A T cell dysfunctional state is rapidly-induced in a murine model of AML

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DISCLOSURES:
NONE

Immune evasion mechanisms in solid versus hematological malignancies

- Multiple putative immune evasion mechanisms have been well-characterized in the setting of solid malignancies
  - We have identified a contribution of Tregs, classical anergy, and PD-1/PD-L1 interactions as key resistance mechanisms
    - Kline et al. Clin. Can. Res. 2008
    - Blank et al. Cancer Res. 2004
- Immune resistance pathways exploited by hematopoietic cancers have not been well-studied
  - Major differences in the growth patterns/rates and stromal networks of solid versus hematological cancers
  - May be important for future development of effective cancer immunotherapy for hematologic malignancies

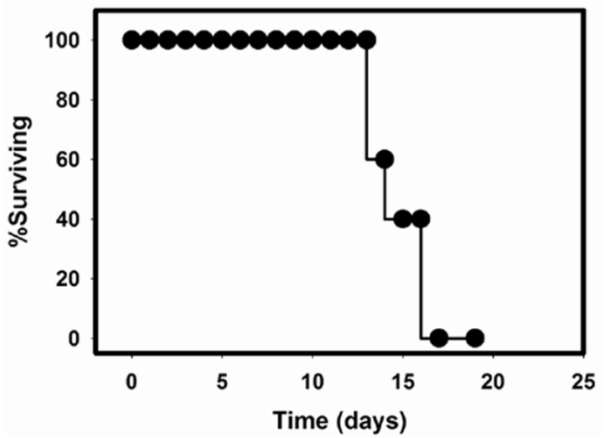
- *Goal – in a mouse AML model, to characterize the host immune response generated against cancer cells which grow as a solid mass versus those which are disseminated systemically (i.e. leukemia)*

Model - C1498 AML

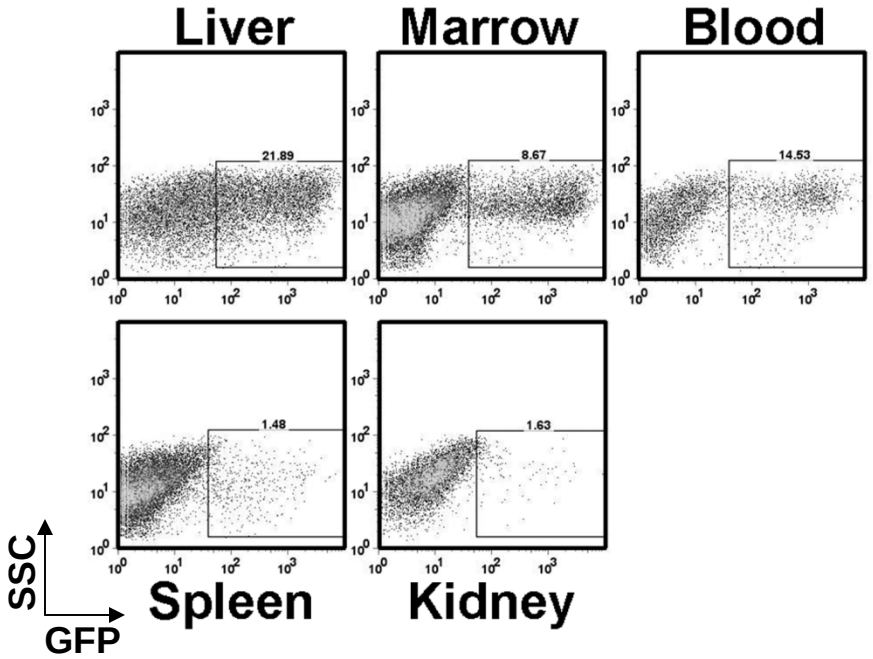
- C1498 is an aggressive, highly-lethal AML cell line which originated spontaneously in a C57BL/6 mouse
- C1498.SIY – engineered to express the model SIY peptide Ag
  - SIYRYYGL – a model K^b-restricted peptide Ag
  - SIY-expression on C1498 cells enables monitoring of endogenous anti-tumor T cell responses
    - (SIY/K^b tetramer, IFN-γ ELISPOT)
    - SIY is the cognate antigen for 2C $CD8^+$ TCR Tg T cells

C1498 cells infiltrate the blood, marrow and liver and are lethal in C57BL/6 mice

A. Survival in C57BL/6 mice

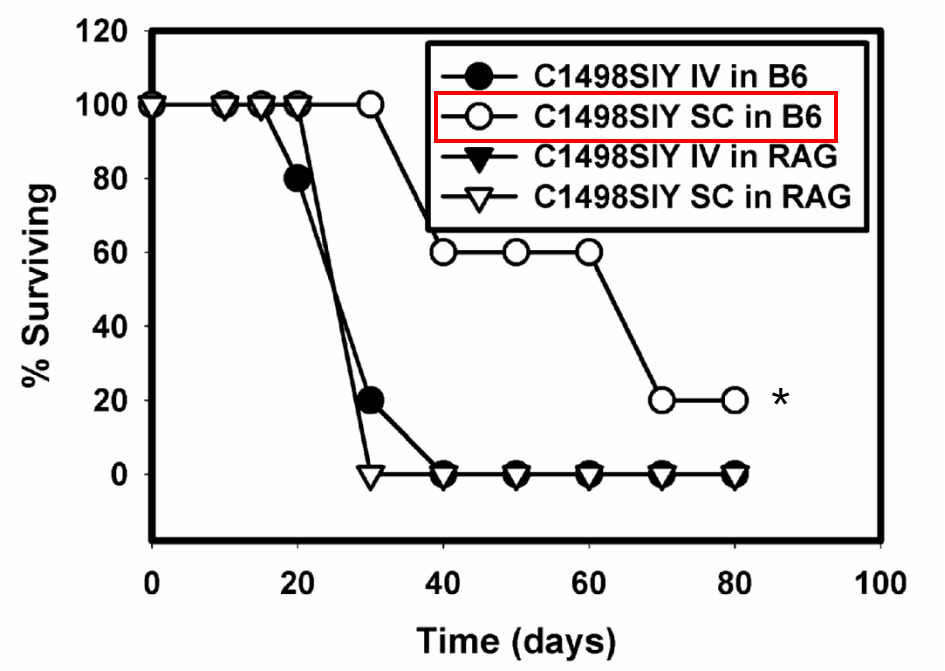


B. End-organ involvement



Zhang et al. Blood 2009

A systemic versus local C1498.SIY cell challenge decreases survival in immunocompetent mice



*p < 0.05

Observations:

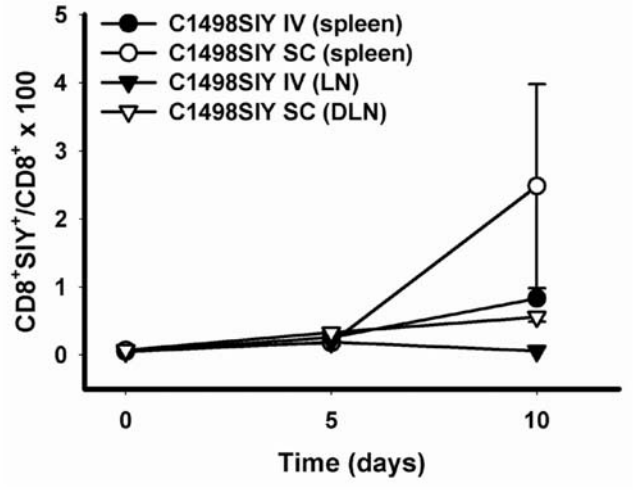
1) Similar survival in RAG$^{-/-}$ and B6 mice challenged with C1498.SIY cells IV
2) In contrast, prolonged survival was observed in B6 mice following SC C1498.SIY cell challenge

