Dual Role for Galectin-3 in CD8 T cell Function

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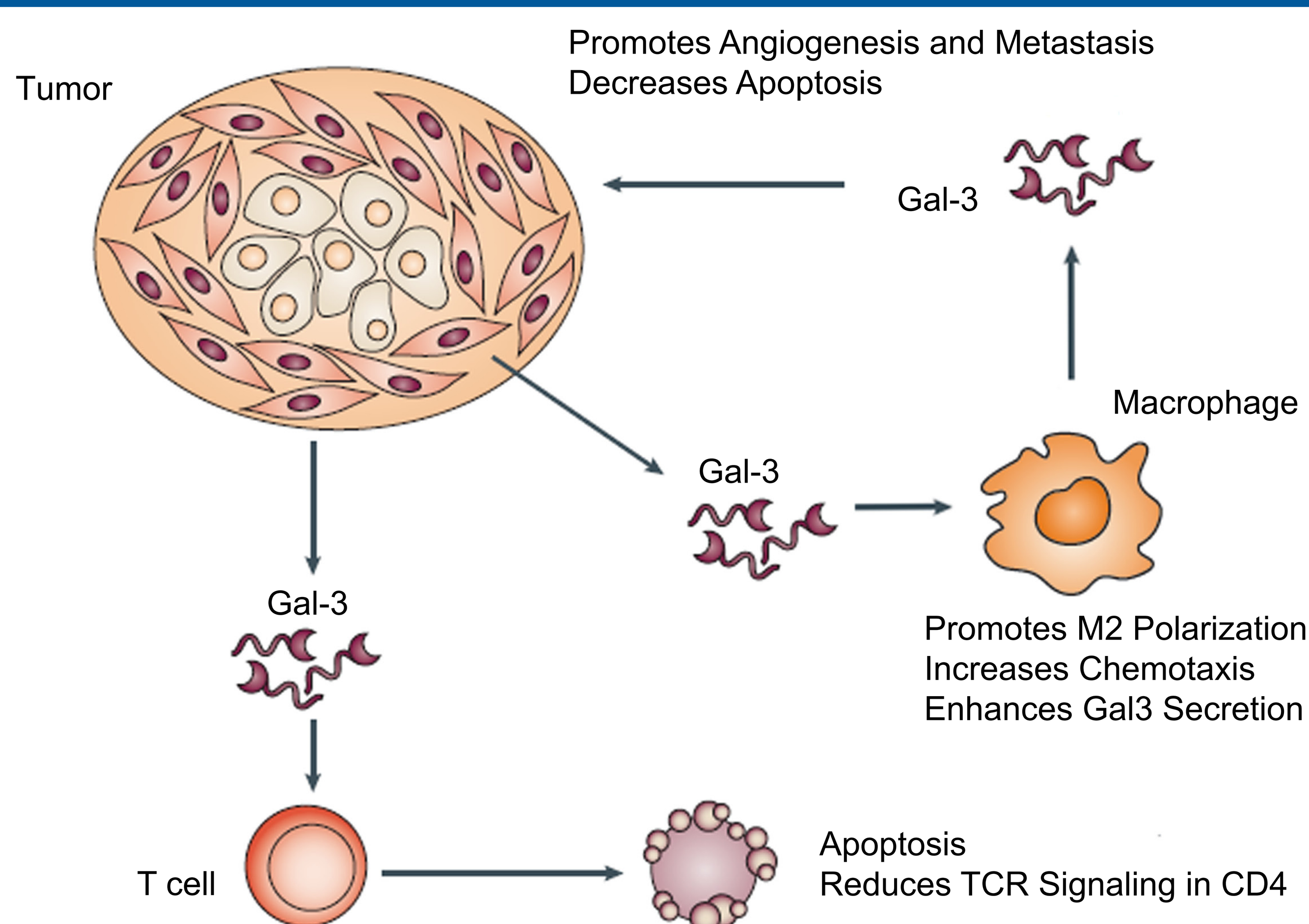
ABSTRACT

