Dysfunctional and tolerant CD8 T cell persistence in premalignant lesions

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Background

- Most studies examining T cell-cancer interactions have been directed toward late-stage tumors, but much less is known about how T cells respond to premalignant lesions
- T cells are present in the early premalignant environment.
- We previously demonstrated that TAG-specific CD8 T cells (TCR-TAG) rapidly become dysfunctional (unable to produce cytokines) after entering premalignant liver lesions, although they persist long-term.

Models to study CD8 T cell responses in early tumorigenesis and peripheral tolerance

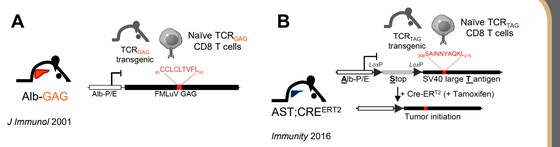


Figure 1: A. Albumin-GAG (Alb-GAG) self-antigen model with the Friend murine leukemia virus (FMLuV) GAG antigen as a liver-specific self-antigen. CD8 T cell GAG epitope shown in red **B.** Inducible ASTxCre-ER^{T2} HCC model with the SV40 Large T Antigen (TAG) as oncogene and tumor neoantigen. CD8 T cell TAG epitope shown in red.

Tumor-specific T cells rapidly become dysfunctional

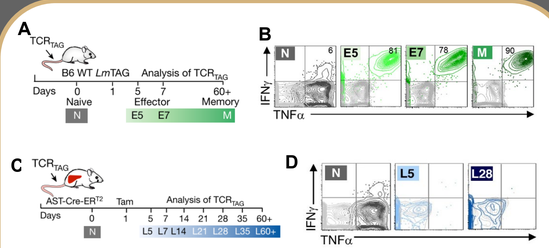
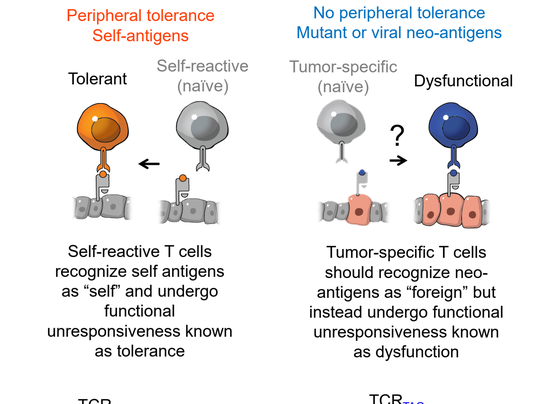


Figure 2: A. Naïve TCR$_{TAG}$ (N) were transferred into B6 mice and inoculated with *Lm*TAG one day later to model infection. **B.** Transferred TCR$_{TAG}$ were reisolated from the spleen at different time points. Flow analysis shows efficient production of effector cytokines (assessed by intracellular cytokine staining after 4-hour *ex vivo* TAG peptide stimulation). **C.** Naïve TCR$_{TAG}$ (N) were transferred into ASTxCre-ER^{T2} mice, and Cre activated by TAM one day later to induce liver carcinogenesis. **D.** Transferred TCR$_{TAG}$ were reisolated from the liver at different time points (L5-60). Flow analysis shows rapid PD1 upregulation (top) and loss of effector cytokine production (bottom; assessed by intracellular cytokine staining after 4-hour *ex vivo* TAG peptide stimulation).

Hypothesis and Approach

Tumor-specific T cell recognition of neoantigen in pre-malignant lesions induces "self-tolerance" program.



Tumor-specific T cell dysfunction is distinct from tolerance to liver self-antigen

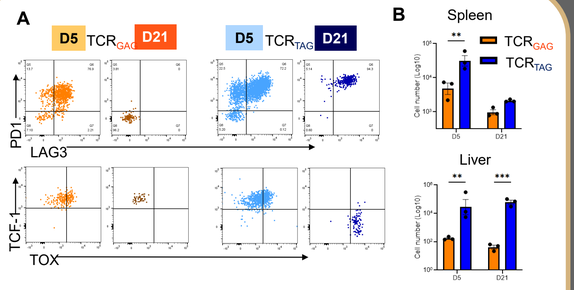


Figure 3: A. PD1, LAG3, and TOX are upregulated at day 5 for both tolerant (TCR$_{GAG}$) and dysfunctional T cells (TCR$_{TAG}$); however, by day 21 tolerant T cells have downregulated these markers while dysfunctional T cells remain high. **B.** Tolerant T cell numbers are significantly lower than dysfunctional T cells.