Questions:

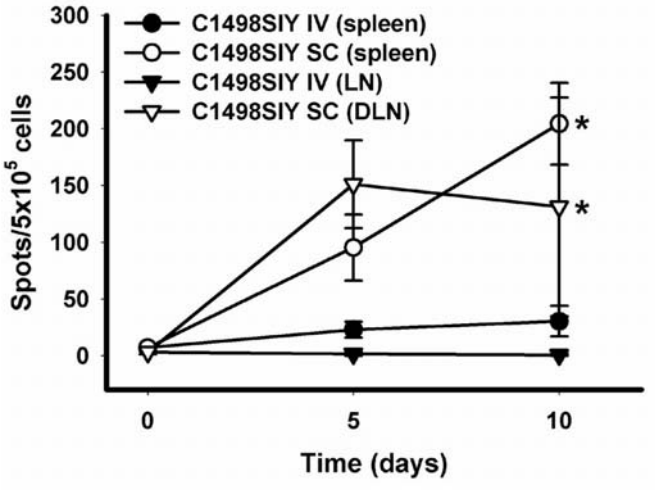
1) Are antigen-specific immune responses generated in B6 mice following IV inoculation of AML cells?
2) Is the prolonged survival in B6 mice following SC compared to IV AML cell challenge mediated by adaptive immunity?

Minimal induction of tumor-specific T cell responses upon IV introduction of AML cells

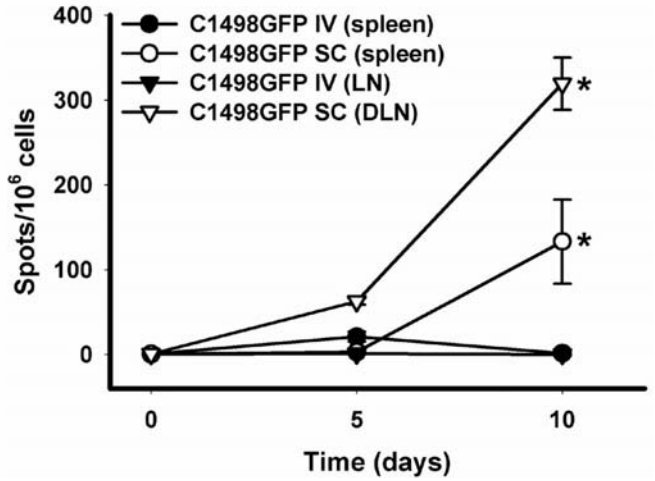
A. SIY tetramer – C1498.SIY



B. IFN-γ ELISPOT – C1498.SIY



C. Tumor cell ELISPOT – C1498.GFP

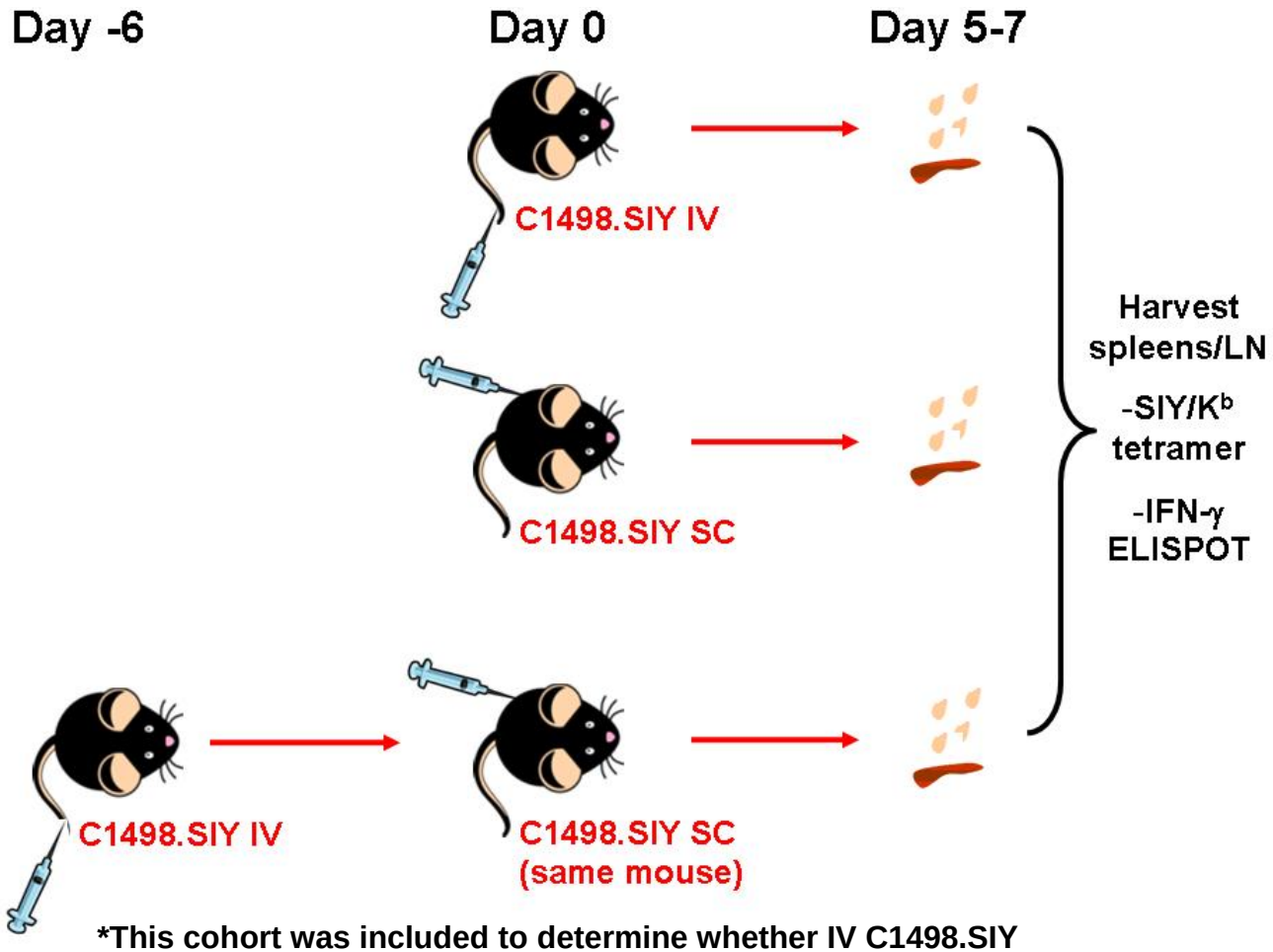


What explains impaired T cell response to AML cells given IV compared to SC?

- Differential diagnosis
  - Failed priming of Ag-specific T cells (immunological ignorance)
  - Extrinsic suppression
    - Regulatory T cells (Treg)
    - Myeloid-derived suppressor cells (MDSC)
  - T cell anergy/deletion

Does an IV C1498.SIY challenge result in poor T cell priming or induce peripheral tolerance?

