Humanized models to study immunity and to accelerate the development of new solutions for human health

HelmholtzZentrum münchen
German Research Center for Environmental Health

A humanized mouse model to study insulin-specific tolerance and islet autoimmunity

Carolin Daniel, Ph.D.
Research Group leader, Immune tolerance in diabetes
Institute of Diabetes Research

April 28th, 2017

Les Pensières
Fondation Mérieux Conference Center
Veyrier-du-Lac - France

HELMHOLTZ ASSOCIATION

Helmholtz Diabetes Center

DZD
Deutsches Zentrum für Diabetesforschung

Incidence trends of childhood Type 1 diabetes

The incidence of childhood Type 1 diabetes is increasing worldwide at an alarming rate

Patterson CC et al., Lancet 2009, **Ziegler AG** et al., JAMA 2013

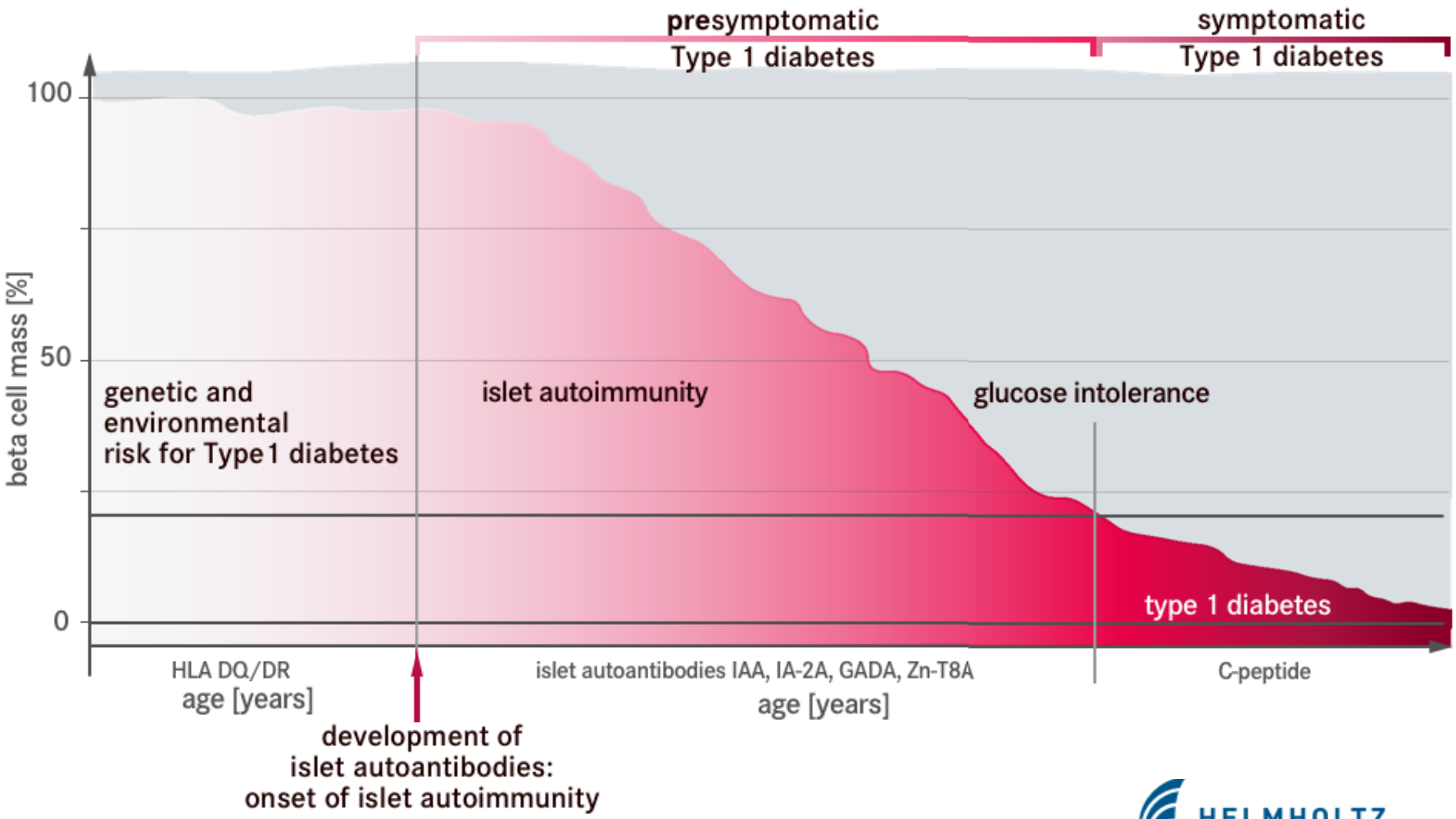
Nearly 90 000 children newly develop Type 1 diabetes each year

