CELLULAR AND MOLECULAR REQUIREMENTS FOR REJECTION OF B16 MELANOMA IN THE SETTING OF REGULATORY T CELL DEPLETION AND HOMEOSTATIC PROLIFERATION

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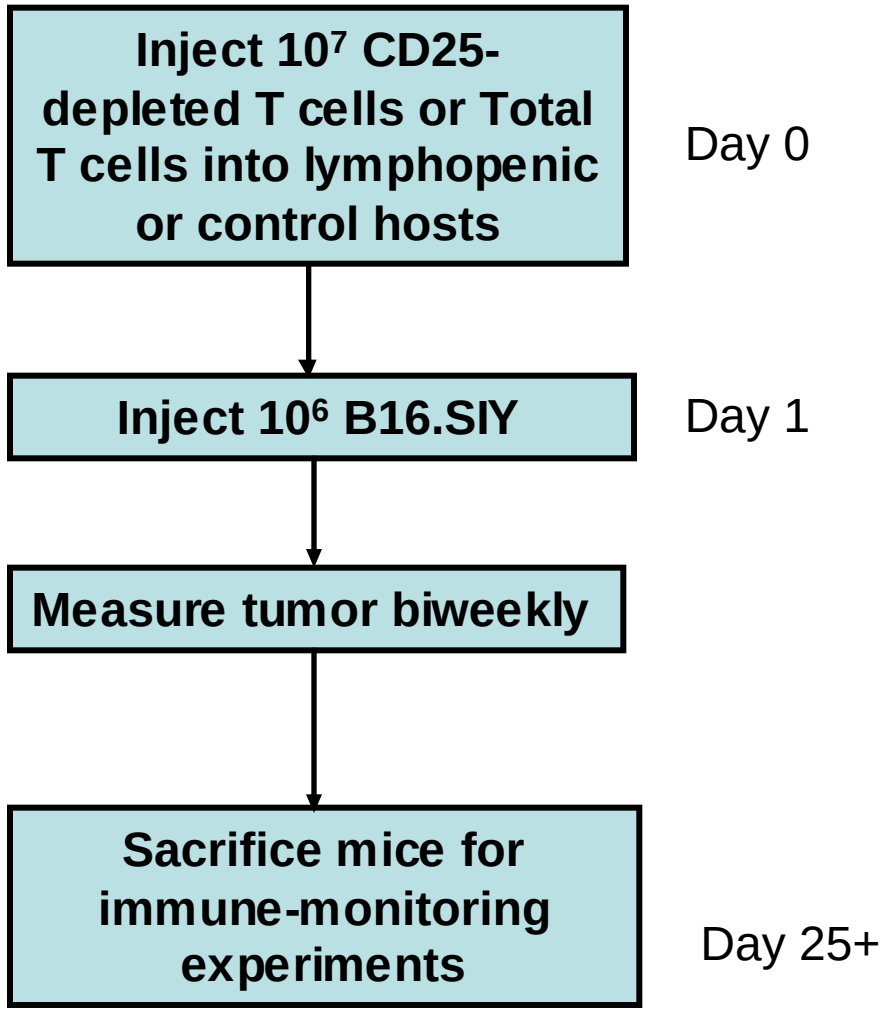
Introduction

- Cancer cells can express peptide antigens against which T cell responses can be generated in tumor-bearing hosts
- Immune evasion mechanisms employed by the tumor microenvironment can inhibit even a successfully primed anti-tumor immune response.
- We have found that the uncoupling of 2 downstream immune suppressive mechanisms can lead to rejection of B16 melanoma.
  - T cell anergy/hyporesponsiveness (through lympopenia-induced homeostatic proliferation (HP) following adoptive T cell transfer)
  - Extrinsic suppression of Tconv by Treg (Ex vivo $CD25^+$ T cell depletion)
- Manipulation of either pathway in isolation does not lead to tumor rejection
- This model system provided a context in which to determine which cellular and molecular elements are required for B16 tumor rejection by the normal host T cell repertoire

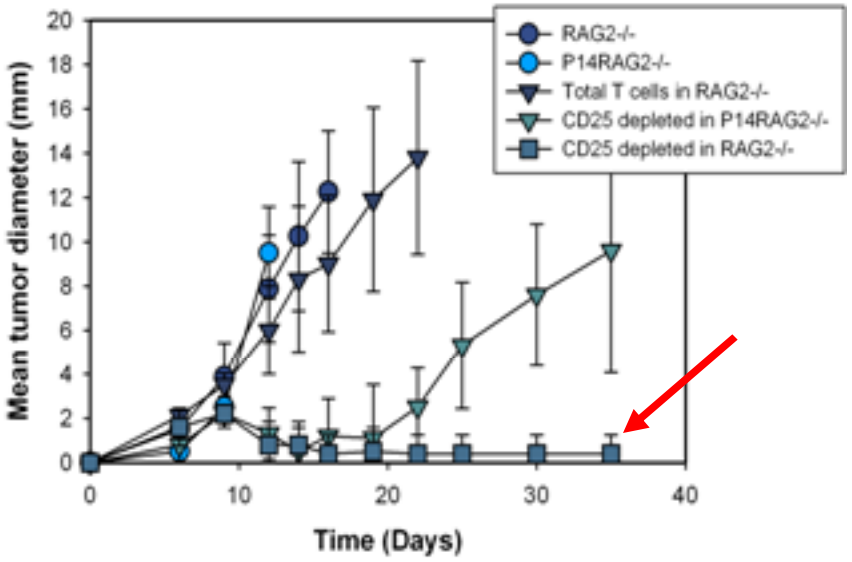
Model

- **Tumor**: B16.SIY = B16 melanoma transduced with vector encoding GFP (tumor cell detection) and the SIY peptide (immune monitoring)
- **Hosts:**
  - **A. RAG2$^{-/-}$ mice**: deficient in T and B cells used as lymphopenic hosts
  - **B. C57BL/6 mice:** lymphodepleted with 600rad TBI
- **T cells:** Bulk T cells purified from spleens of C57BL/6 mice and depleted of CD25^{+} T cells prior to adoptive transfer
- **Result: 90-100% protection**