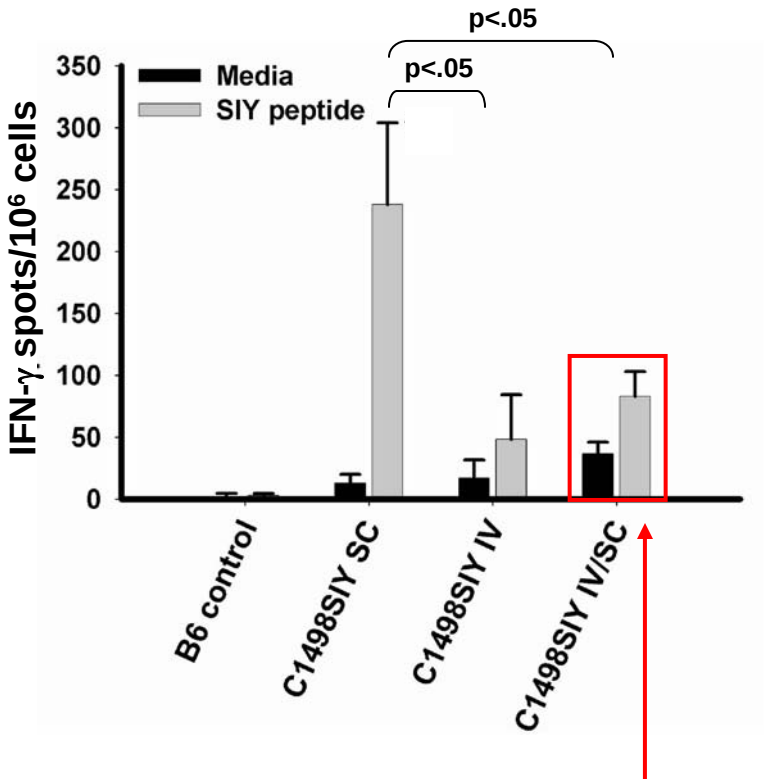
Experimental Design



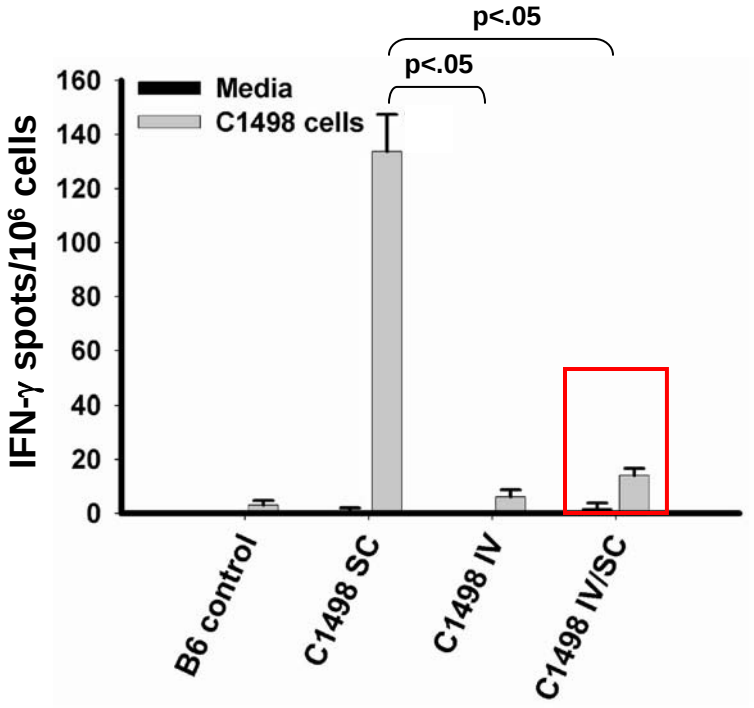
*This cohort was included to determine whether IV C1498.SIY was associated with passive ignorance versus active tolerance

A state of immune tolerance is rapidly induced in hosts following IV C1498 inoculation

A. IFN-γ ELISPOT – C1498.SIY

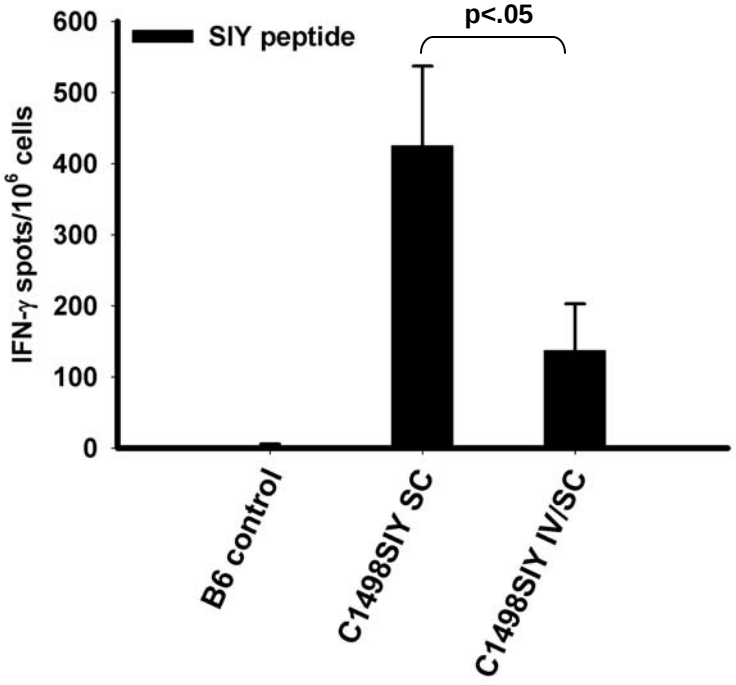


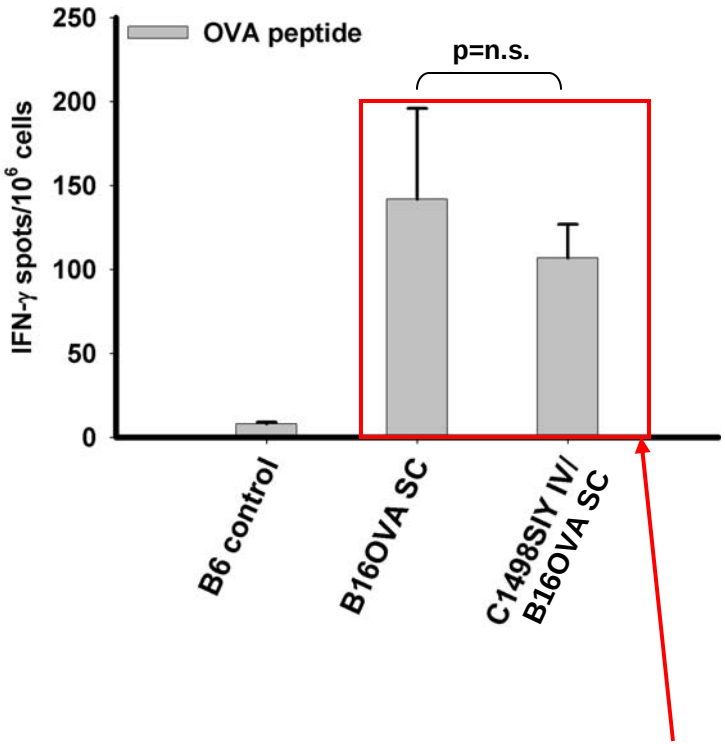
B. IFN-γ ELISPOT – Control C1498



Blunted SIY-specific T cell response in IV/SC dual-challenged mice argue that IV C1498 cells actively promote peripheral tolerance

