Insulin B9-23 is a diabetes-initiating epitope for human immune systems

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Introduction

- InsB:9-23 is a MHC class II-restricted antigen recognized by the majority of insulin-reactive T cell clones and serves as a key autoantigenic target in NOD mice (*Simone, et al. PNAS 1997; Nakayama, et al. Nature 2005*).

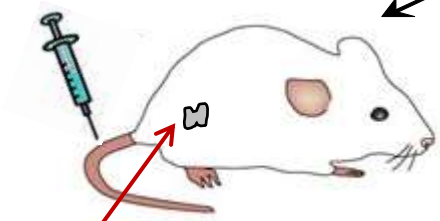
- T cell response to InsB:9-23 peptide is also highly associated with T1D in humans, but direct evidence for its role in destruction of pancreatic β cells in humans is lacking (*Alleva, et al. JCI 2001; Yang, et al. PNAS 2014; Michels, et al. Diabetes 2016*).

- **Goal**: to determine the potential of InsB:9-23-specific human CD4 T cells to induce diabetes in humanized mice.

HiRN
HUMAN ISLET RESEARCH NETWORK

1-3 Gy

CD34⁺ FLCs
(i.v.)

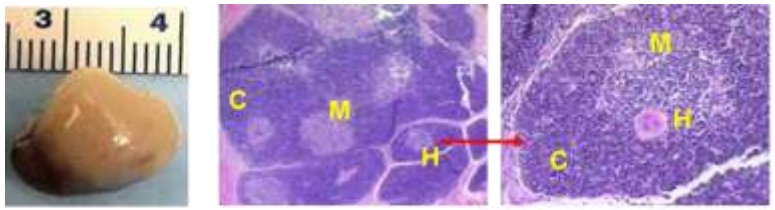


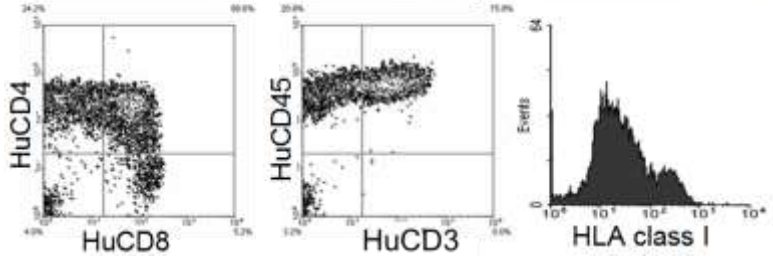
Thy/~~Liv~~ tissue
(kidney capsule)

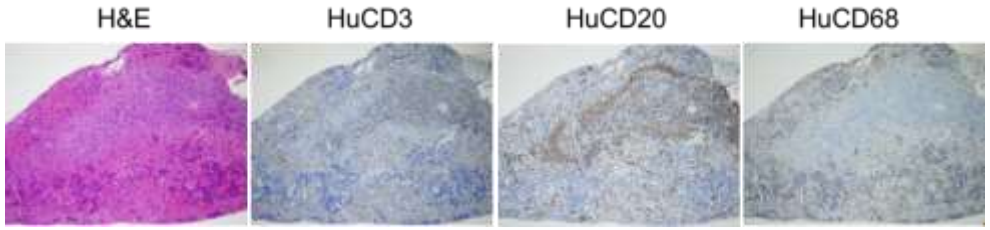
NOD/SCID
or NSG

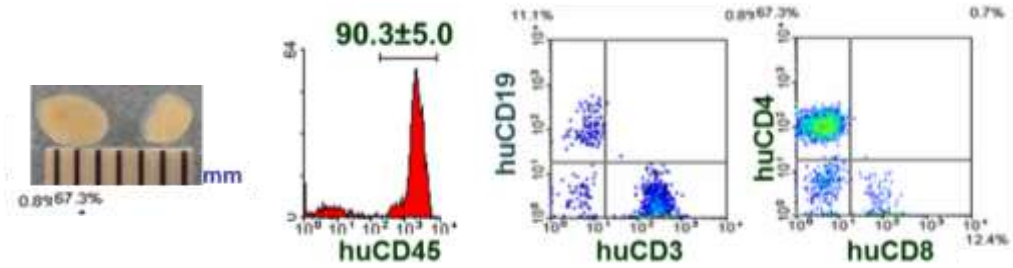
McCune JM, Science 1988)