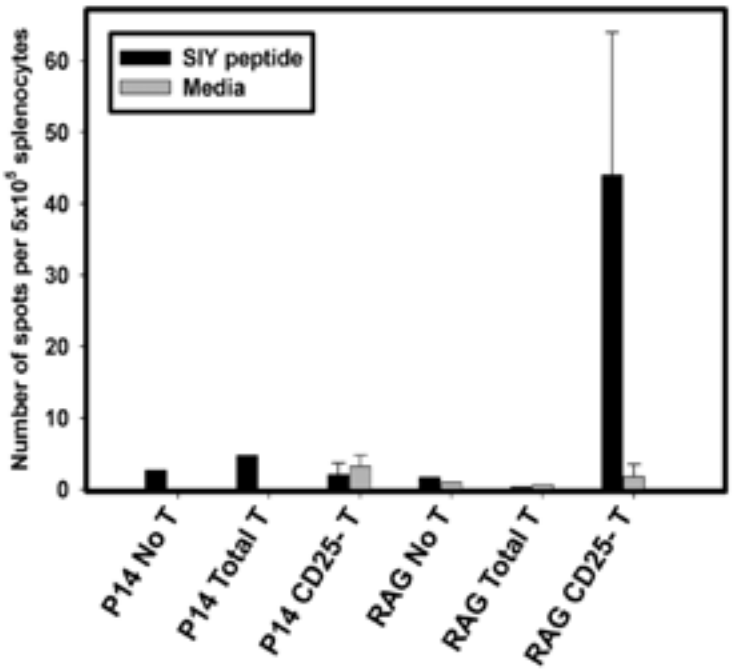


Uncoupling immune suppressive mechanisms: Combined Treg depletion and anergy reversal supports rejection of B16 melanoma

Tumor growth



T cell response

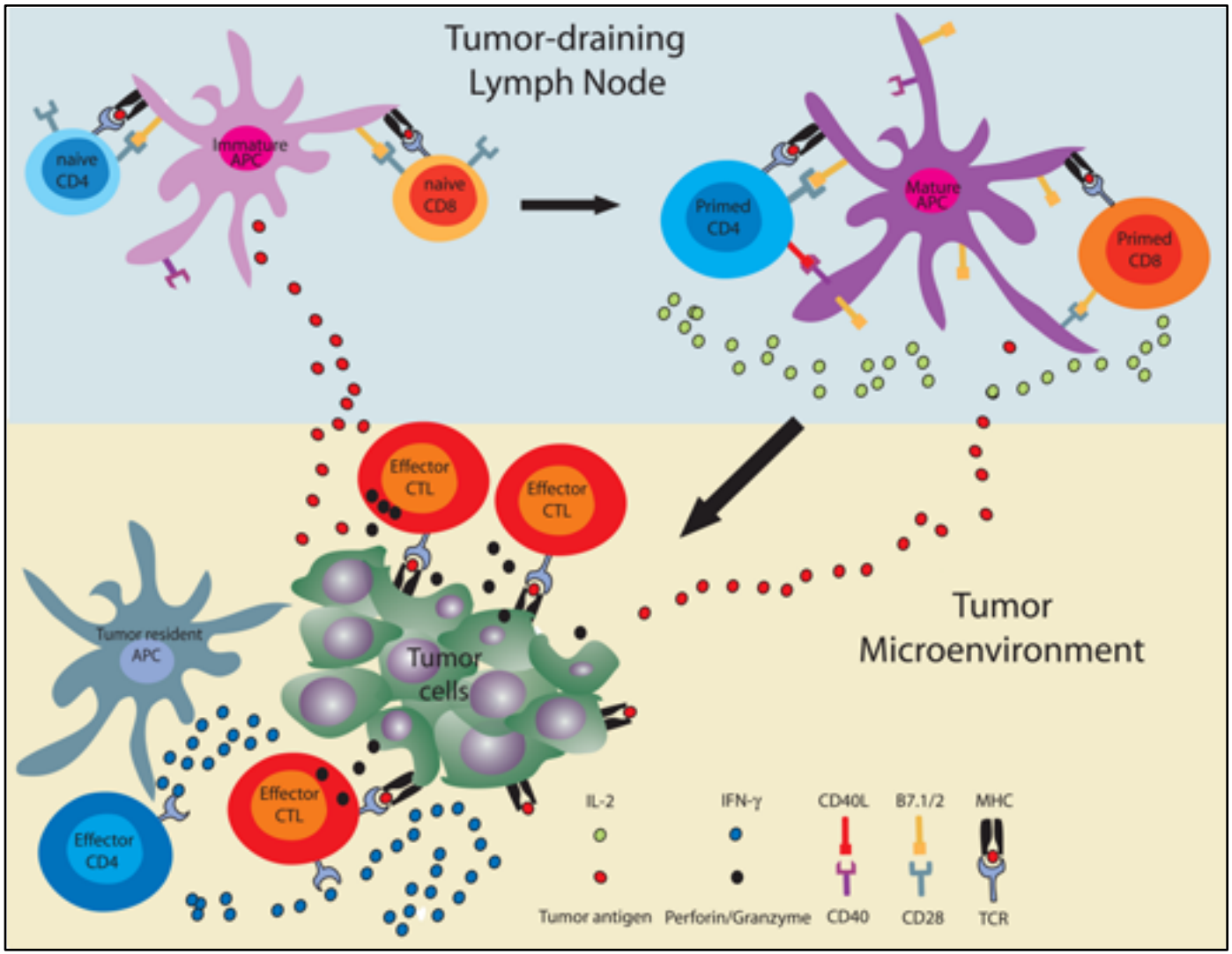


Kline et al., Clin. Can. Res. 2008

Question: What are the requirements for rejection of B16.SIY within the setting of $CD25^+$ T cell depletion and lymphopenia-induced homeostatic proliferation?