IDF Diabetes Atlas 2015, 7ed. Brussels, Belgium

The number of children affected with Type 1 diabetes is doubling every 12 years

IDF Diabetes Atlas 2015, 7ed. Brussels, Belgium,
Ziegler AG et al., JAMA 2013

Pathogenesis of autoimmune Type 1 diabetes

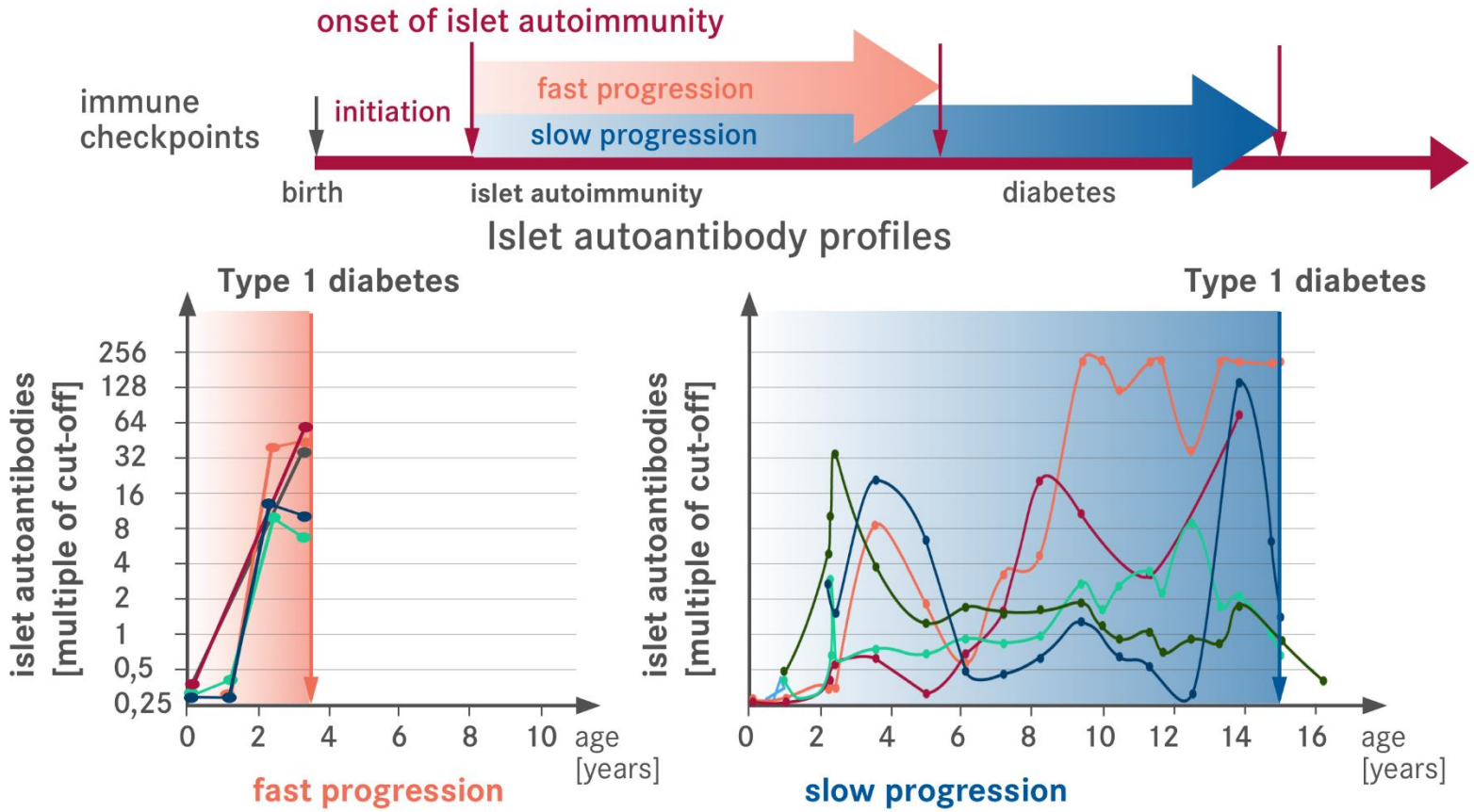


modified from Ziegler AG et al., JAMA 2013
Eisenbarth GS, N Engl J Med 1986
Atkinson MA, Eisenbarth GS, Lancet 2001, Lancet 2014