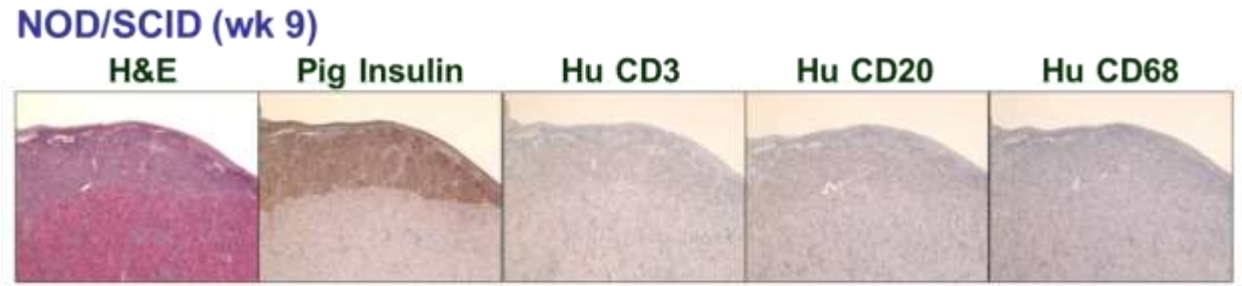
Lan P, et al. Blood 2004; Blood 2006

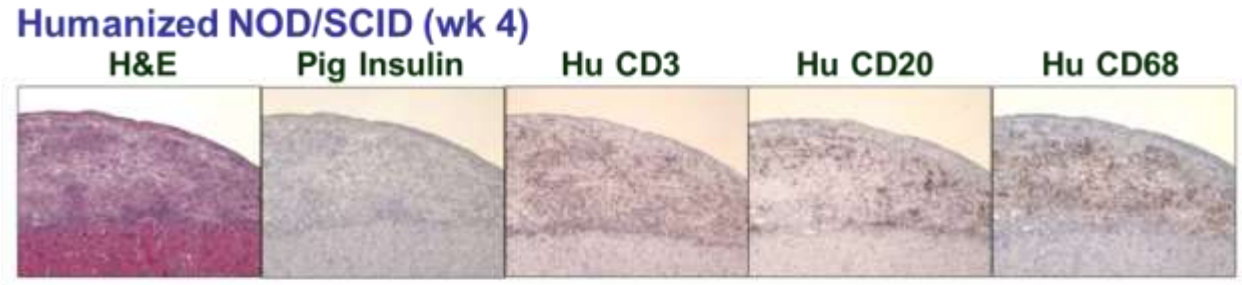




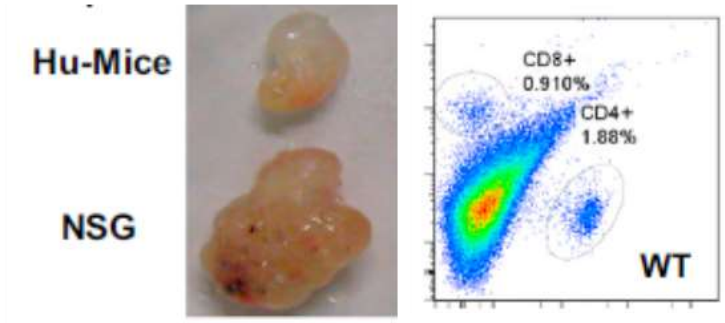


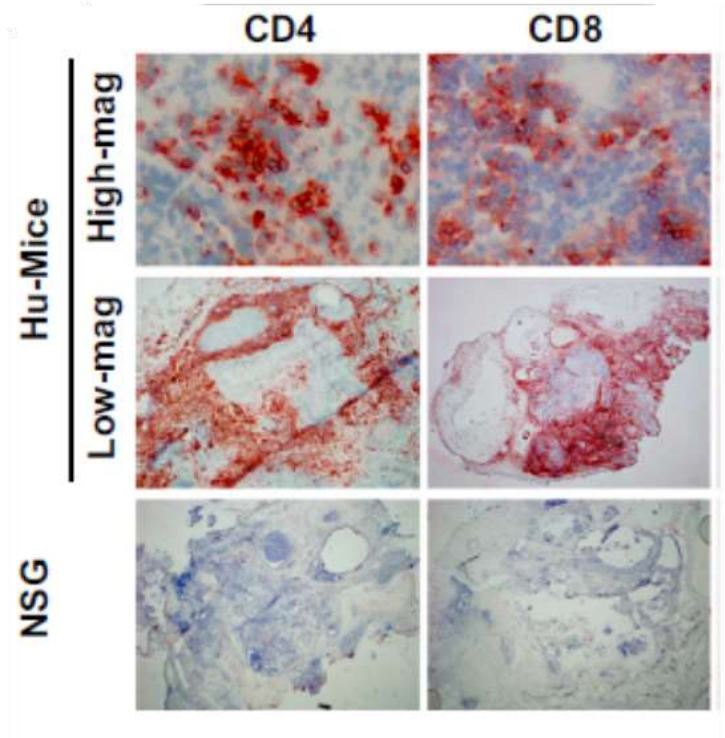






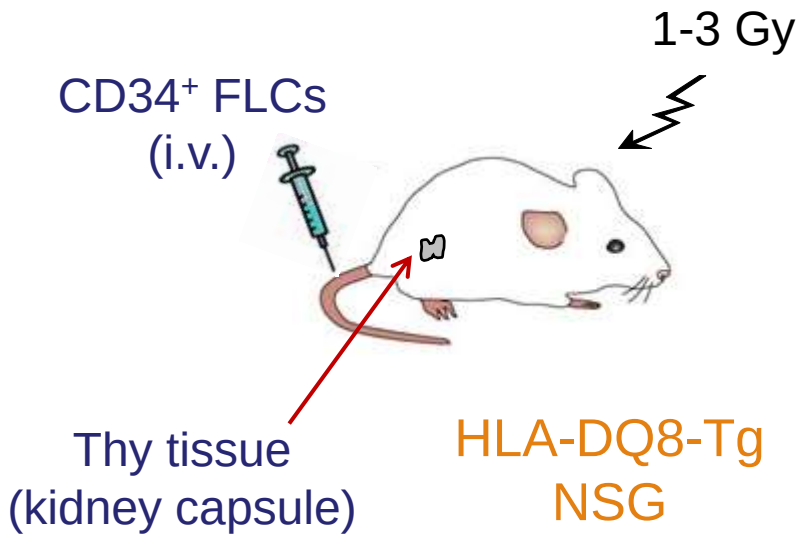
Tonomura et al, *Xenotransplantation* 2008