- Cellular requirements for rejection: CD4, CD8, NK
- 2 phases of the anti-tumor immune response
  - **Priming**
    - Tumor Ag presentation/cross-presentation
    - Costimulation (e.g. B7-1/B7-2)
    - APC-derived cytokines (e.g. IL-12)
    - $CD4^+$ T cell "help" (IL-2 versus CD40L/DC arming)
    - Innate immune mediators (e.g. IFN-α/β)
  - **Effector**
    - Extrinsic apoptosis (FasL)
    - Cytolysis (perforin, granzyme B)
    - Cytokines (IFN-γ, TNF-α)

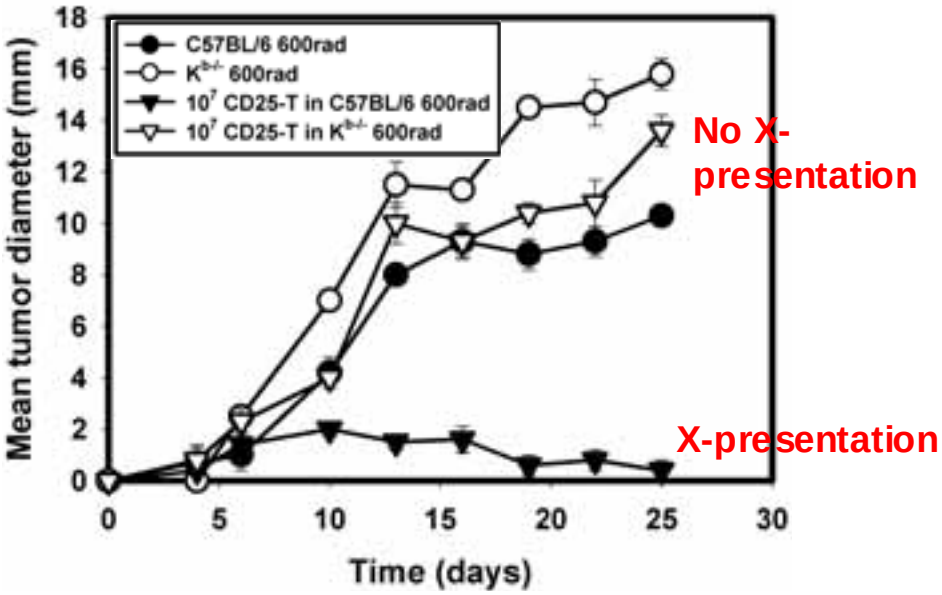
Classical model of a successful anti-tumor immune response



1. Priming phase

Tumor antigen cross-presentation by host cells is required for rejection of B16.SIY