T cell dysfunction induced by C1498.SIY is antigen-specific

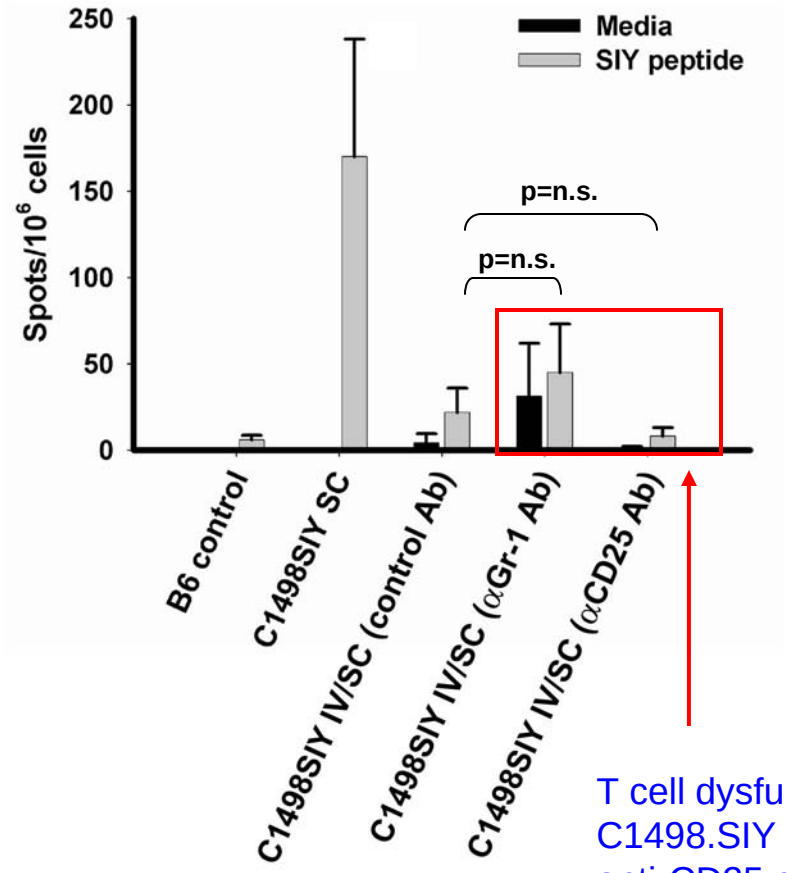




OVA-specific T cell responses to a SC challenge were similar following a preceding IV C1498.SIY challenge

T cell dysfunction is not reversed following Treg or MDSC depletion

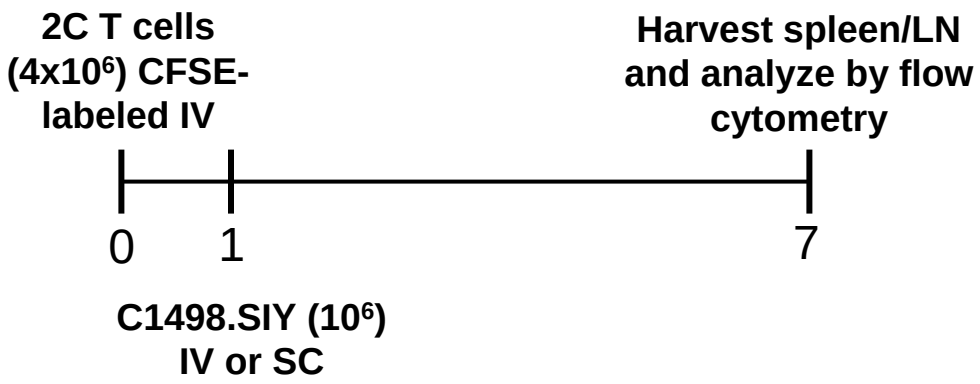
IFN-γ ELISPOT – Treg/MDSC depletion



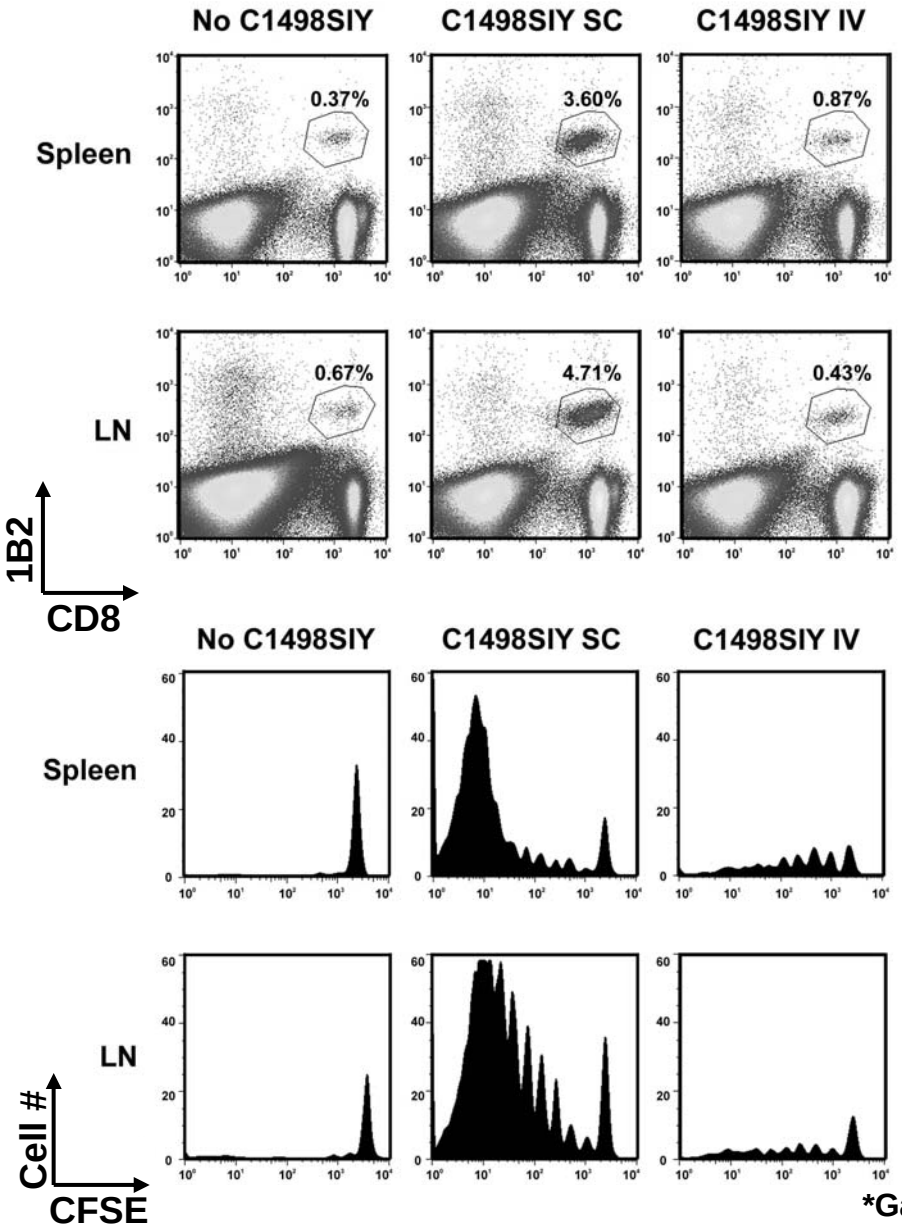
T cell dysfunction following dual IV/SC C1498.SIY challenge not reversed following anti-CD25 or anti-Gr1 mAb

Experimental approach to dissect the mechanism(s) of T cell tolerance in C1498

- C57BL/6 2C Tg mice
- Contain monoclonal population of “2C” $CD8^+$ T cells
- Recognize SIYRYYGL (SIY) peptide when presented by K^b
- Adoptively transfer purified 2C T cells into WT C57BL/6 mice challenged with C1498.SIY IV vs SC
- Study priming and function of 2C cells harvested at specific time points.