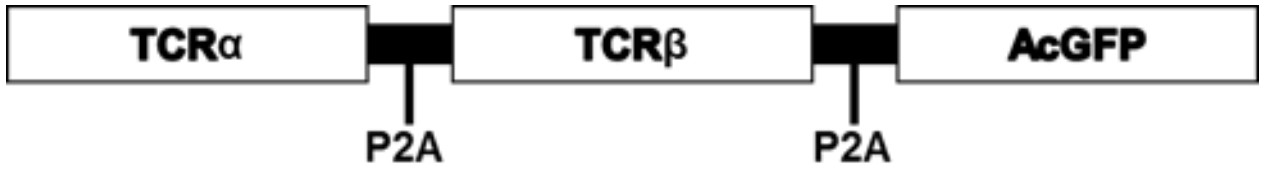
Rong et al, *Cell Stem Cell* 2014
Zhao et al, *Cell Stem Cell* 2015

Experimental Design



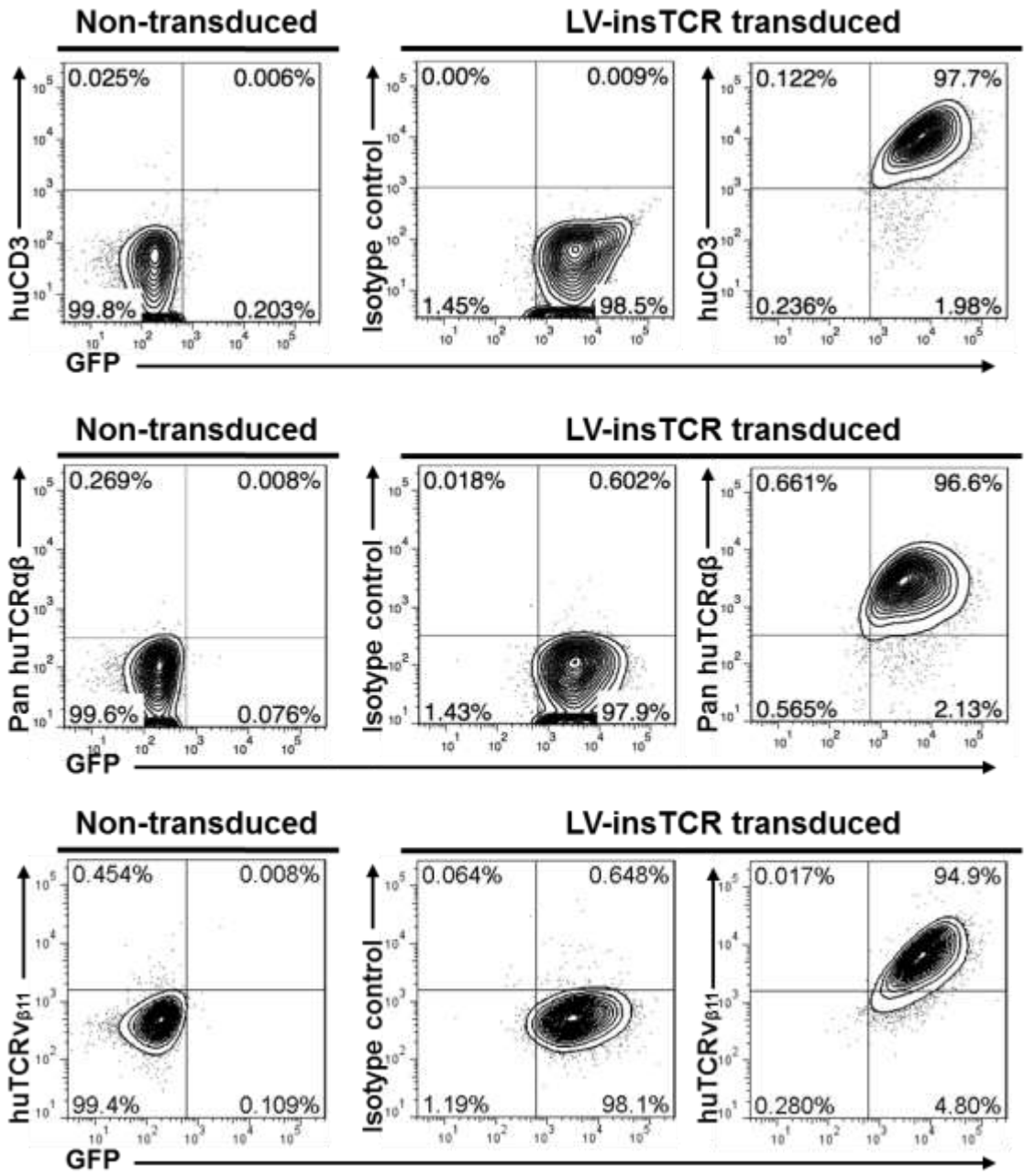
1. Human T cells from hu-mice
2. Lentiviral transduction to express InsB:9-23-specific TCR
3. Ex vivo expansion
4. Infusion to conditioned hu-mice (with an autologous immune system) to determine diabetogenic potential