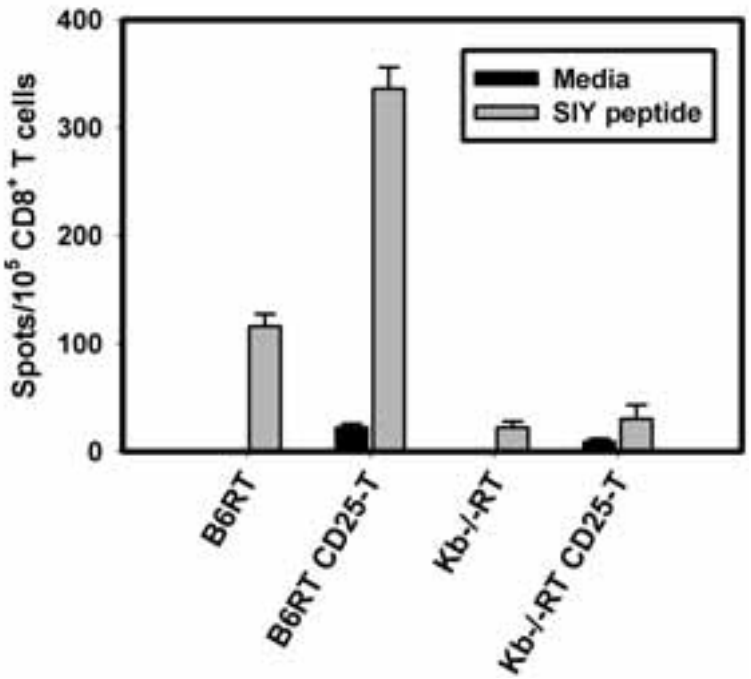
Tumor growth



No X-presentation

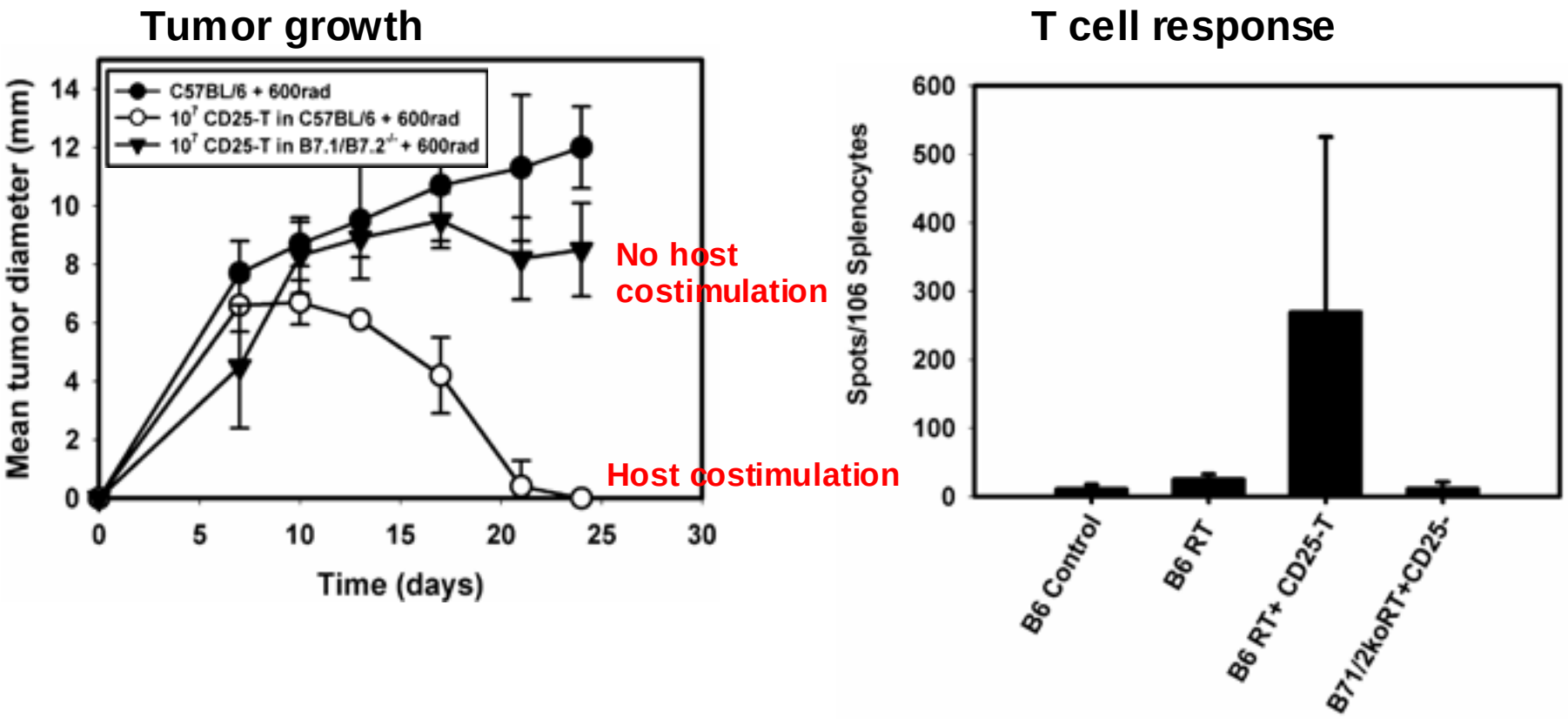
X-presentation

T cell response

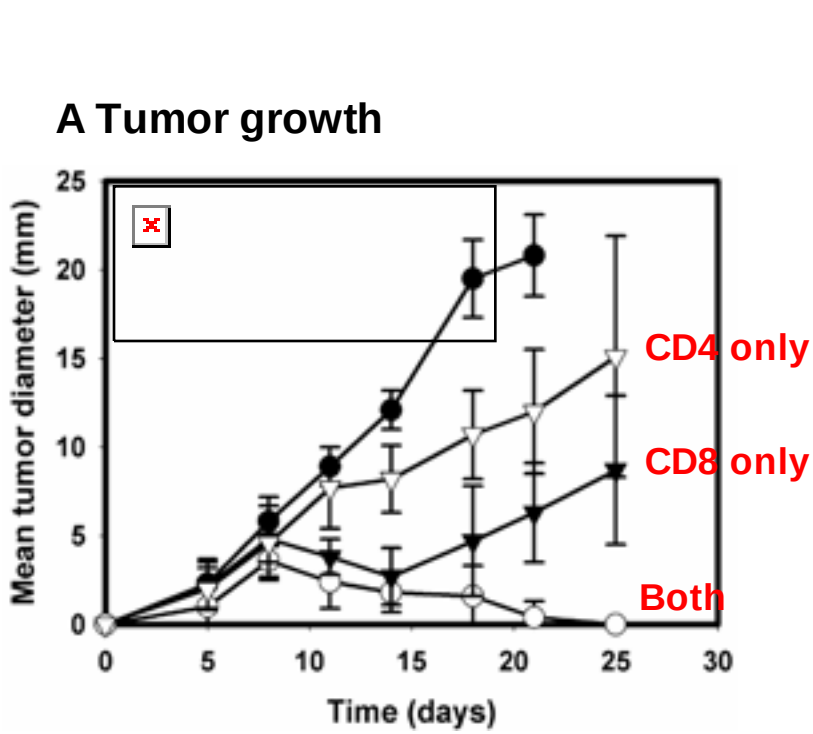


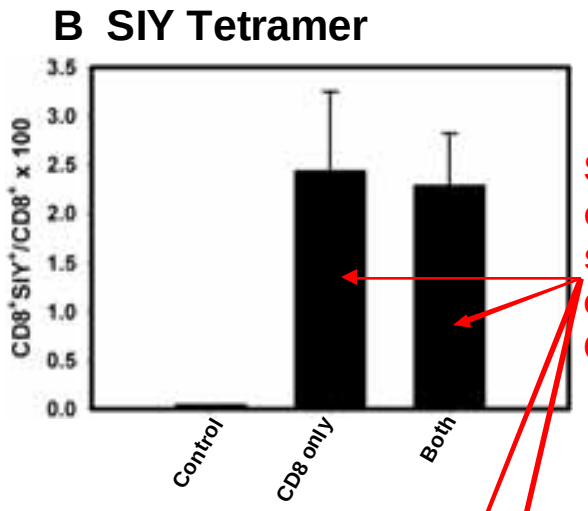
Direct presentation of antigen by tumor cells to T cells alone is NOT sufficient for tumor rejection

Rejection of B16.SIY is lost in irradiated B7.1/B7.2$^{-/-}$ hosts following CD25-depleted T cell transfer