Immunosuppression and reduced cytolytic function of tumor infiltrating lymphocytes are major obstacles to creating effective therapies for patients. Galectin-3 (Gal3), a lectin family member, is expressed in numerous cancers including breast and prostate. Moreover, it is expressed ubiquitously by prostate epithelia, macrophages, and activated lymphocytes. Endogenous Gal3 promotes alternative macrophage activation and limits TCR-mediated CD4 T cell activation, which limit antitumor immunity. However, the regulatory effects of Gal3 on inflammation and CD8 T cell function remain unknown. We hypothesized that Gal3 within the tumor microenvironment promotes tumor progression by negatively regulating the function of CD8 T cells. To test this, we first examined the effects of endogenous Gal3 deletion in CD8 T cells. In vivo, antigen-specific Gal3-/- CD8 T cells exhibited decreased effector function (decreased proliferation, granzyme B, IFN-gamma, and IL-2) compared to wildtype controls. We also analyzed differential gene expression in antigen-specific Gal3-/- or Gal3+/+ CD8 T cells and found that granzyme B, CD25, KLRG-1, and Blimp-1 were reduced in Gal3-/- CD8 T cells as compared to controls. In vitro studies demonstrated that antigen-specific Gal3-/- CD8 T cells had a significant reduction in CD25 and OX40 expression. Interestingly, Gal3 inhibition in vivo augmented CD8 T cell expansion and CD62L expression, suggesting dual roles for Gal3 in CD8 T cell function. Future studies will examine Gal3 inhibition directly on CD8 T cell function and macrophage polarization within the tumor microenvironment and on tumor growth in prostate tumor-bearing mice.

BACKGROUND



Adapted from Rabinovich and Liu, Nat Rev Imm, 2005.

RESULTS

Figure 1. Phenotype comparison of naive Galectin-3 deficient CD8 T cells vs. WT CD8 T cells by flow cytometry.

