SUPPORTING INFORMATION

Title: Bystander activation of iNKT cells occurs during conventional T cell alloresponses

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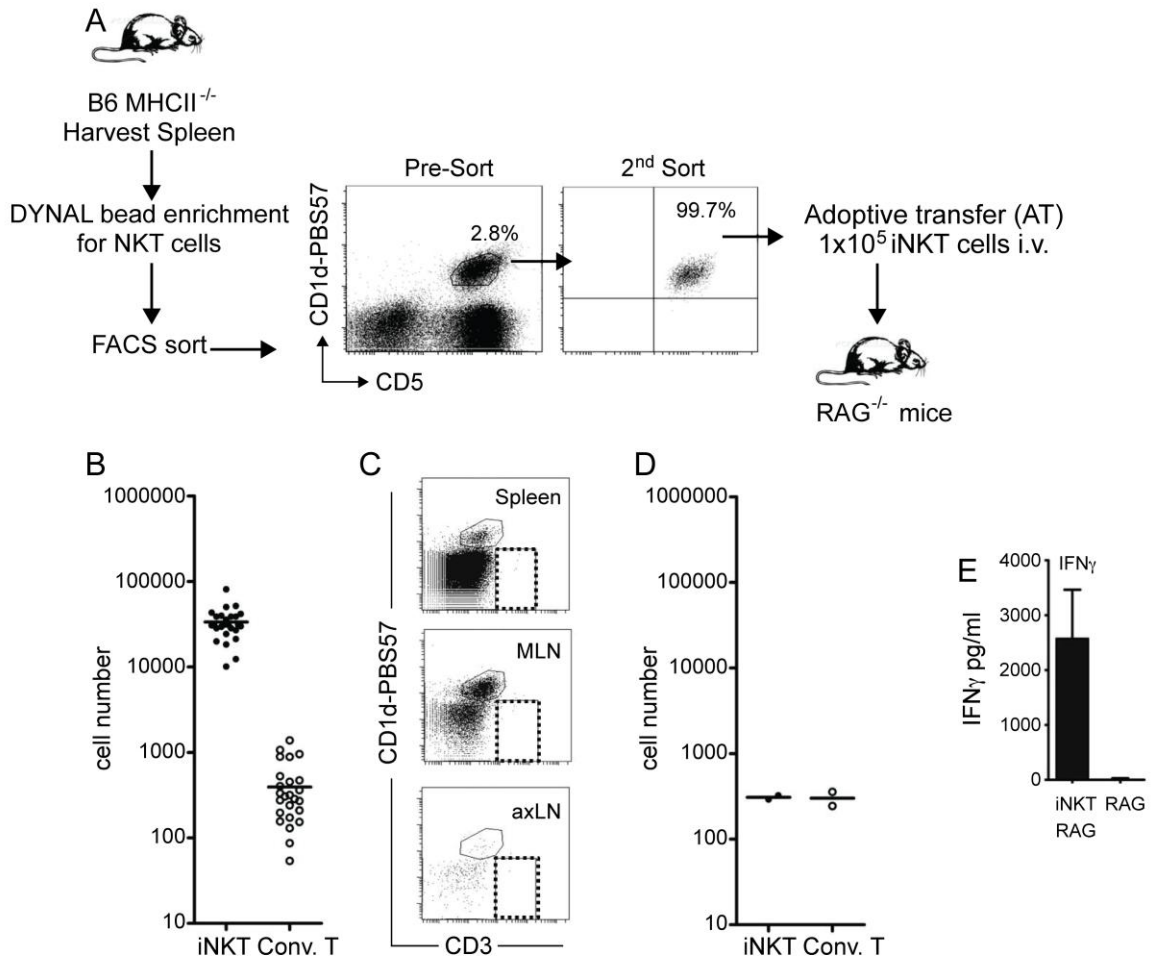
Running Title: Alloreactive T cells can mediate iNKT cell activation