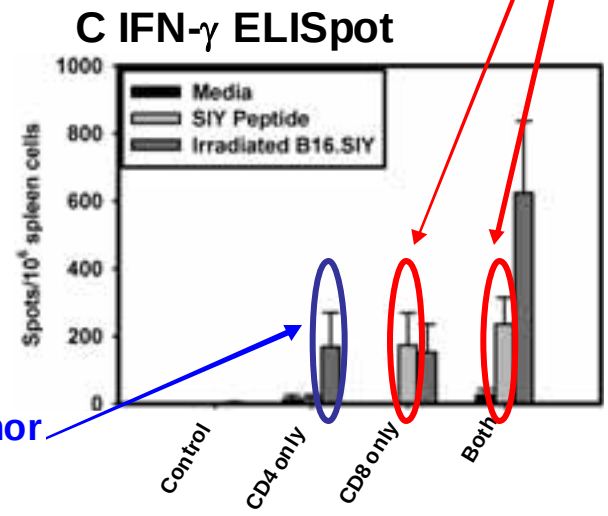


Both conventional CD4+ and CD8+ T cells are necessary for rejection of B16.SIY



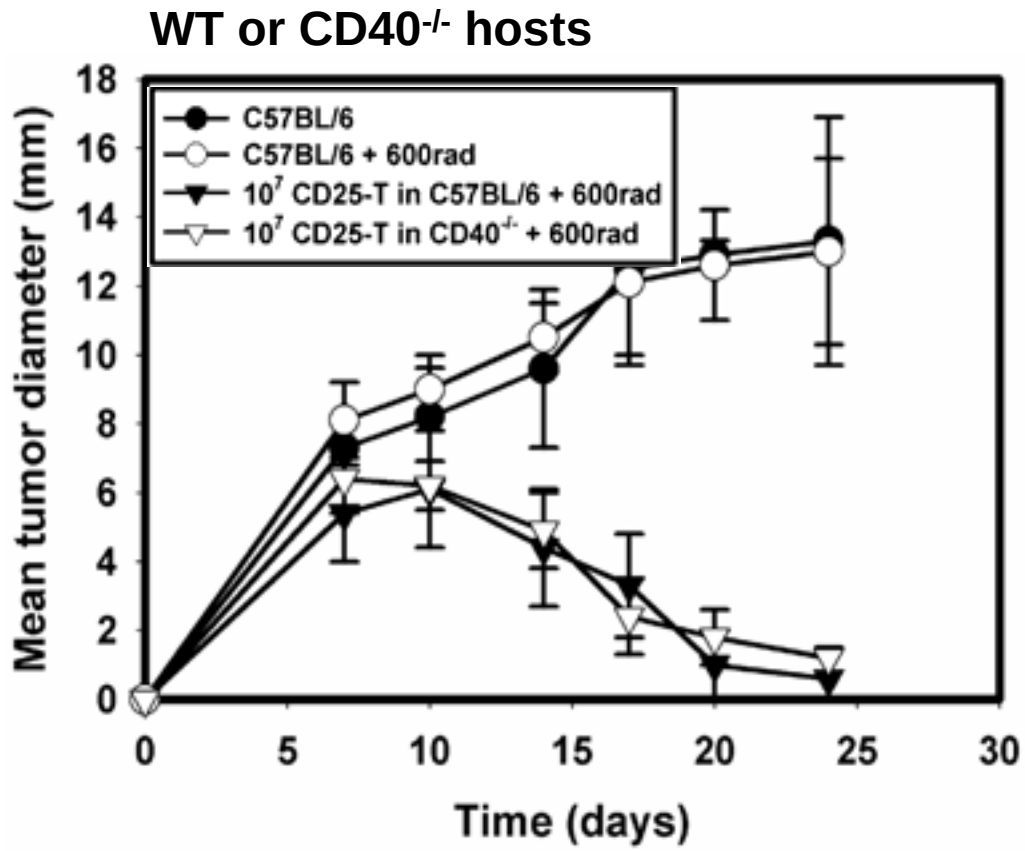


Similar priming of tumor-specific CD8+ T cells w/ or w/o CD4+ T cells

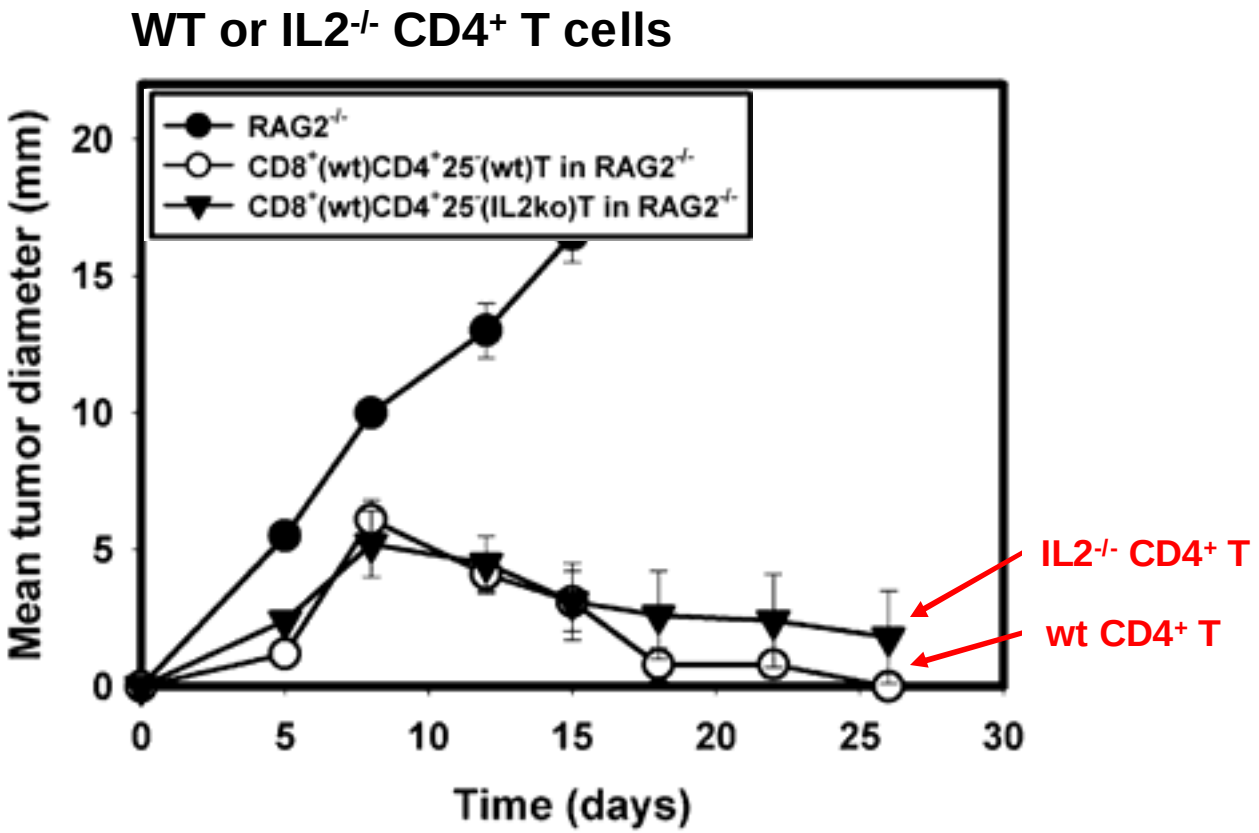


Strong IFN-γ response by CD4+ T cells following tumor cell restim

CD40 is dispensable for rejection of B16.SIY in lymphopenic hosts receiving CD25-depleted T cells