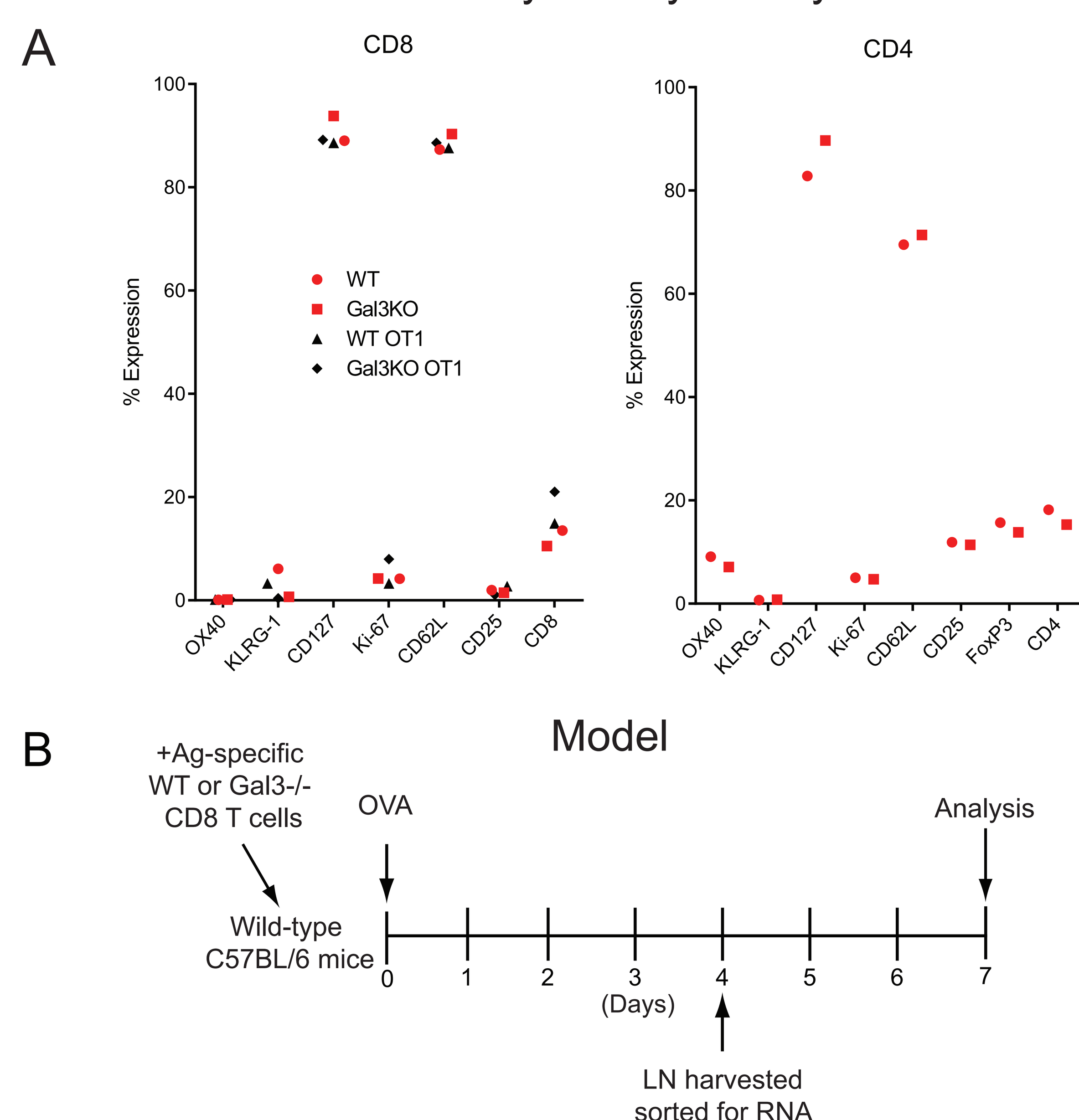


Figure 1. A) Baseline expression of phenotypic markers on naive CD8 and CD4 in WT, Gal3-/-, WT OT-1, or Gal3-/- OT-1 mice was assessed on untreated splenocytes. For CD8 and CD4, the % expression listed is the % of Live cells expressing either CD8 or CD4. B) Model. Wild-type C57BL/6 mice received 3x10e6 naive WT or Gal3-/- OT-I CD8 T cells (iv) on day-1. Donor OT-I T cells were stimulated with 500 mcg soluble OVA (sq; d0).

RESULTS

Figure 2. Galectin-3 deficient CD8 T cells exhibit reduced effector function following antigen stimulation in vivo.