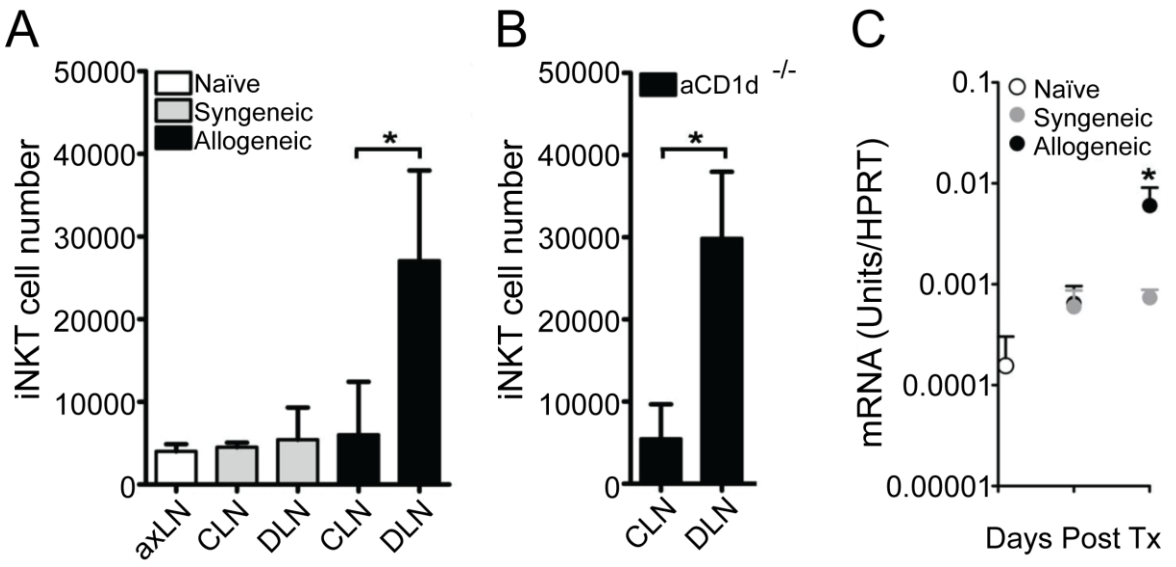
Keywords: iNKT cells, Allorecognition, Bystander Activation, Transplantation

Supporting Information: 2 figures

Supporting Figures



S1. Experimental plan and analysis to generate iNKT-RAG mice. MHCII$^{-/-}$ mice were used as a source of splenocytes and DYNAL enrichment for iNKT cells (A). iNKT cells were sorted and adoptively transferred into RAG$^{-/-}$ mice (B). Mice received either no α-GalCer (n=2; D) or an injection of α-GalCer on day+1 and +14 (n=27; B), relative to adoptive transfer. All mice were sacrificed 30-80 days after adoptive transfer and the spleen (B, D), mesenteric lymph node (C) and axillary lymph nodes (C) analysed for the presence of iNKT cells or conventional T cells. A cohort of iNKT-RAG were injected with α-GalCer at day +30 and serum analysed for the presence of IFNγ 12 hours later (n=3, E). *Dots represent individual animals (B/D). Data in E represented as the mean IFNγ concentration ± s.d.*



S2. iNKT cells accumulate at sites of T cell priming following transplantation. iNKT cell ($CD3^+CD1d\text{-}PBS57^+$) numbers were assessed on day 10 following skin transplantation of syngeneic and allogeneic skin grafts in the draining axillary lymph node (DLN) and compared to those in the non-draining contralateral axillary lymph node (CLN) and untreated mice (axLN) by flow cytometry (n=3-5/group; A). The influence of allogeneic CD1d on iNKT cell recruitment was determined using skin grafts that lacked CD1d (n=3; $aCD1d^{-/-}$) and the number of iNKT cells in the CLN and DLN assessed 10 days post transplant (B). The recruitment of iNKT cells to grafts following skin transplantation was determined using qRT-PCR on day 5 and 10 using syngeneic and allogeneic donors (n=3/group; C). Data in A and B shown as mean iNKT cell number ± *s.d. Data in C shown as the mean mRNA expression (Units/HPRT) ± s.d. *p=<0.05.*