IL-2 produced by conventional CD4$^+$ T cells is dispensable for tumor rejection



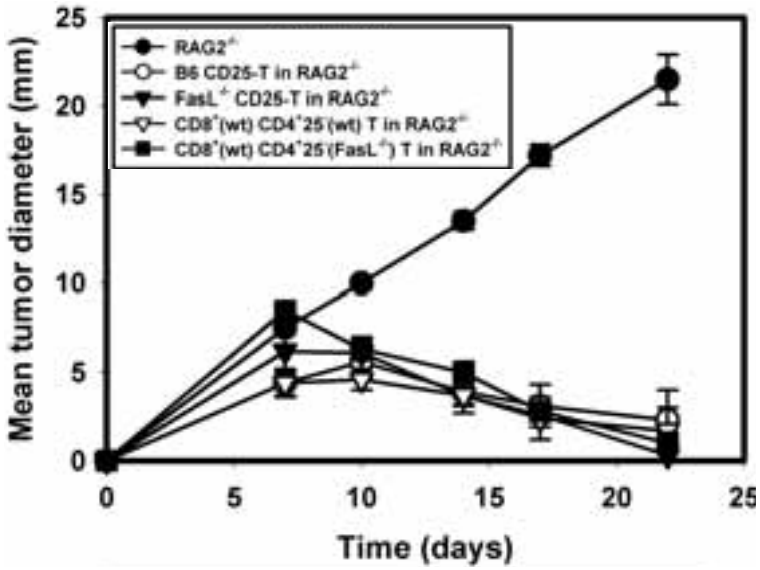
Priming phase conclusions

- T cell priming and subsequent tumor rejection in this model absolutely depends on host cross-presentation and costimulation
- Conventional $CD4^+$ T cells are required for tumor rejection, but apparently not to provide help for priming $CD8^+$ cells ➔ may be required at effector phase

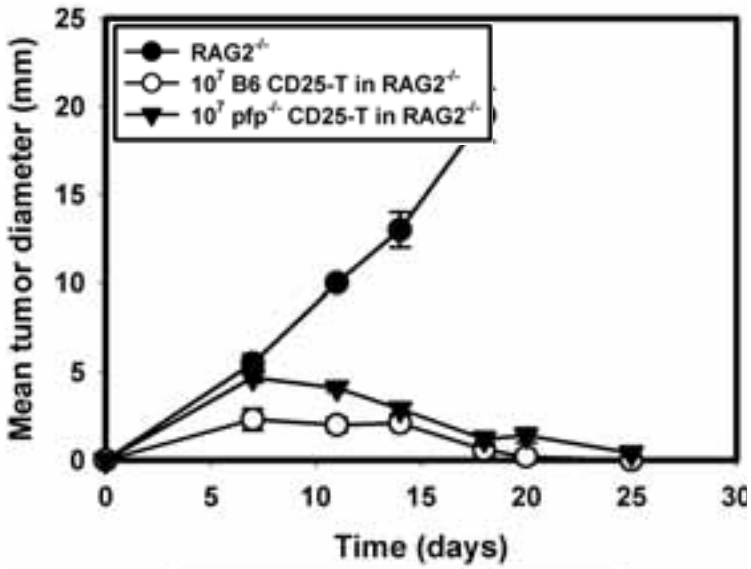
2. Effector phase

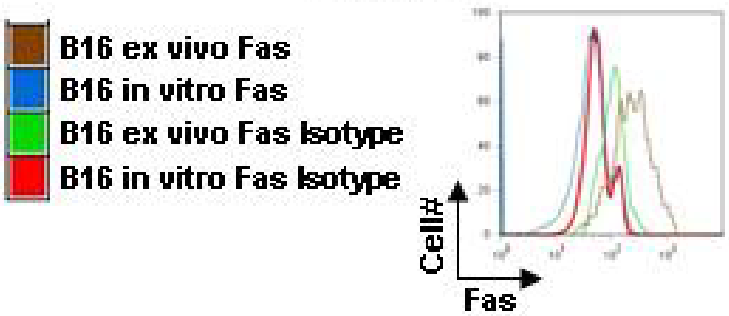
T cell expression of FasL and perforin are not required for B16.SIY rejection