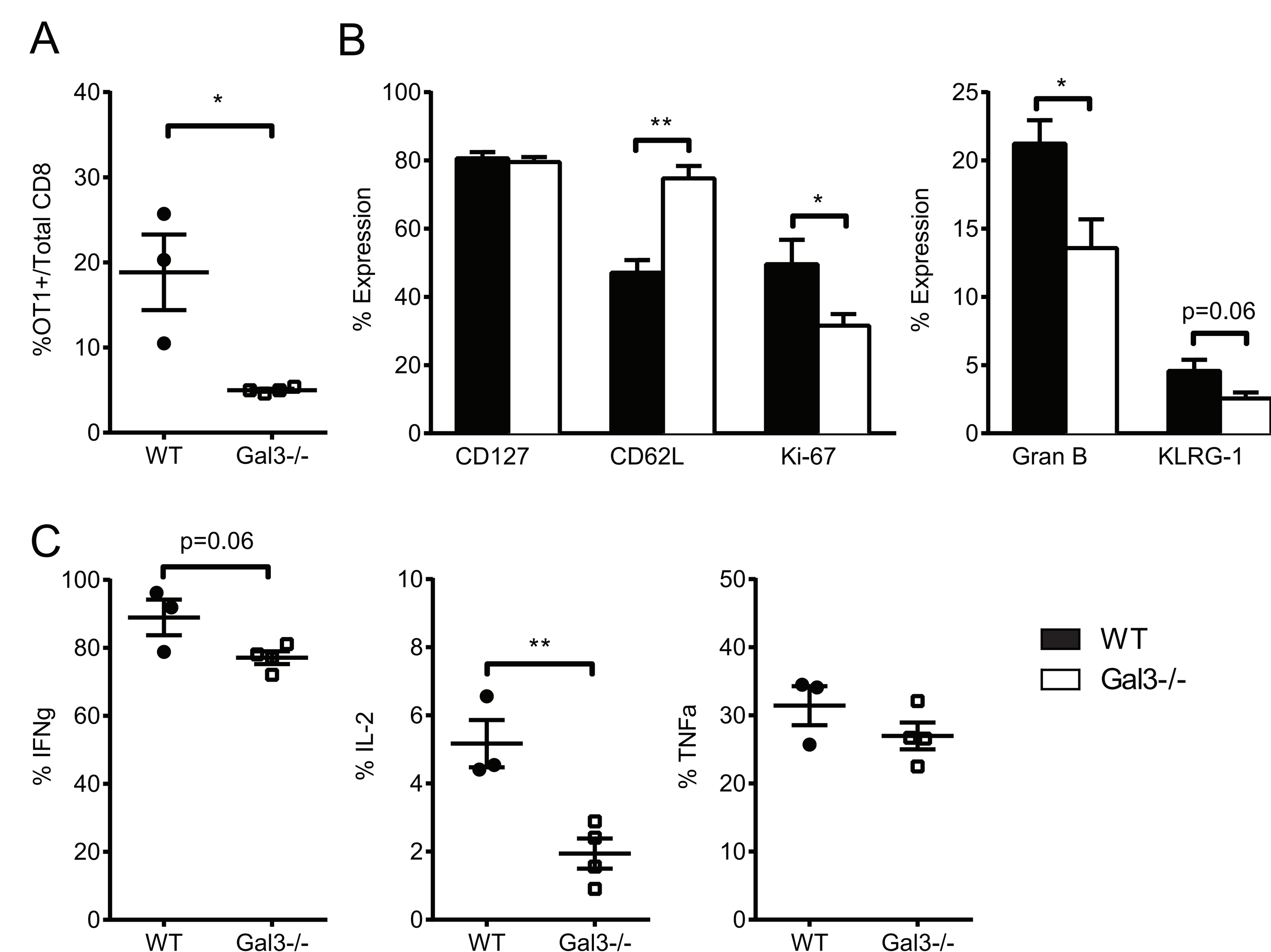
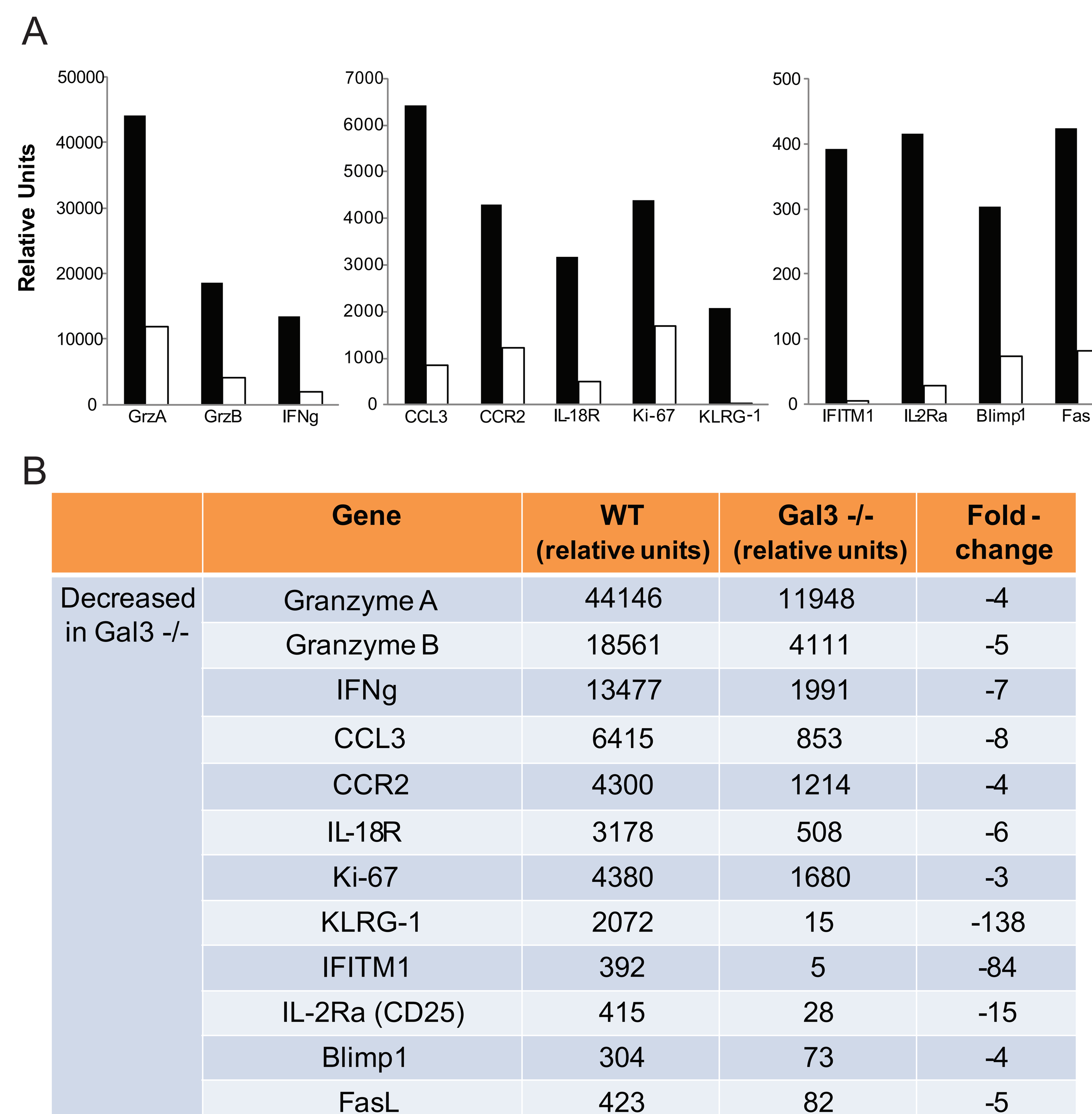