HelmholtzZentrum münchen
German Research Center for Environmental Health

HELMHOLTZ
ASSOCIATION

Progression from islet autoimmunity to clinical Type 1 diabetes

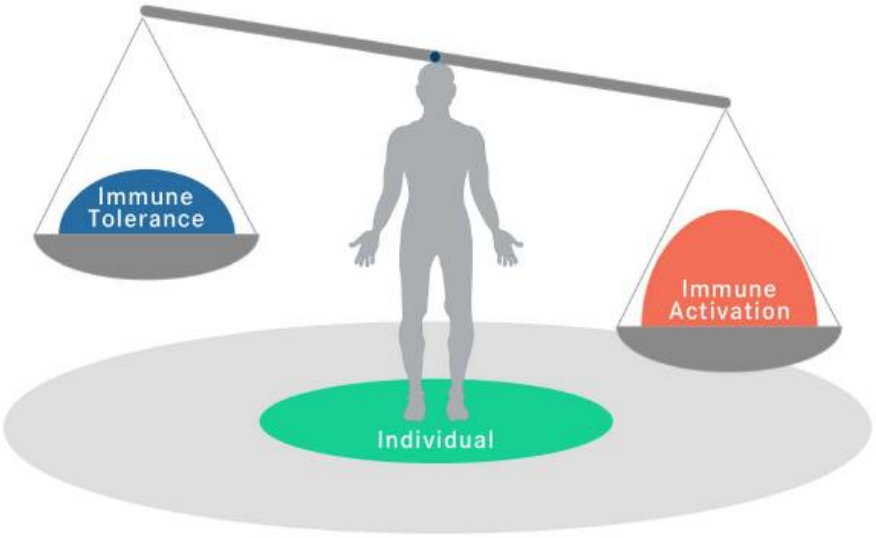


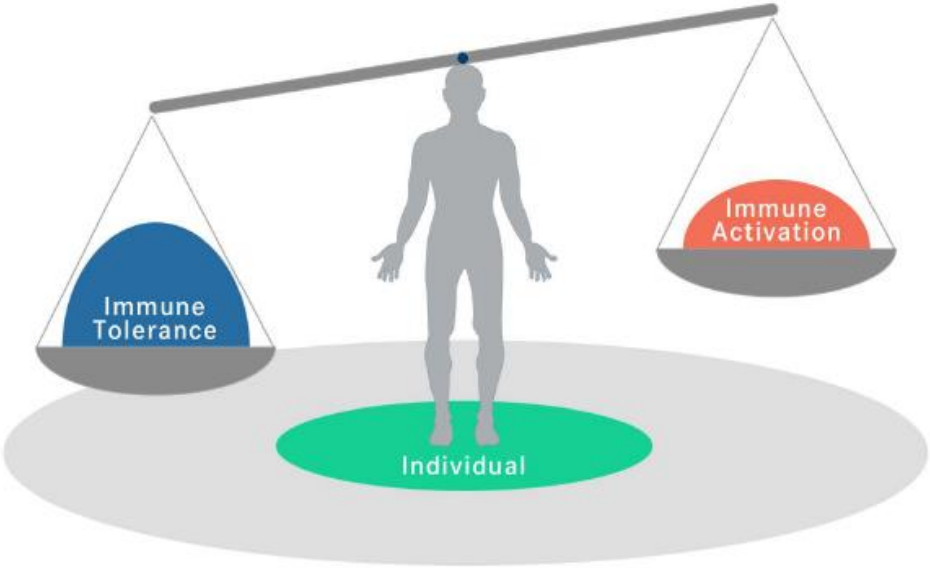
Plasticity in immune activation and impairments in immune tolerance