A: FasL$^{-/-}$ T cells

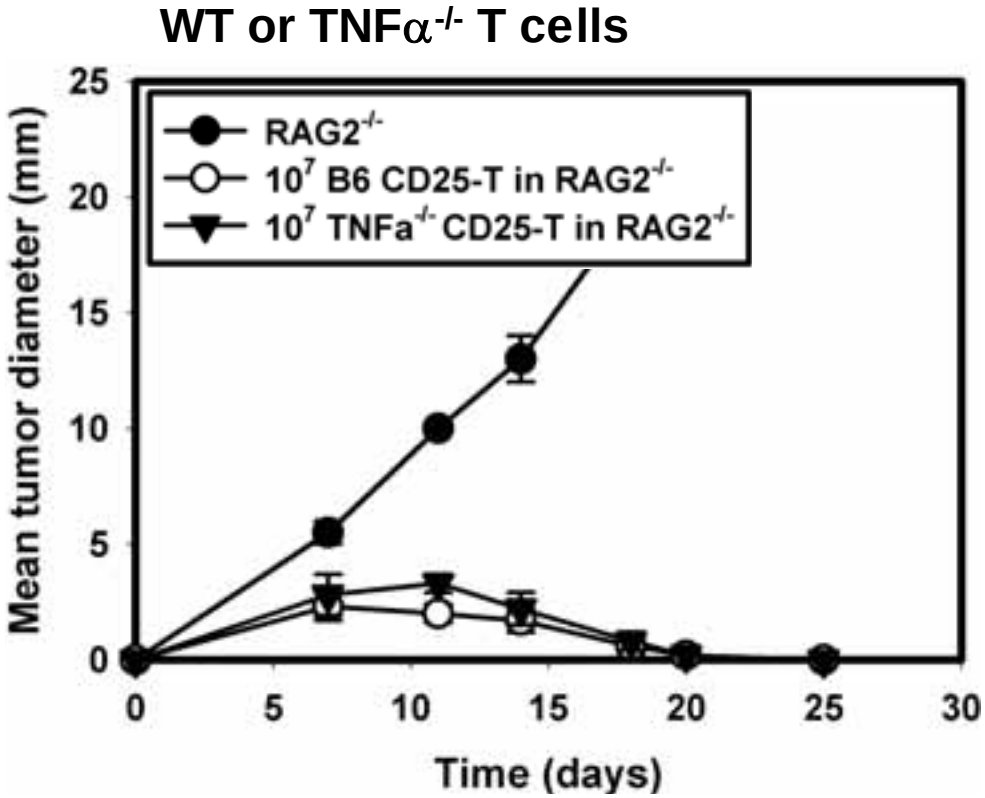


B: Perforin$^{-/-}$ T cells



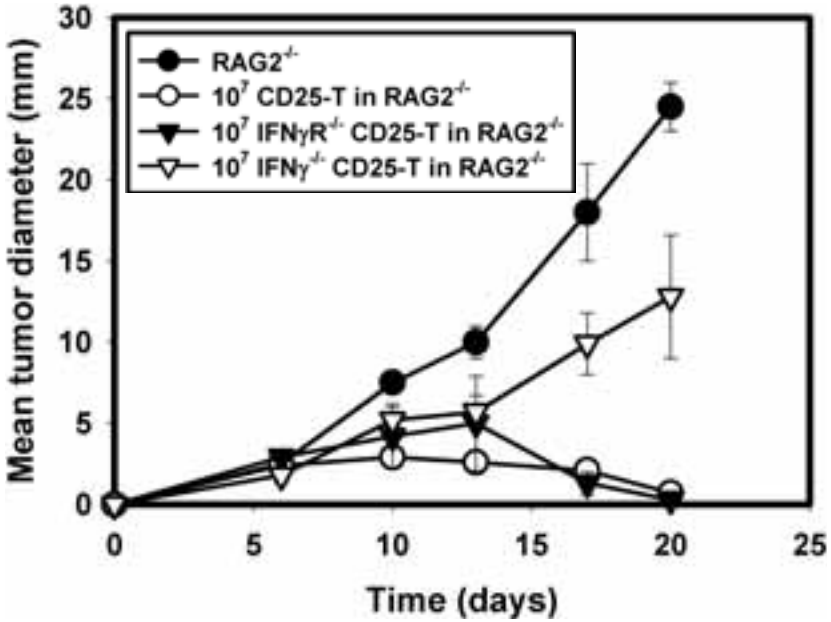


Donor T cell-produced TNF-α is not necessary for tumor rejection

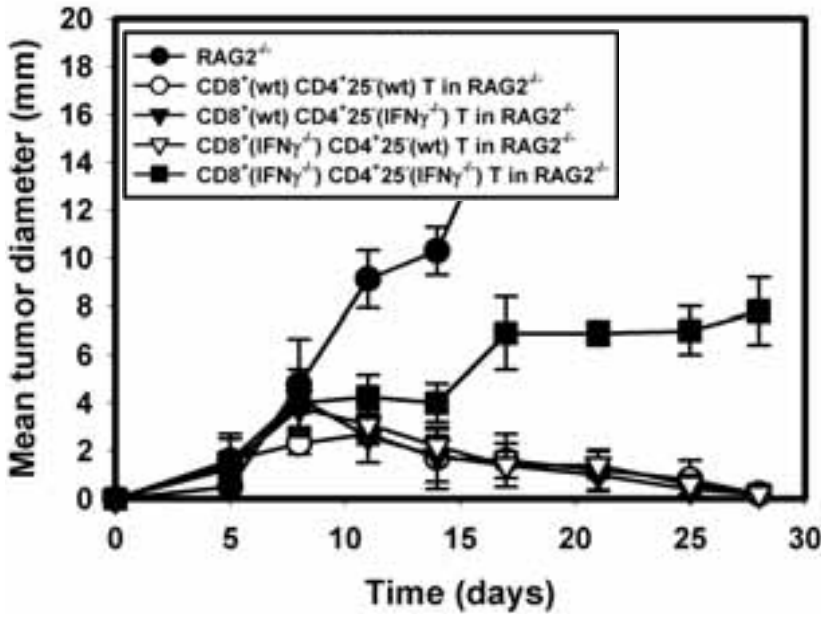


Donor T cell-produced IFN-γ is necessary for optimal tumor rejection

A. IFNγ$^{-/-}$ or IFNγR$^{-/-}$ T cells



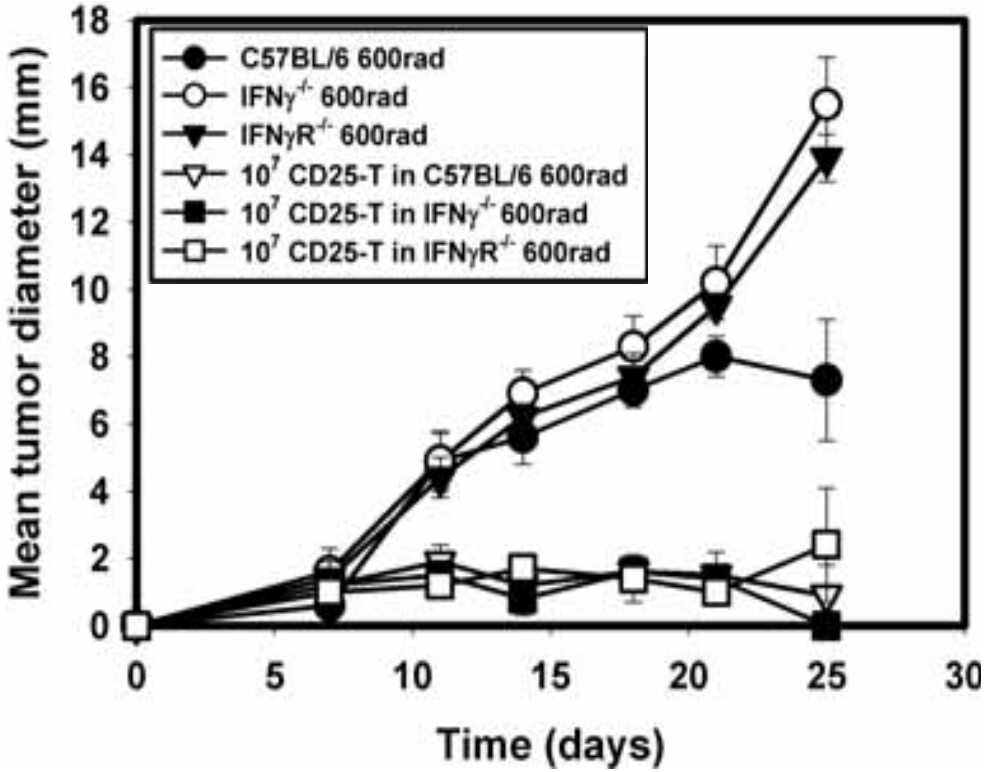
B. IFNγ$^{-/-}$ CD4$^+$ or CD8$^+$ T cells



IFN-γ produced by either CD4$^+$ or CD8$^+$ T cells is sufficient for rejection

IFN-γ production or sensitivity on host cells is dispensable for B16.SIY rejection

IFNγ$^{-/-}$ or IFNγR$^{-/-}$ hosts