Figure 2. A-C) The phenotype of donor OT-I T cells in the spleen was determined by flow cytometry on day 7 post-stimulation. Graphs depict the mean from individual mice (n=4/group) from 1 of 3 independent experiments. *P<0.05; **P<0.01

Figure 3. Selected genes down-regulated in Galectin-3 deficient CD8 T cells



	Gene	WT (relative units)	Gal3 -/- (relative units)	Fold - change
Decreased in Gal3 -/-	Granzyme A	44146	11948	-4
	Granzyme B	18561	4111	-5
	IFNg	13477	1991	-7
	CCL3	6415	853	-8
	CCR2	4300	1214	-4
	IL-18R	3178	508	-6
	Ki-67	4380	1680	-3
	KLRG-1	2072	15	-138
	IFITM1	392	5	-84
	IL-2Ra (CD25)	415	28	-15
	Blimp1	304	73	-4
	FasL	423	82	-5

Figure 3. A, B) Wild-type or Gal3-/- OT-1 T cells were adoptively transferred into wild-type hosts and then stimulated with OVA. Four days later, lymph nodes were harvested and donor CD8 T cells were purified by cell sorting (as seen in Fig. 1A). RNA was extracted from these cells and changes in gene expression were assessed using Affymetrix DNA microarray. A) Graphical representation of several genes found to be down-regulated in Gal3-/- OT-1 over WT. B) Relative units and fold change are shown for selected genes.