HelmholtzZentrum münchen
German Research Center for Environmental Health

HELMHOLTZ | ASSOCIATION

Restoration of immune tolerance in Type 1 diabetes

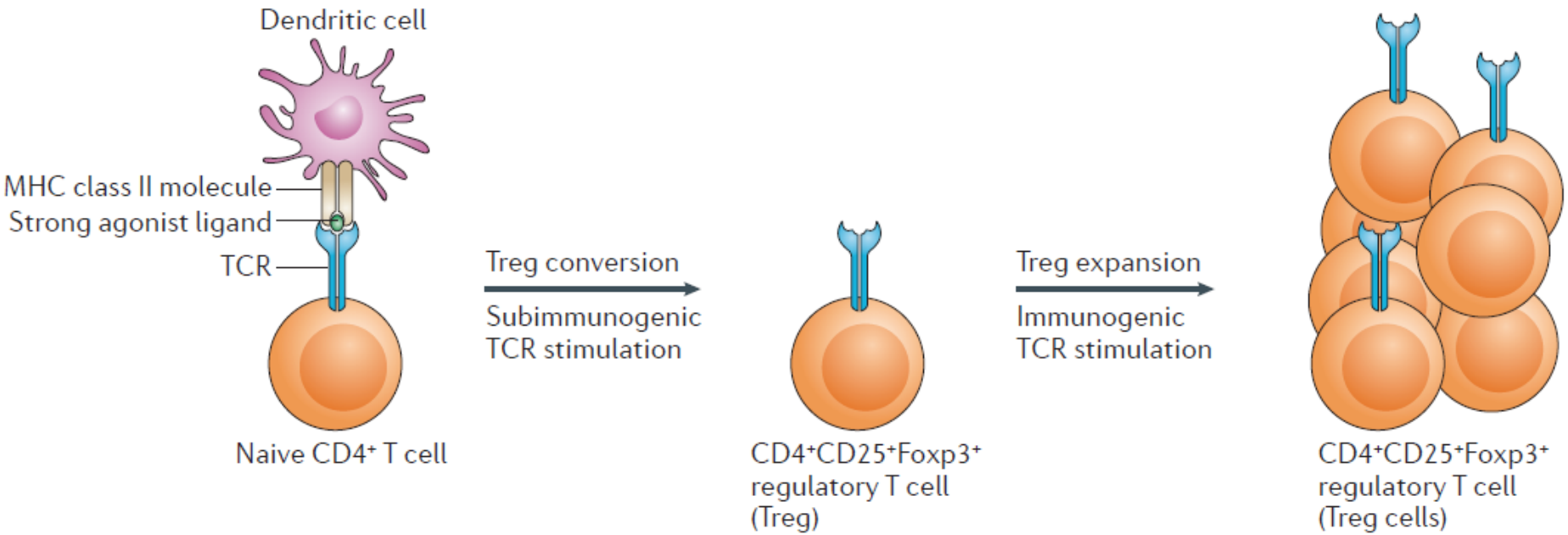




Mediators of tolerance:
Foxp3^{+} regulatory CD4^{+} CD25^{+} T (Treg) cells

Blue helmets
- peacekeeping troops -
of the immune system

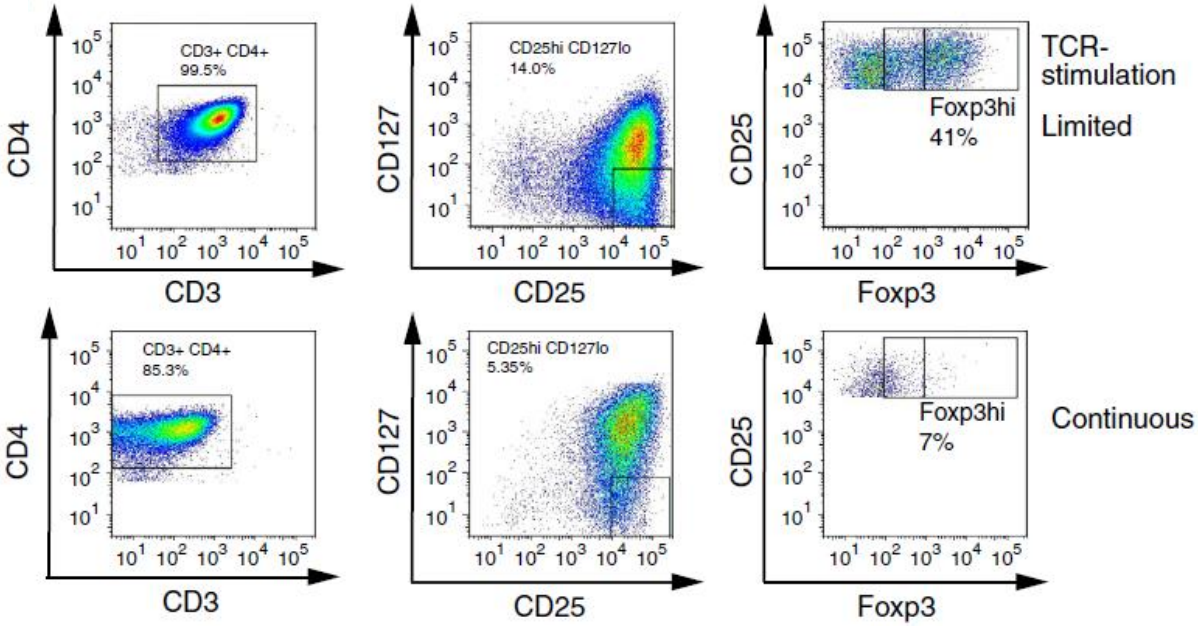