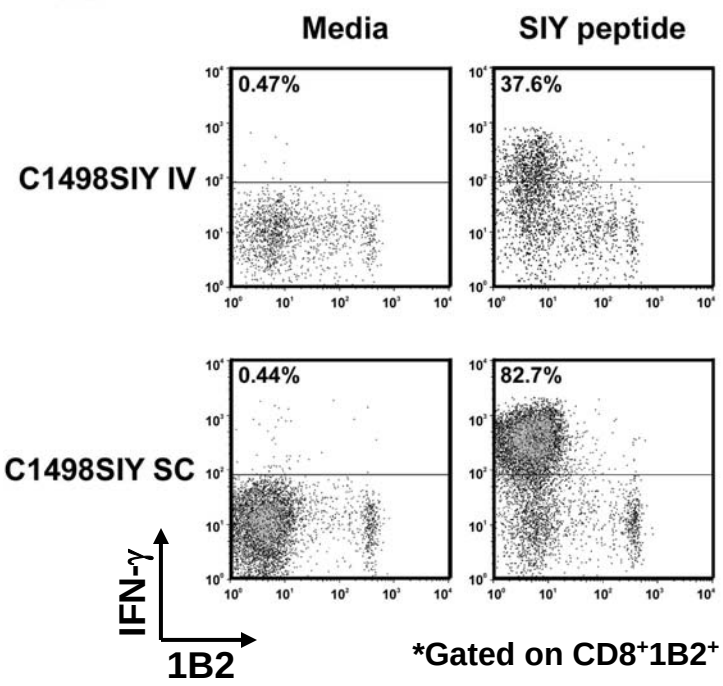
Diminished accumulation of 2C T cells in mice challenged with C1498.SIY IV



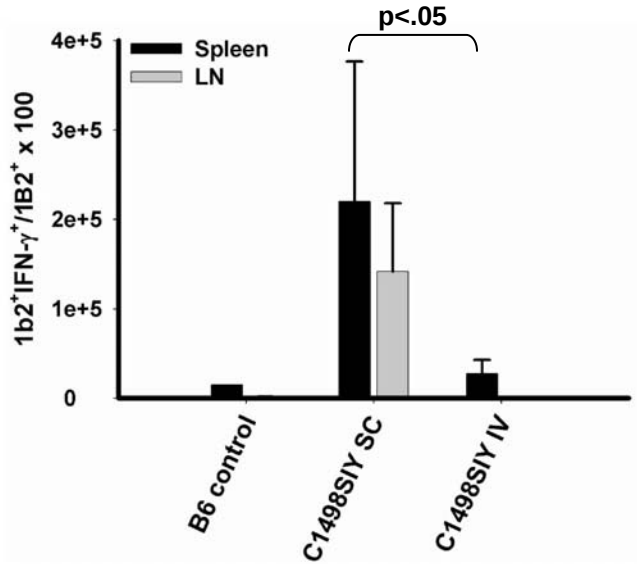
SIY-specific 2C T cells proliferate but fail to accumulate in hosts with IV C1498.SIY

*Gated on $CD8^{+}1B2^{+}$ 2C T cells

Diminished function of 2C T cells in mice bearing IV vs SC C1498.SIY



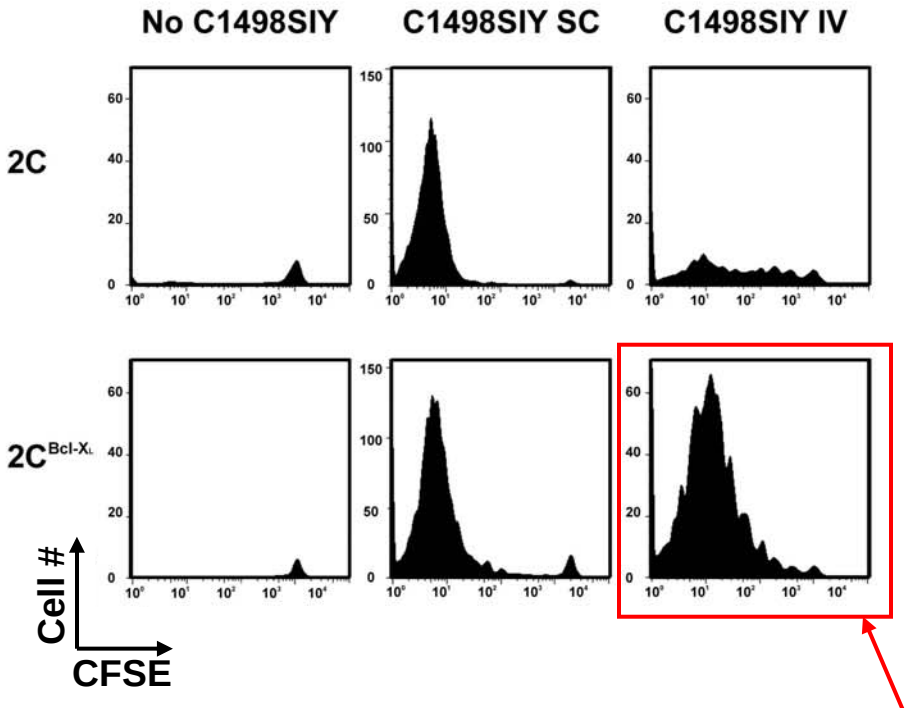
*Gated on $CD8^+1B2^+$ 2C T cells

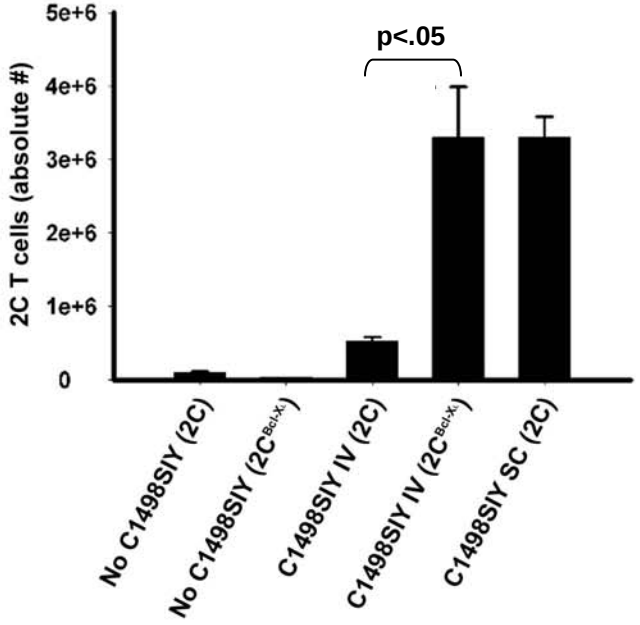


Collectively, these data suggest that antigen-specific T cells in mice with systemic AML do not accumulate and demonstrate poor effector function ex vivo

WHY?