RESULTS

Figure 4. Galectin-3 deficient CD8 T cells have reduced CD25 and OX40 expression following antigen stimulation

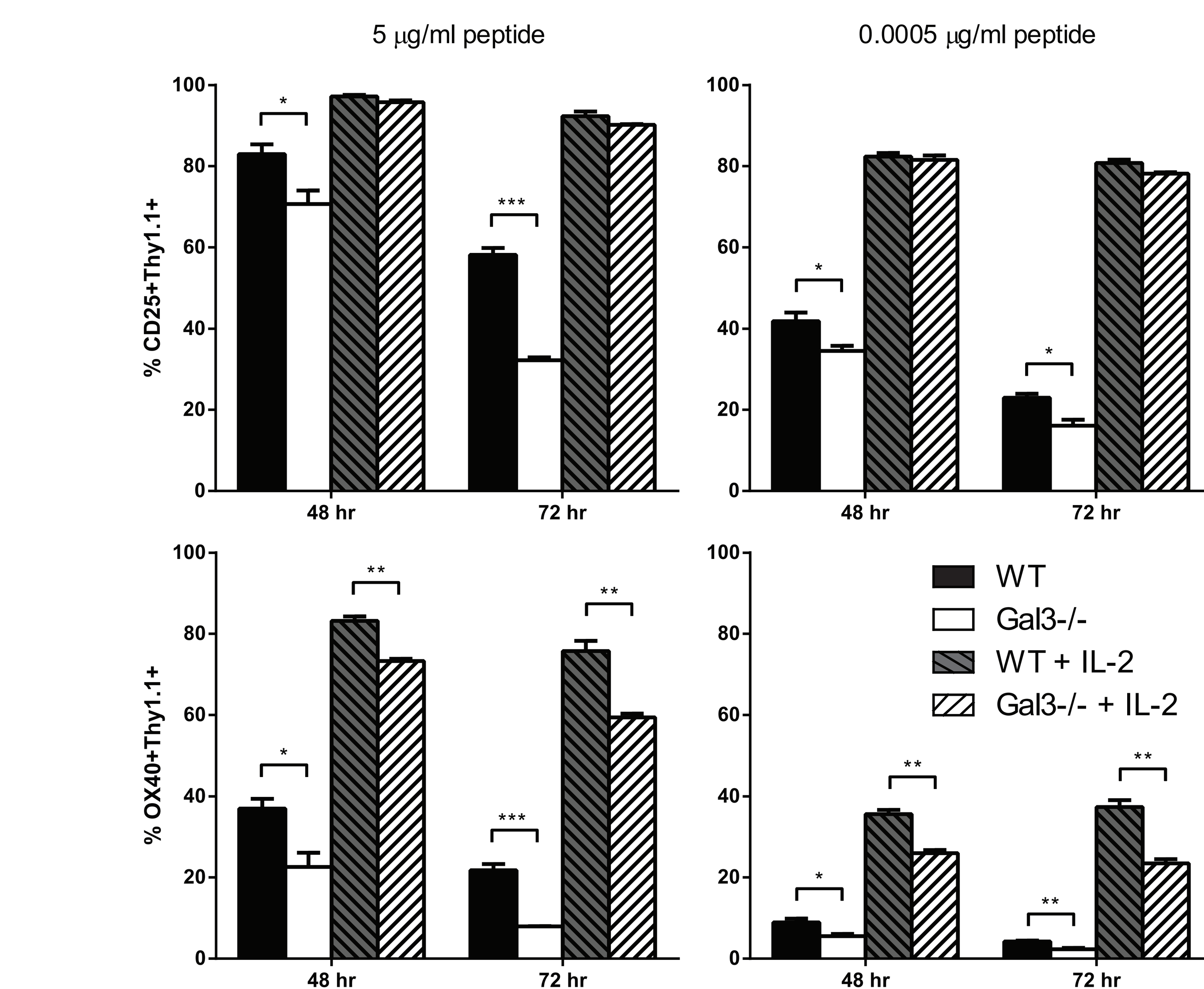


Figure 4. In vitro, naive purified wild-type or Gal3-/- OT-1 CD8 were stimulated with peptide-pulsed (either 5 or 0.0005 micrograms/ml) DC2.4 dendritic cells with or without IL-2 (100 ng/ml). Cells were harvested at 48 or 72 hrs later to examine expression of CD25 (IL-2Ra) or OX40 by flow cytometry.

Figure 5. Galectin-3 inhibition augments CD8 T cell effector function in vivo.