Murine antigen-specific Treg induction

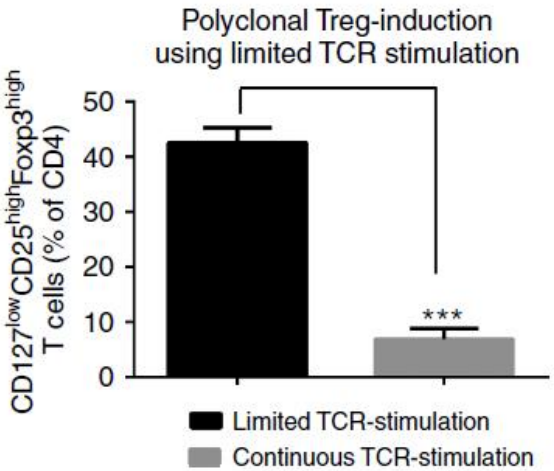


von Boehmer H and ***Daniel C***, *Nat Rev Drug Disc. 2013;* **Serr I** et al., *Nat Comm 2016*

Daniel C *et al., PNAS 2010;* ***Daniel C*** *et al., Meth in Mol Biol 2011;*
Daniel C *et al., J Exp Med 2011,* ***Daniel C*** *et al., Semin Immunol 2011*
Daniel C *et al., Adv Immunol 2011*