Schematic of the LV-insTCR vector

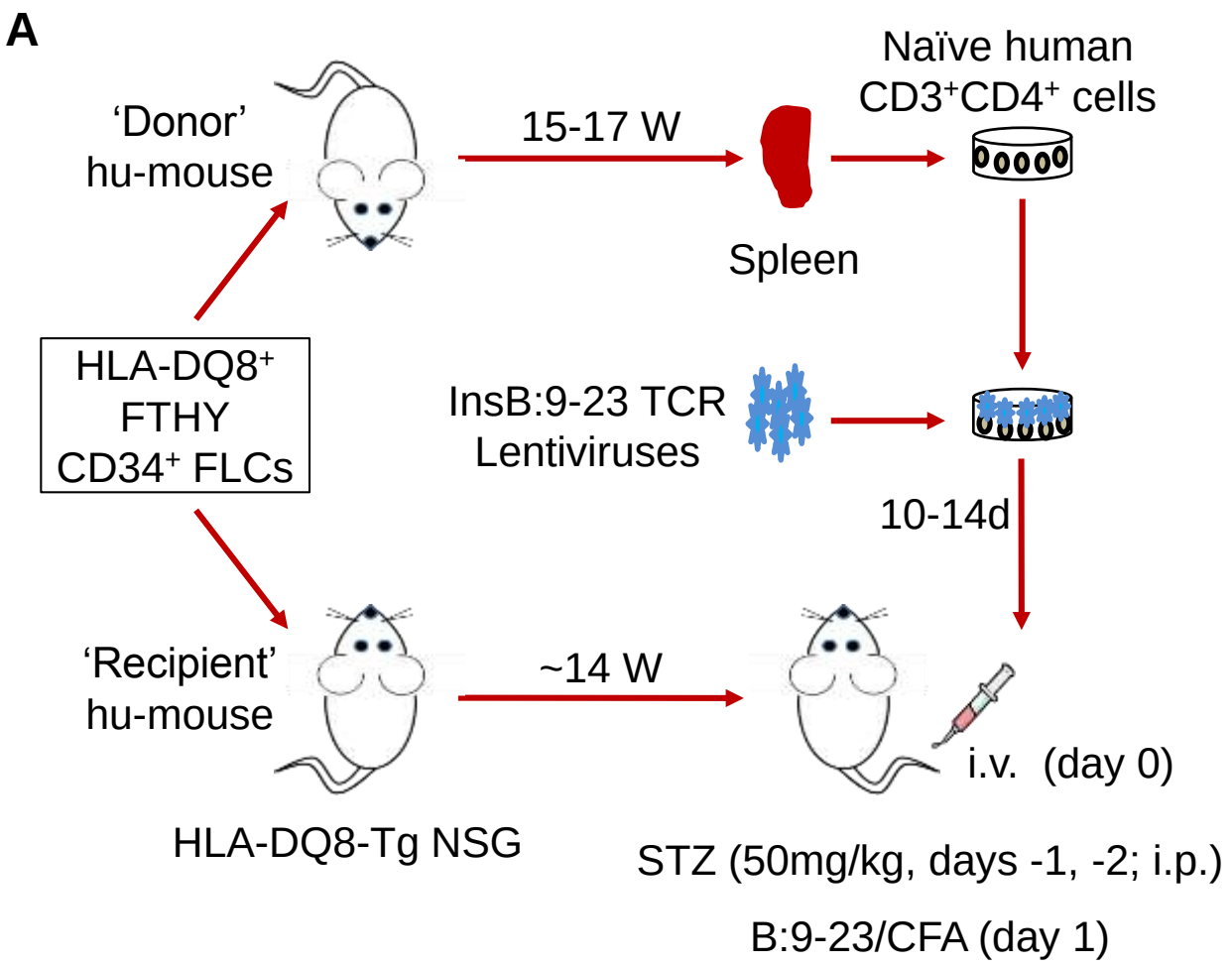


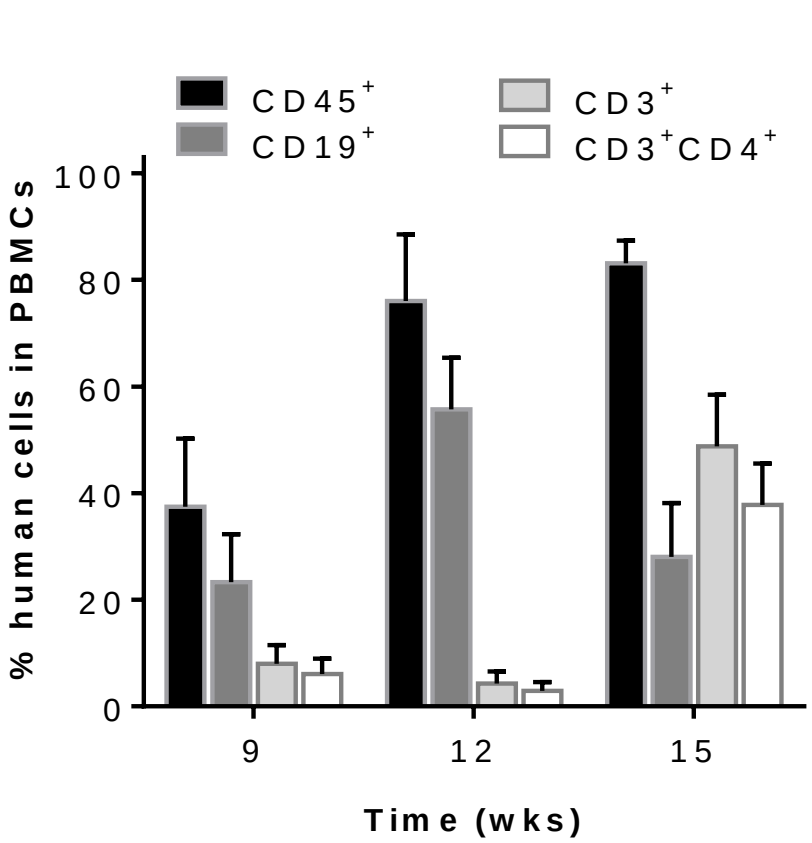
- InsB:9-23-specific T cell line (clone #5) was established from a HLA-DQ8/8 homozygous male patient with T1D (Bart Roep);
- The **TCRα ($V_{α21}$)**-P2A-**TCRβ ($V_{β11}$)**-P2A-**GFP** gene fragment was constructed and cloned into a lentiviral vector, pRRLSIN under MSCV promoter (LV-insTCR).