Transgenic expression of Bcl-X_L in 2C T cells restores their accumulation in mice harboring IV C1498.SIY

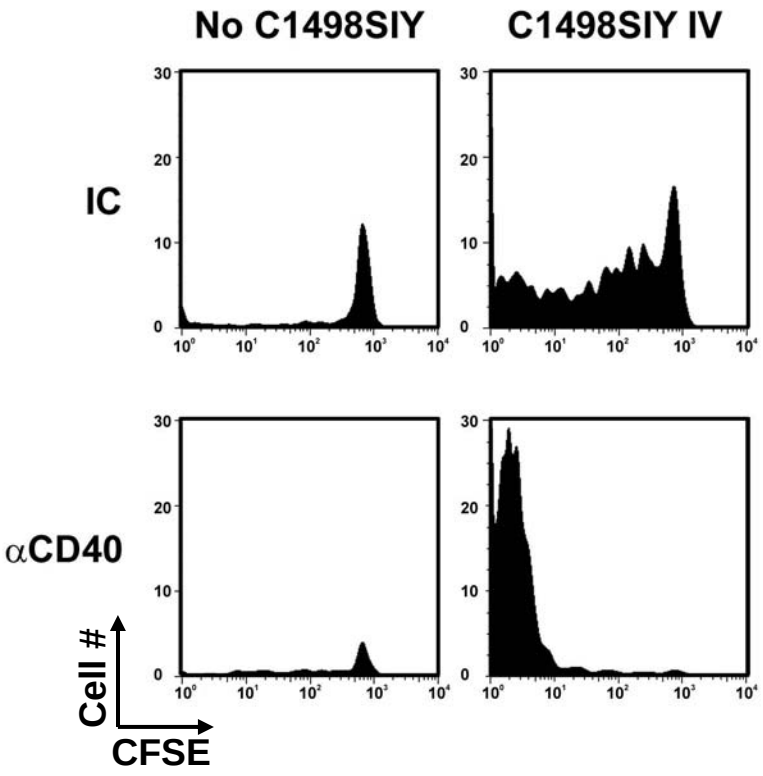




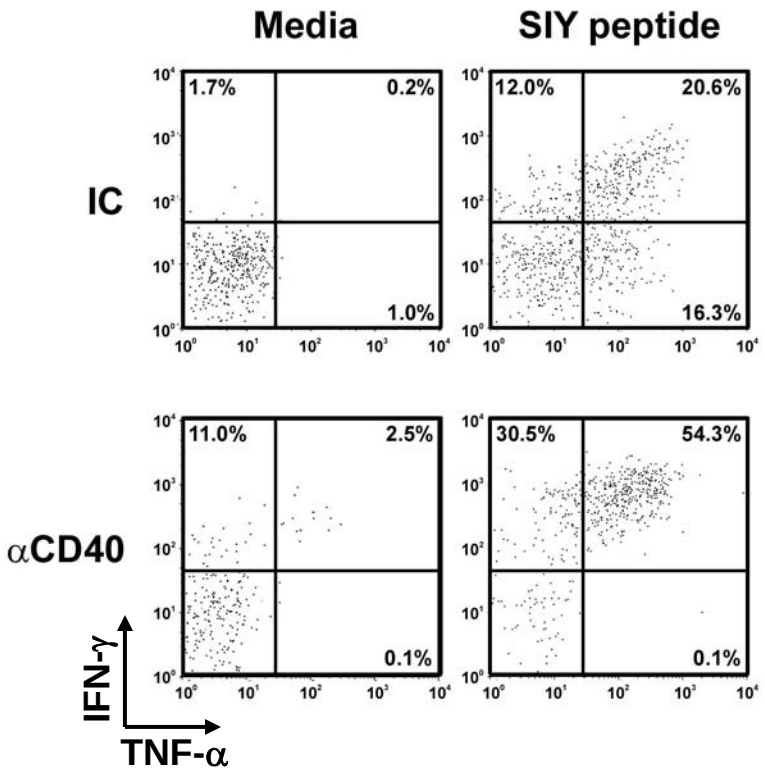
Argues that T cell deletion may be one mechanism regulating T cell tolerance in mice with IV C1498.SIY

Activation of host APC through CD40 ligation restores accumulation and function of 2C T cells in mice with IV C1498.SIY

A. 2C T cells – CFSE dilution



B. 2C T cells – cytokine production



Conclusions

- Endogenous antigen-specific T cell responses are poorly induced in the setting of IV C1498 cell challenge
- C1498.SIY cells induce a state of T cell dysfunction unique to the IV setting which appears antigen-specific
- 2C T cells are unable to accumulate and demonstrate poor effector function in mice harboring C1498.SIY cells systemically
- Recovery of 2C T cell accumulation upon Bcl-X_L expression argues for T cell deletion as one mechanism of immune evasion
- Whether T cell deletion is regulated directly by AML cells or indirectly through tolerigenic host APC is not clear
  - Hypothesize that leukemia antigens cross-presented by quiescent host APC in the IV setting due to lack of local inflammation
    - Supported by anti-CD40 data demonstrating that agonistic CD40 mAb may prevent T cell dysfunction in hosts bearing IV AML
- Future work will focus on identifying the specific APC populations which regulate T cell dysfunction in mice with AML
- Implications:
  - Important differences in immune regulation exist in hosts harboring cancer cells systemically versus locally
  - Immunotherapy for AML should be most effective in the minimal residual disease state

Acknowledgments

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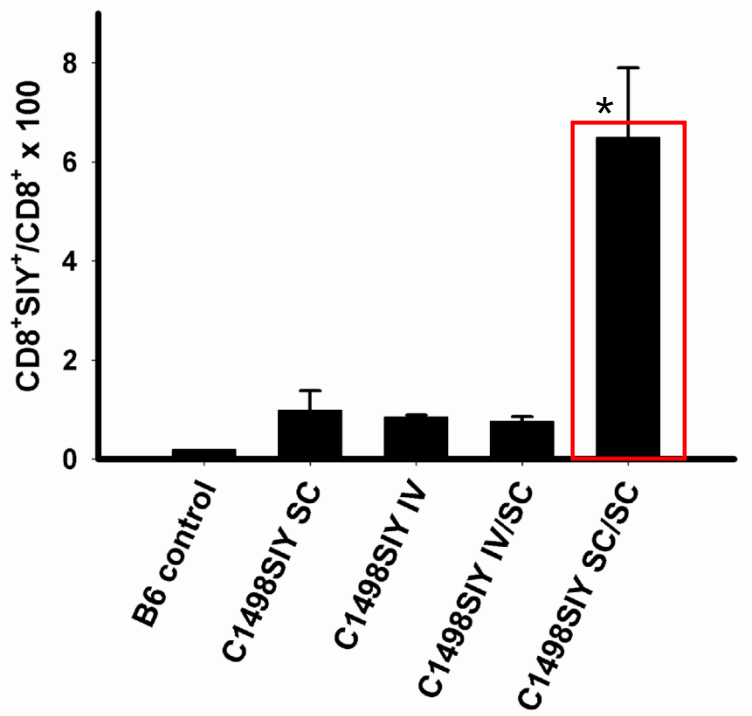
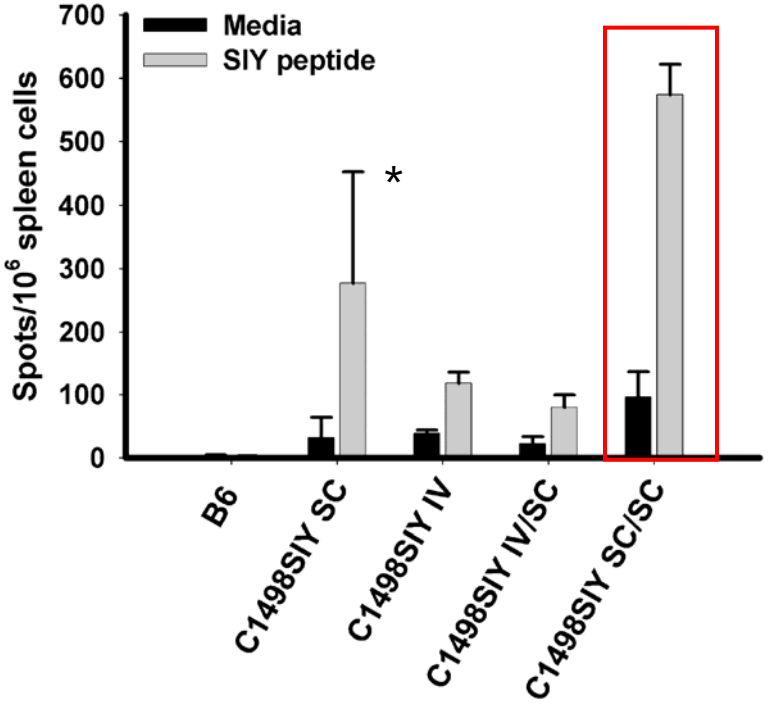
- Leukemia Research Foundation - YIA