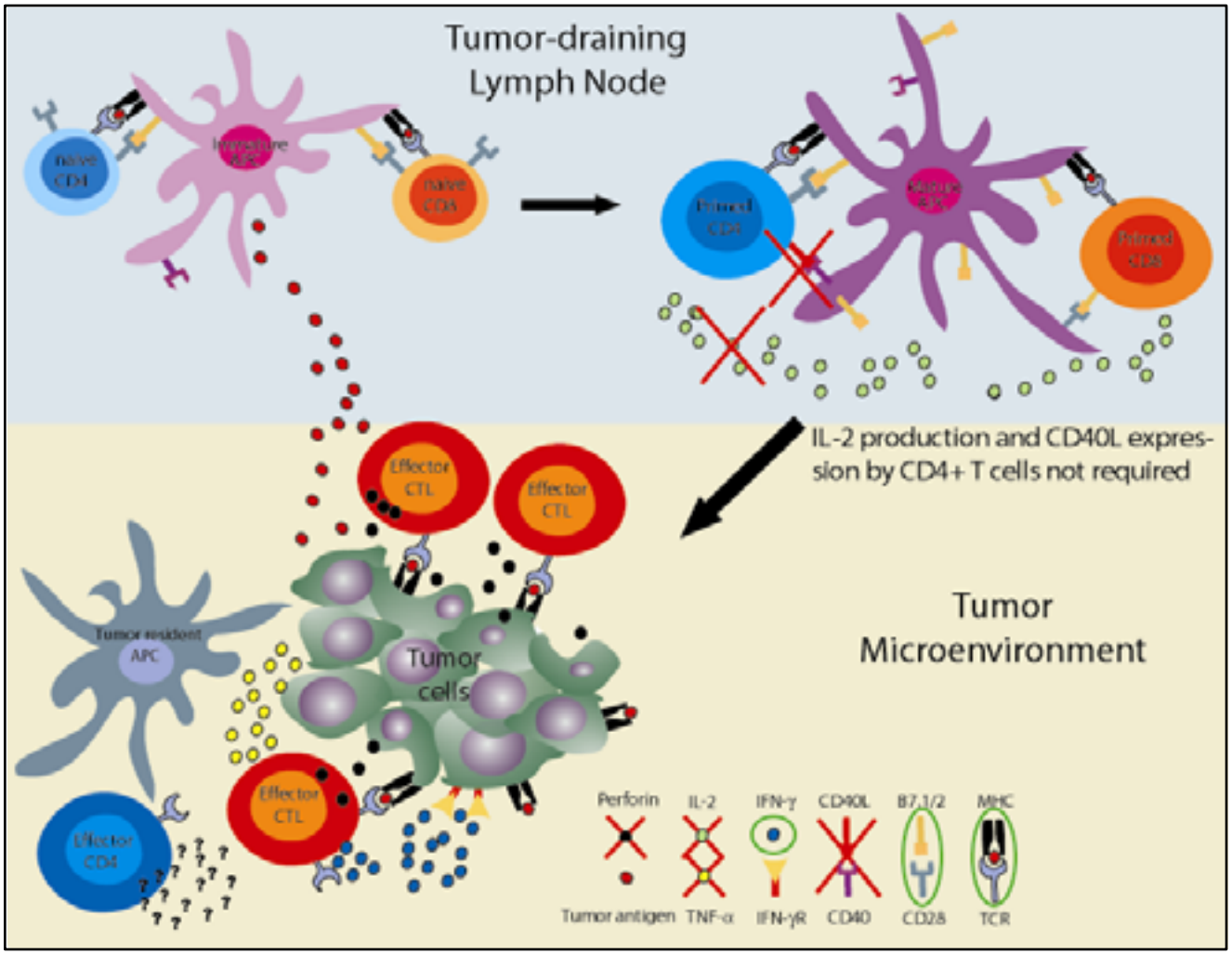


Implication: IFN-γ signaling on tumor cells directly is required

Effector phase conclusions

- Tumor rejection in this model does not require perforin, FasL, or TNF-α expression by transferred T cells
- IFN-γ production by transferred T cells is required for tumor rejection
- IFN-γ signaling on transferred T cells or on host cells is dispensable ➔ likely must act directly on tumor cells

Modified working model



Acknowledgements

- Thomas Gajewski
- Long Zhang
- Gregory Driessens
- Yan Zheng
- Fred Locke
- Michelle Gao

- This work was supported by K23 CA133196-01 (Justin Kline)