Transgenic TCR expression in transduced TCR-deficient T cell line cells



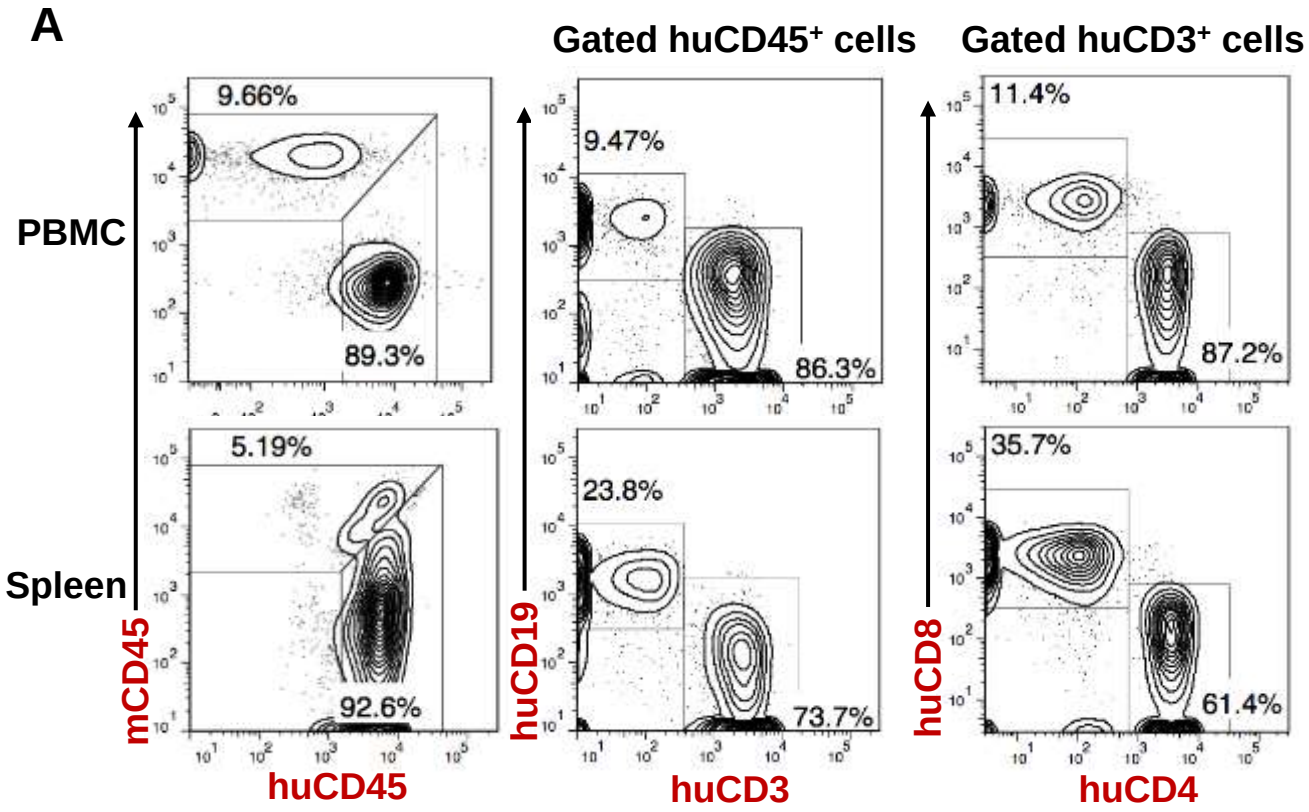
Preparation of hu-mice for generating InsB:9-23-specific T cells (“donor”) and for induction of diabetes (“Recipient”)