T cell dysfunction induced by C1498SIY is unique to mice harboring IV tumors

A. SIY tetramer



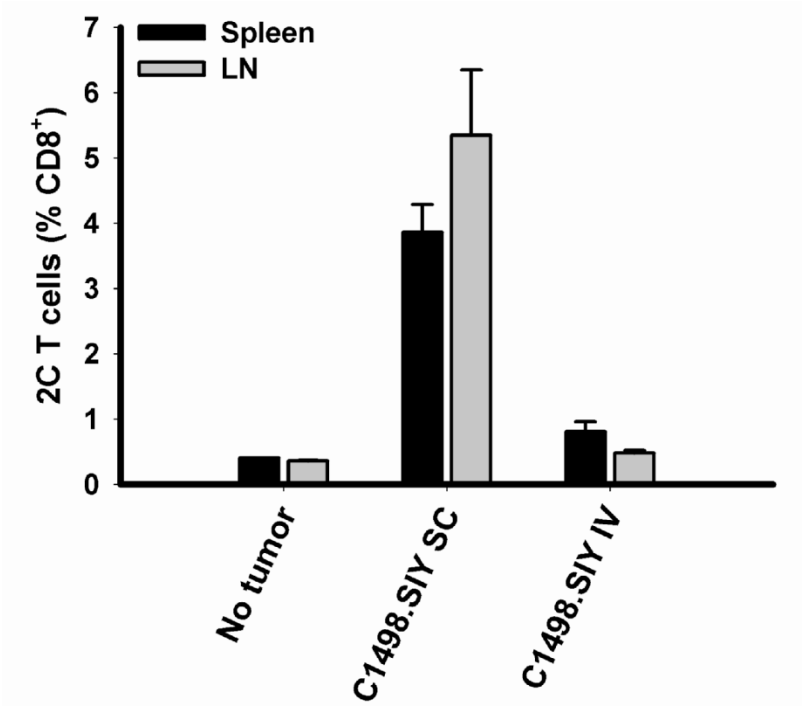
B. IFN-γ ELISPOT



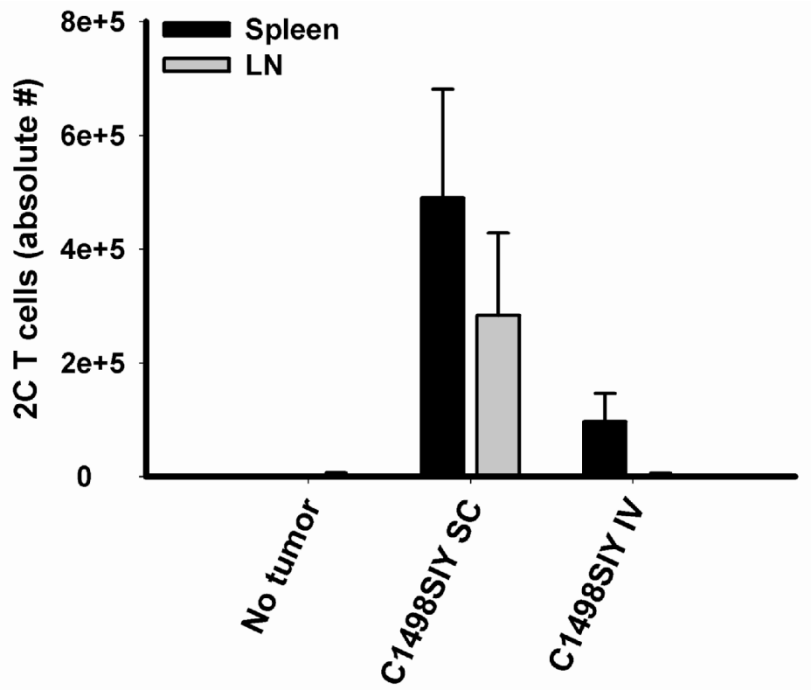
Augmented SIY-specific CD8+ T cell responses in the SC/SC group suggests tolerance is specific to the IV setting

