone inflation for more than 3 months. The concentration of SIINFEKL peptide necessary to achieve 50% of the maximal IFN-γ response (EC50) was assessed for each animal using titrations.

Figure 7

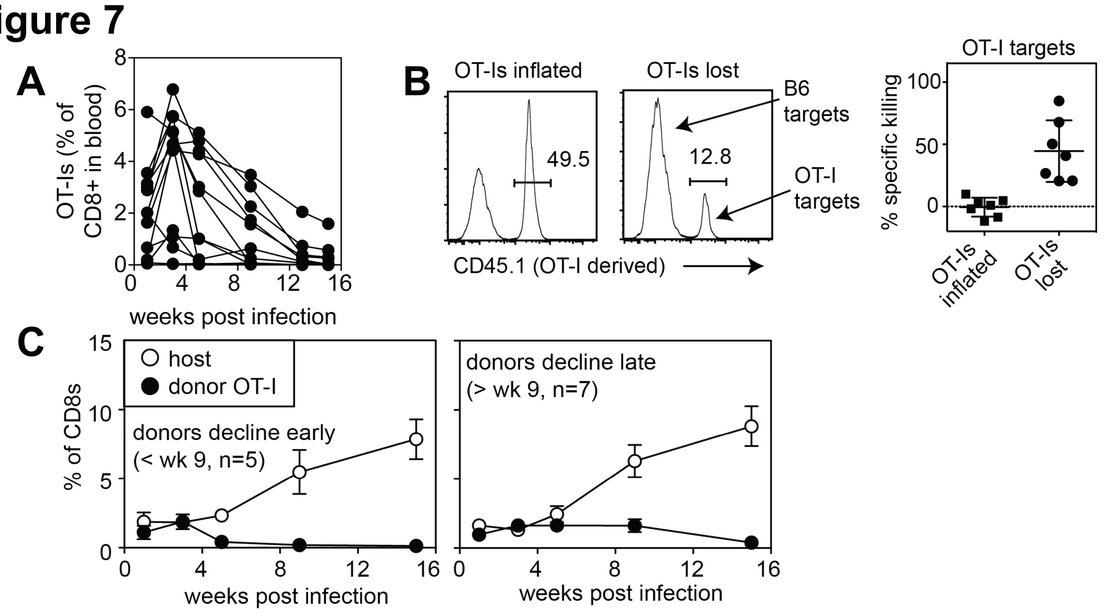


Figure 7: Rejection of transferred OT-I T cells allows host-derived SIINFEKL-specific T cells to inflate. A) 60 or fewer OT-Is were transferred as in Figure 5. Shown is the frequency of OT-I T cells in the blood in mice that lost OT-Is. **B)** To demonstrate immunological rejection, in vivo killing assays were conducted in which mice received a mixture of CFSE-labeled B6 splenocytes (CD45.2+ controls) and CD45.1/.2+ OT-I splenocytes (targets). Representative FACS plots and specific killing are shown. C). The donor and host SIINFEKL-specific response was measured by intracellular cytokine staining in animals in which OT-I T cells were lost by week 9 (left) or declined but comprised more than 0.3% of all CD8+ T cells at least 9 weeks post infection (right).