Self-reactive T cells undergo limited proliferation, in contrast to tumor-specific T cells

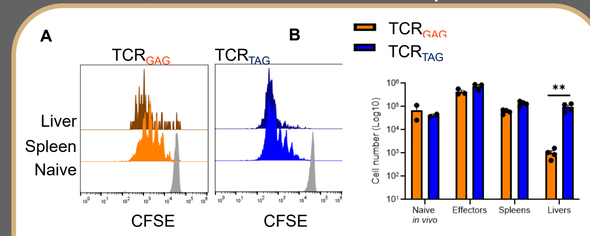


Figure 4. A. CFSE, a proliferation dye to track proliferation, staining of T cells at 72 hours after antigen encounter. In both liver and spleen, tolerant (TCR$_{GAG}$) T cells begin to lose cell numbers whereas dysfunctional T cell (TCR$_{TAG}$) increase at later divisions. **B.** At 72 hours, numbers of tolerant and dysfunctional t cells are the same except in the liver, where tolerant T cells persist at much lower numbers than dysfunctional T cells.

Self-reactive T cells exit cell cycle prematurely as compared to tumor-specific T cells

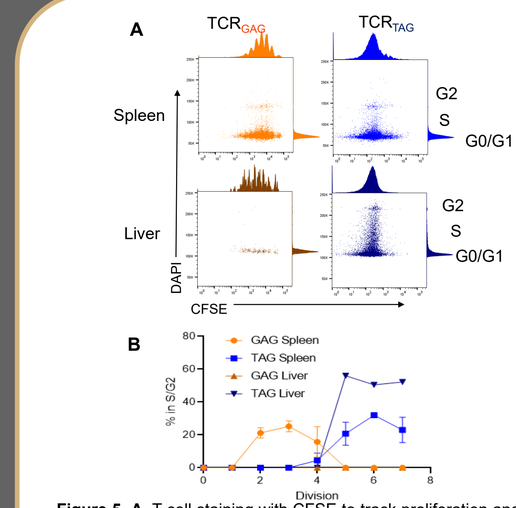


Figure 5. A. T cell staining with CFSE to track proliferation and DAPI to assess cell cycle (stages labeled) at 72 hours post-antigen encounter. Tolerant T cells exit cell cycle at division 4 in the spleen while dysfunctional are cycling at divisions 5 and 6. In the liver, however, tolerant T cells are not cycling at all while over 50% of dysfunctional T cells are actively cycling. **B.** Quantification of cell population percentages actively cycling as defined by those in S and G2 phases.

Lower self-antigen level in liver could lead to premature cell cycle exit by self-reactive T cells

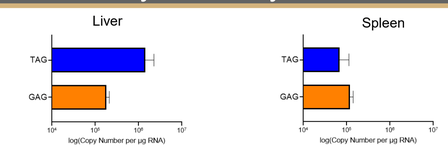


Figure 6: Expression levels of antigen message as measured by RT-qPCR. Within the spleen, expression levels of TAG and GAG are similar; however, within the liver, TAG message is ten-fold higher than GAG.

Self-reactive T cells primed by *Listeria* do not exit cell cycle prematurely, but do not persist long-term

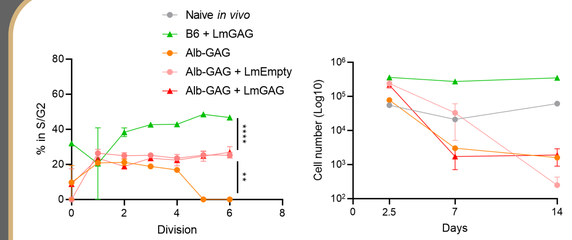


Figure 7: A. Quantification of cell population percentages actively cycling as defined by those in S and G2 phases. **B.** Within the spleen, numbers of TCR$_{GAG}$ are similar at 60 hours in AlbGAG and B6, but quickly drop by day 7, regardless of optimal antigen stimulation provided by *Listeria*-GAG.

Conclusions and Future Directions

- Self-tolerance and tumor-specific T cell dysfunction are distinct states.
- Despite being in the same environment, TCR$_{GAG}$ T cells fail to persist in a liver tumor while TCR$_{TAG}$ expand.
- The failure to express TOX does not explain the lack of TCR$_{GAG}$ persistence (data not shown).
- TCR$_{GAG}$ T cells begin to exit cell cycle, regardless of a premalignant lesion environment.
- Antigen expression levels may be a factor in the differences seen in liver-specific tolerant T cells and liver tumor-specific T cells.
- TCR$_{GAG}$ and TCR$_{TAG}$ undergo cell death and apoptosis at the same rate (data not shown), indicating that cell cycle exit is the primary way tolerant T cells disappear
- We will explore whether regulatory T cells are responsible for the lack of TCR$_{GAG}$ persistence by depleting CD4 T cells.

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