Decreased recovery of 2C T cells from C57BL/6 mice harboring IV C1498.SIY

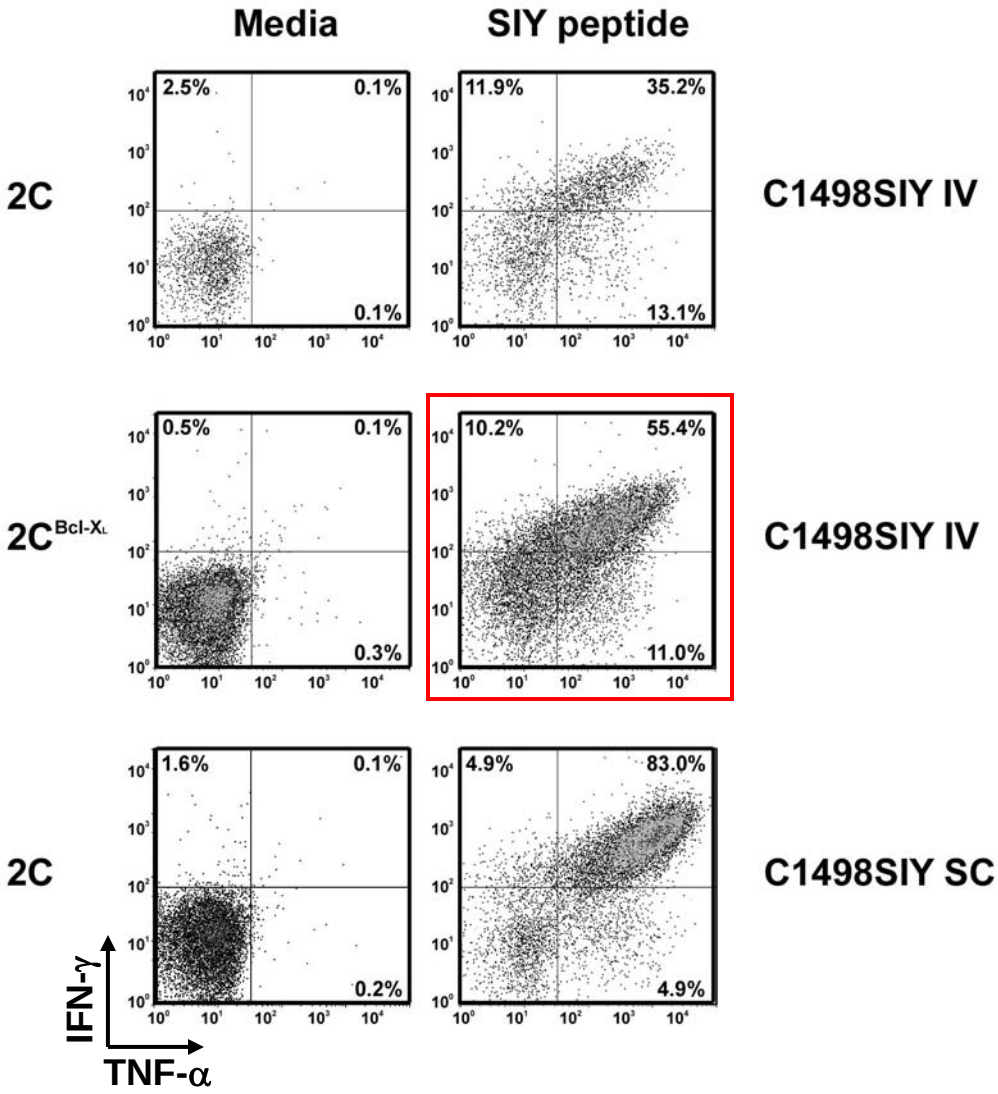
2C T cells - percentages



2C T cells – absolute #s

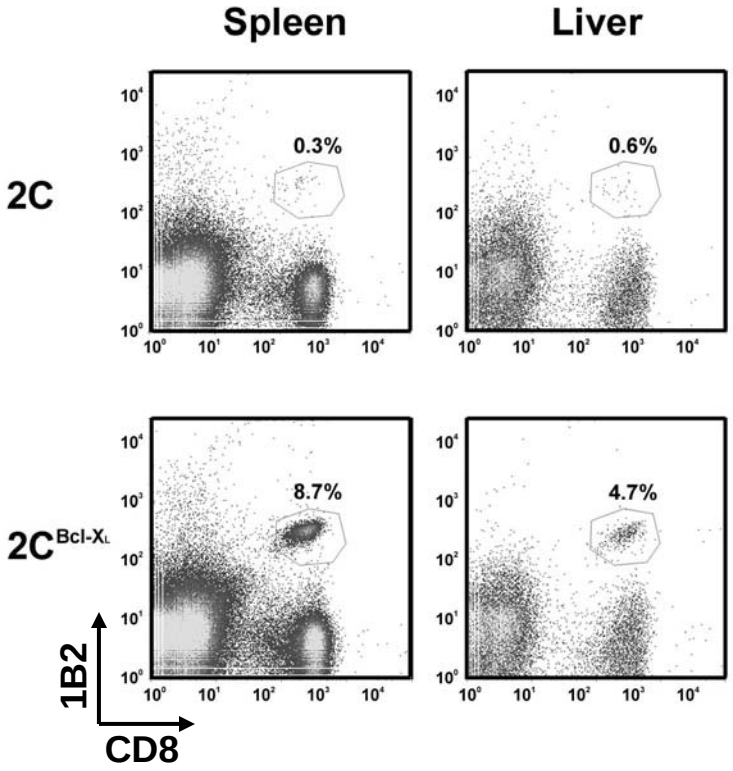


Transgenic expression of Bcl-X_L in 2C T cells partially restores effector function in mice harboring IV C1498.SIY

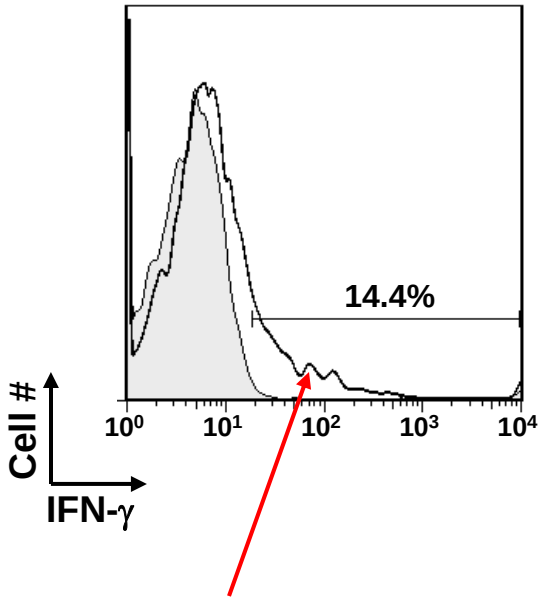


Bcl-X_L transgenic 2C T cells persist in mice harboring IV C1498.SIY

A. 2C T cells – Frequency at 25d



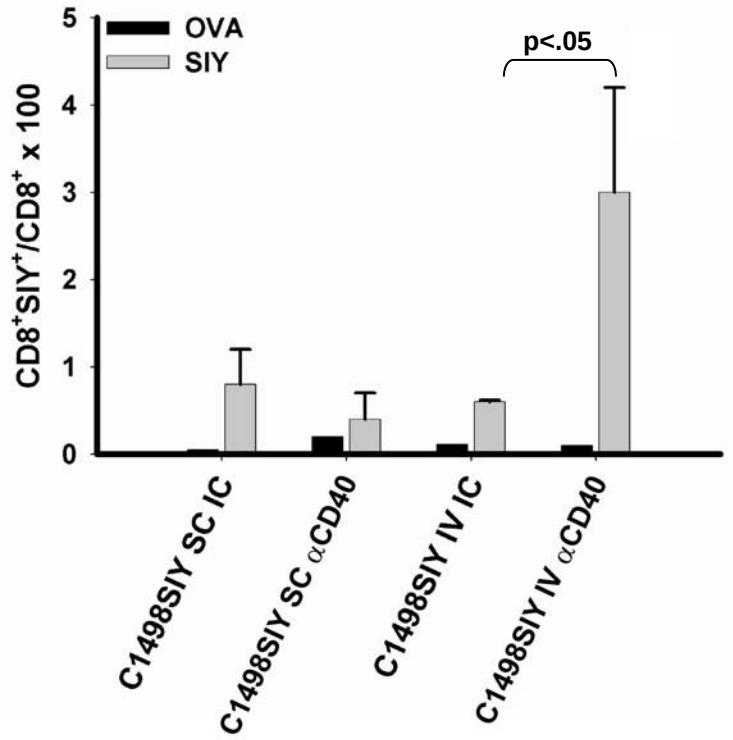
B. 2C$^{Bcl\text{-}XL}$ T cells – IFN-γ (spleen)



Poor IFN-γ production by 2C$^{Bcl\text{-}XL}$ T cells at later time points argues that they are susceptible to additional negative regulation at later time points

In vivo CD40 ligation augments the priming and function of endogenous SIY-reactive CD8$^+$ T cells in mice with IV C1498.SIY

A. SIY tetramer – αCD40



B. IFN-γ ELISPOT – αCD40