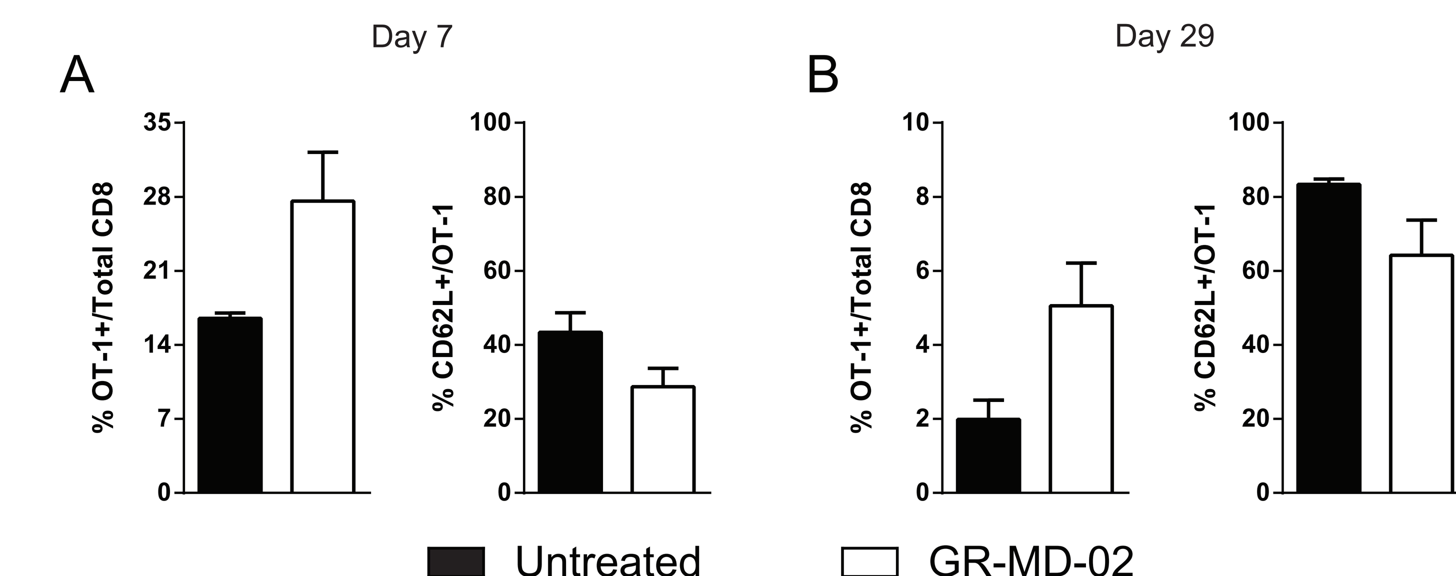


Figure 5. Wild-type C57BL/6 mice received 3x10e6 naive WT OT-I CD8 T cells (iv) on day-1. Donor OT-I T cells were stimulated with 500 mcg soluble OVA (sq; d0). Mice were either untreated (black bars) or administered a Gal-3 inhibitor, GR-MD-02 (white bars) (sq; d0, 1). Seven (A) or 29 (B) days later the phenotype of donor cells in the peripheral blood or spleen, respecitvcely, was determined by flow cytometry. There were no differences between groups for expression of Ki-67, Granzyme B, or KLRG-1.

CONCLUSIONS

- Endogenous Gal3 deficiency decreased CD8 T cell proliferation and activation in response to antigen, and decreased cytokine production
- Gal3 deficient CD8 T cells have reduced KLRG-1, CD25, IFNg, granzyme B, and FasL, which are all increased in effector CD8
- Gal3 deficient CD8 T cells have reduced CD25 and OX40 expression in vitro, and CD25 expression can be rescued by the addition of IL-2, while OX40 expression cannot
- Gal3 inhibition in vivo enhances CD8 T cell proliferation and activation in response to antigen.

ACKNOWLEDGEMENTS

- Dan Haley, Jennifer Wilk, Tacy Brotherton, Amanda Lyon
- V Foundation Scholar Award
- NIH/NCI Pathway to Independence Award
- Safeway Foundation
- Providence Portland Medical Foundation