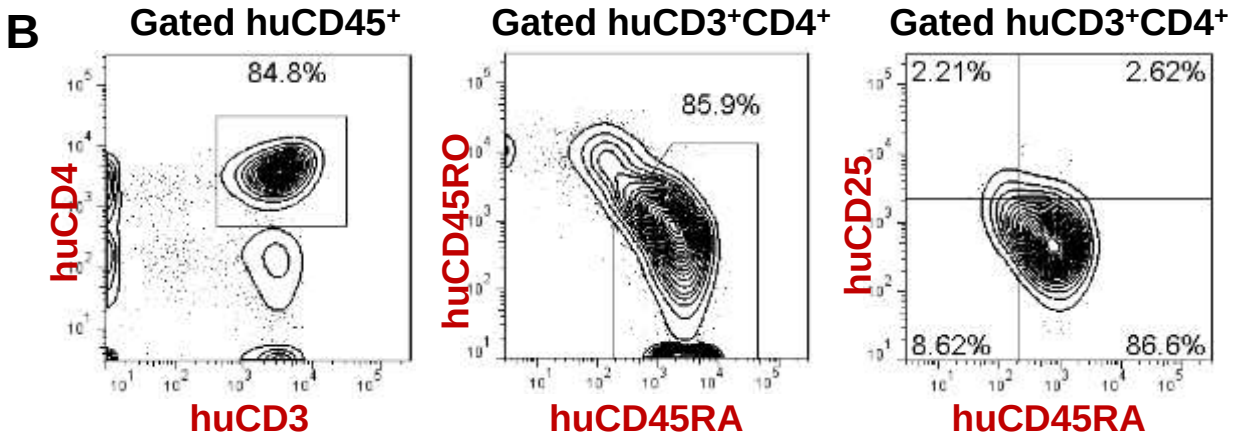


Purification and lentiviral transduction of hu-mouse-derived human $CD3^+CD4^+CD45RO^-CD25^-$ naïve T cells

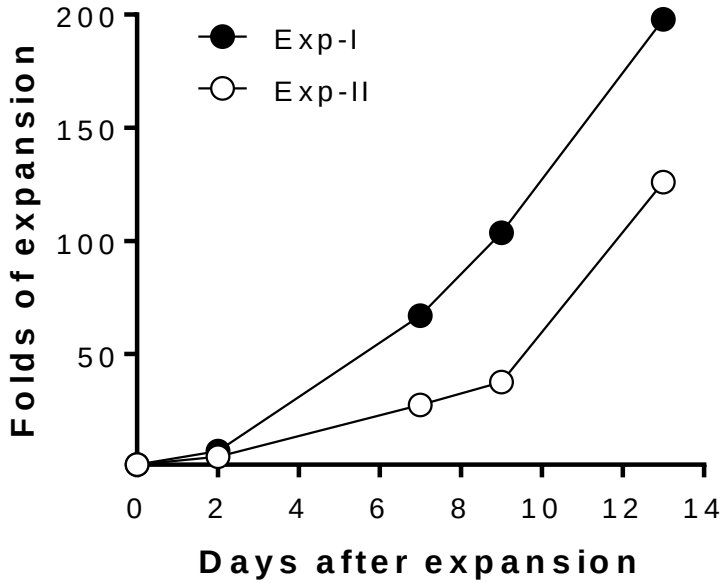
Human Immune cell reconstitution in hu-mice



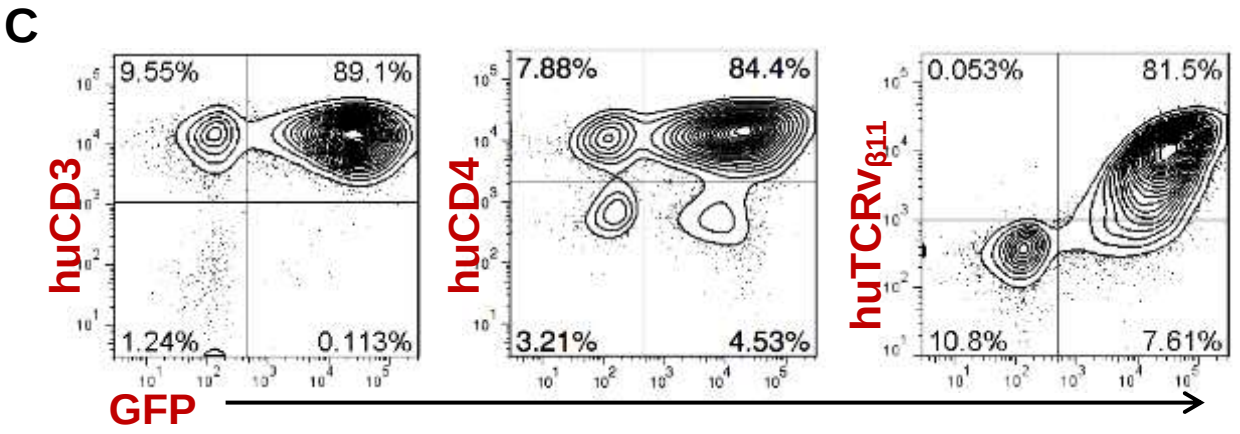
MACS-selected splenic naïve $huCD4^+$ T cells



Expansion of lentivirally transduced human CD4⁺ T cells in vitro



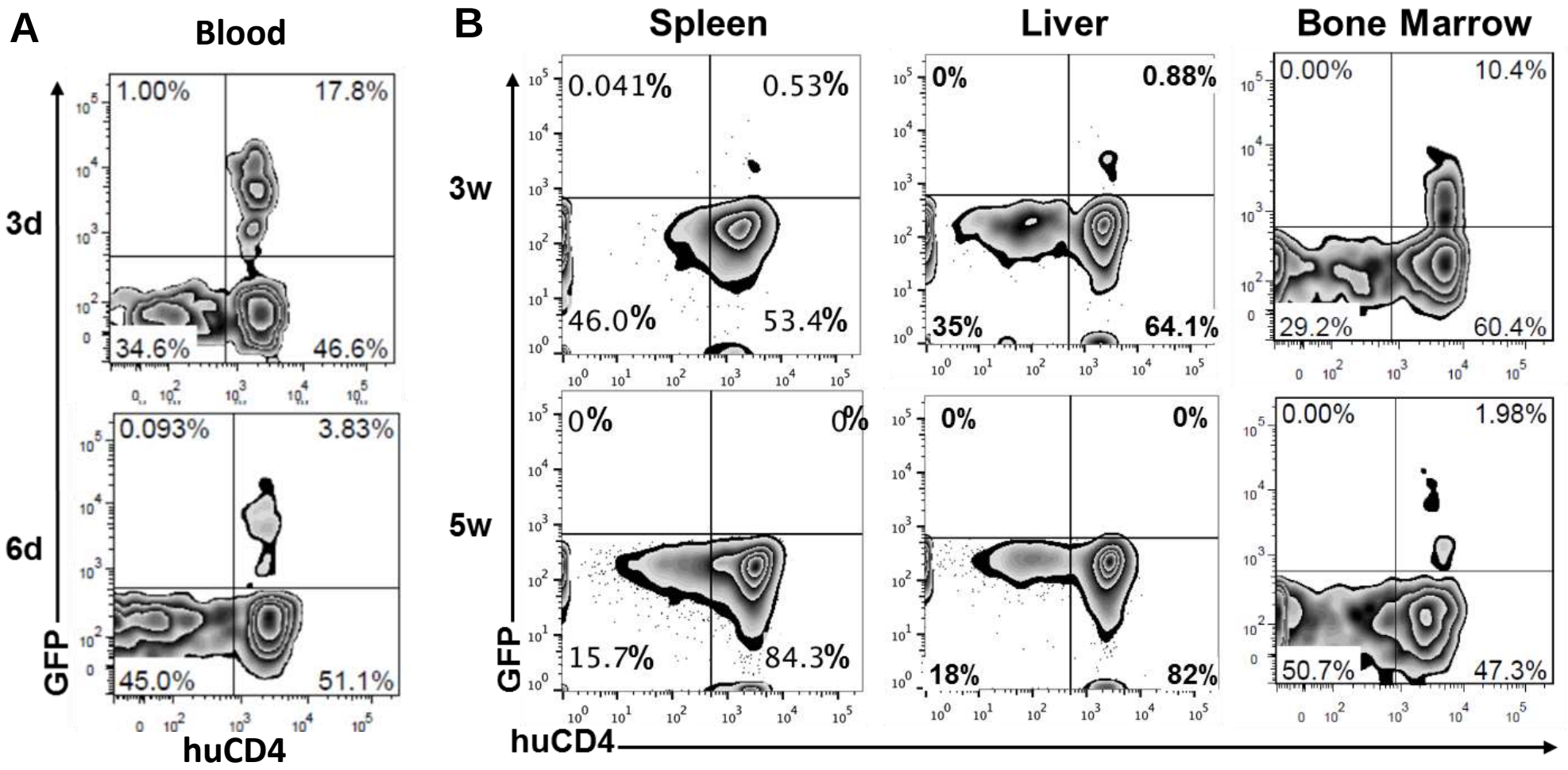
CD3, CD4 and TCRvβ11 expression on *ex vivo* expanded LV-insTCR-transduced GFP⁺ cells



10% AB serum, irradiated feeder cells, recombinant human cytokines (20U/ml IL-2, 10ng/ml IL-7, 10ng/ml IL-15), plus PHA (1.5µg/ml) or anti-CD3 mAb (OKT3; 30ng/ml)

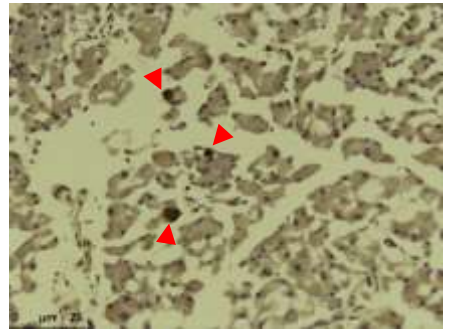
Induction of diabetes in huFTHY/CD34 FLC-grafted HLA-DQ8-Tg hu-mice conditioned by STZ and InsB:9-23 peptide immunization

Survival of infused GFP$^+$CD4$^+$ T cells

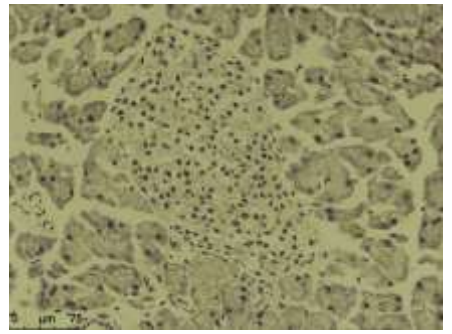


C

LV-Ins-TCR

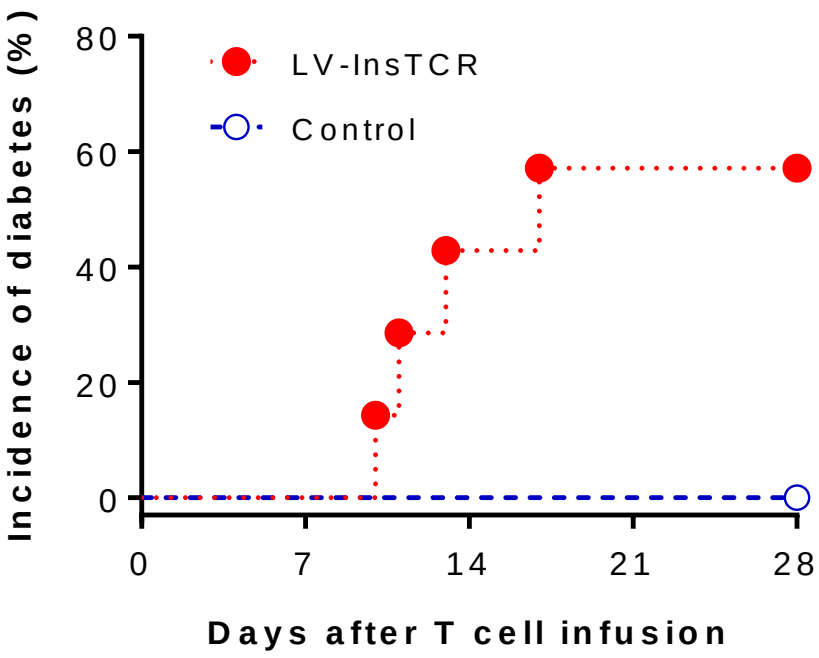


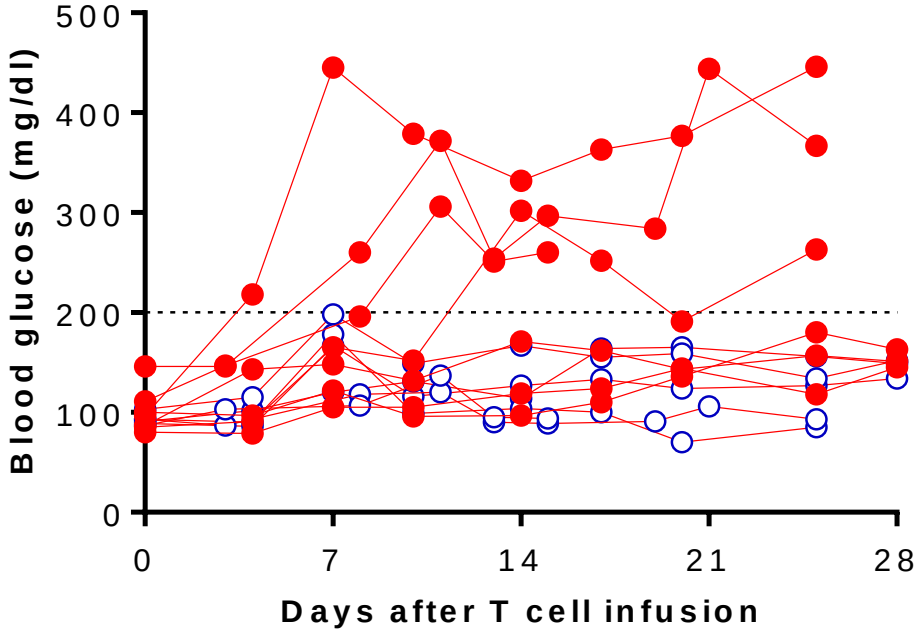
Control T cells



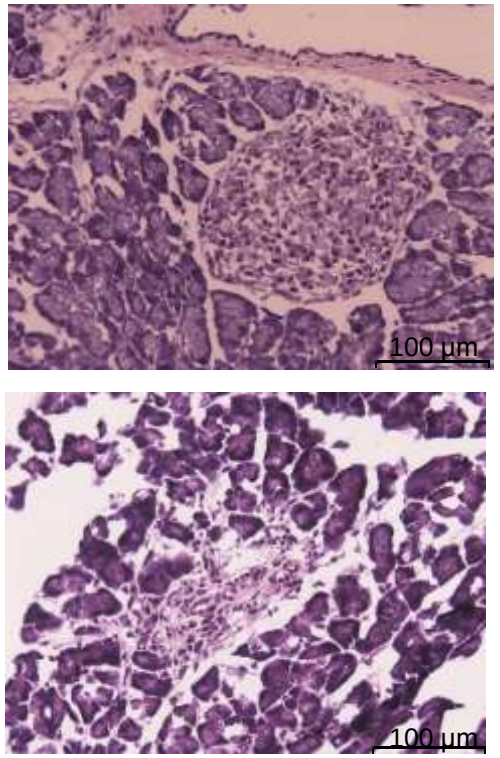
LV-insTCR^{+} T cells induce diabetes in huFTHY/CD34 FLC-grafted HLA-DQ8-Tg hu-mice conditioned by STZ and InsB:9-23 peptide immunization

A





B



Summary & conclusion

- **Induction of T1D in hu-mice in the absence of allo- or xeno-GVHR:** Streptozotocin-conditioned HLA-DQ8-Tg hu-mice develop hyperglycemia and diabetes following transfer of autologous huCD4 T cells expressing HLA-DQ8/InsB:9-23-specific TCR and immunization with InsB:9-23;
- **APCs** are important in facilitating the survival, expansion and phenotypic conversion of human T cells in hu-mice when xeno-GVH reactivity is absent;
- **Peptide immunization** may play an important role in activating the transferred InsB:9-23-reactive human CD4 T cells (Type B clone);
- **Endogenous human immune cells** may also contribute to T1D development in hu-mice receiving IsnB:9-23-TCR+ T cells;
- **The data suggest an important pathogenic role** of CD4 T cell responses to the InsB:9-23 epitope in T1D induction in humans;
- **The hu-mouse model offers an useful tool** for assessing the diabetogenic potential of human T cells.

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Nakayama Lab

Maki Nakayama

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Survival of infused human T cells and blood glucose levels in DQ8-Tg hu-mice (CD34+ FLCs only) and NSG mice following infusion of human CD4 T cells