Human Treg induction using subimmunogenic TCR stimulation *in vitro*

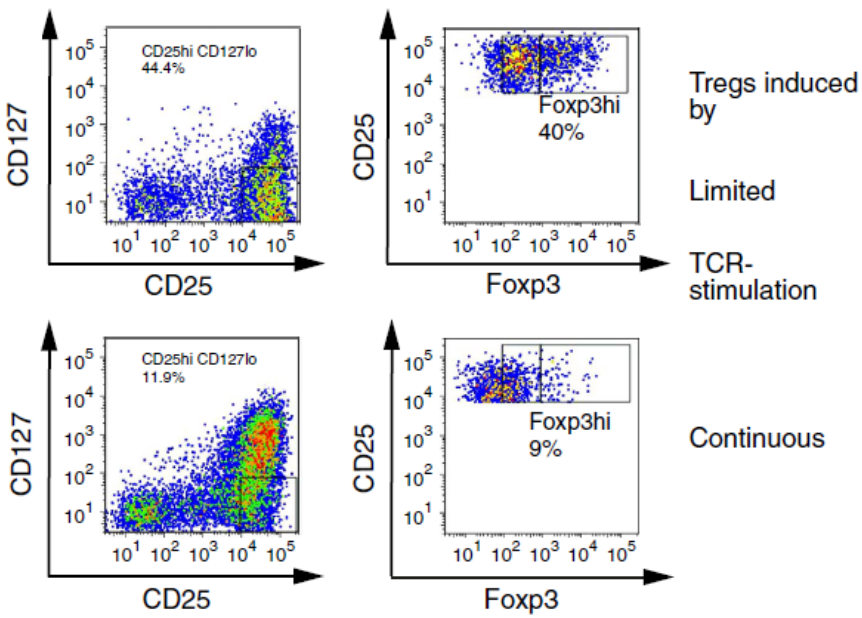


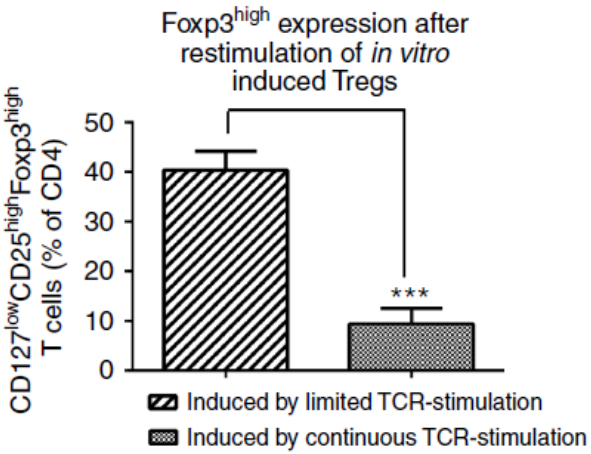


Serr et al., Nat Comm 2016

Human Treg induction using subimmunogenic TCR stimulation *in vitro*

Restimulation of human Tregs induced *in vitro*





Serr et al., Nat Comm 2016

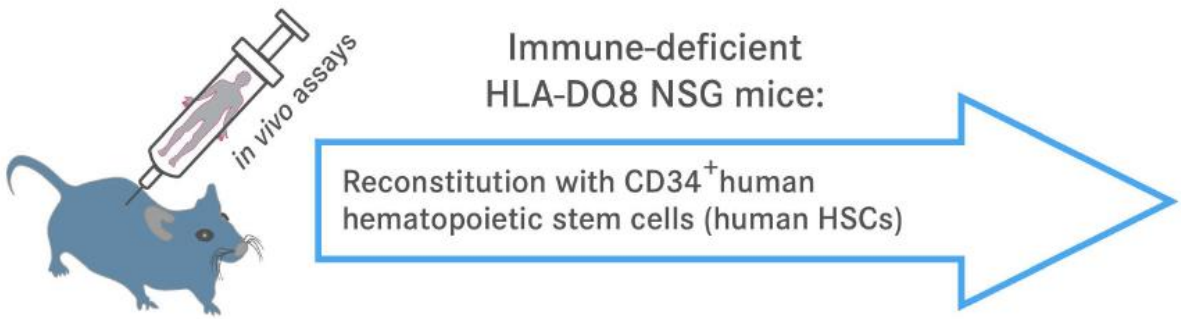
From mouse to man: Human antigen-specific Treg induction relevant for autoimmune Type 1 diabetes

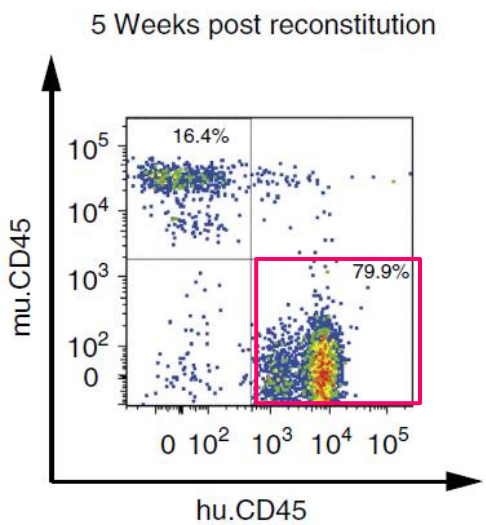
insulin autoantibodies are often the first to appear in children
→
insulin-specific autoimmunity present in the earliest stages of islet autoimmunity

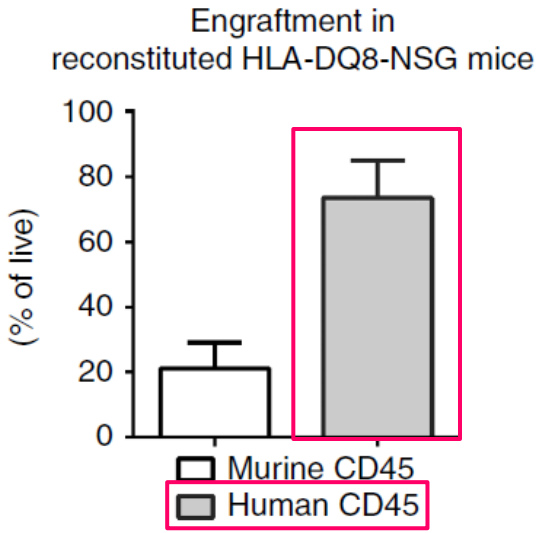
insulin epitopes function as a main target of the autoimmune attack
insulin B:9-23 in mice and humans

insulin B:9-23 epitopes are presented by human HLA-DQ8 to human $CD4^{+}$T cells

How to induce human Foxp3$^+$Tregs *in vivo*? Use of HLA-DQ8 Tg NSG mice



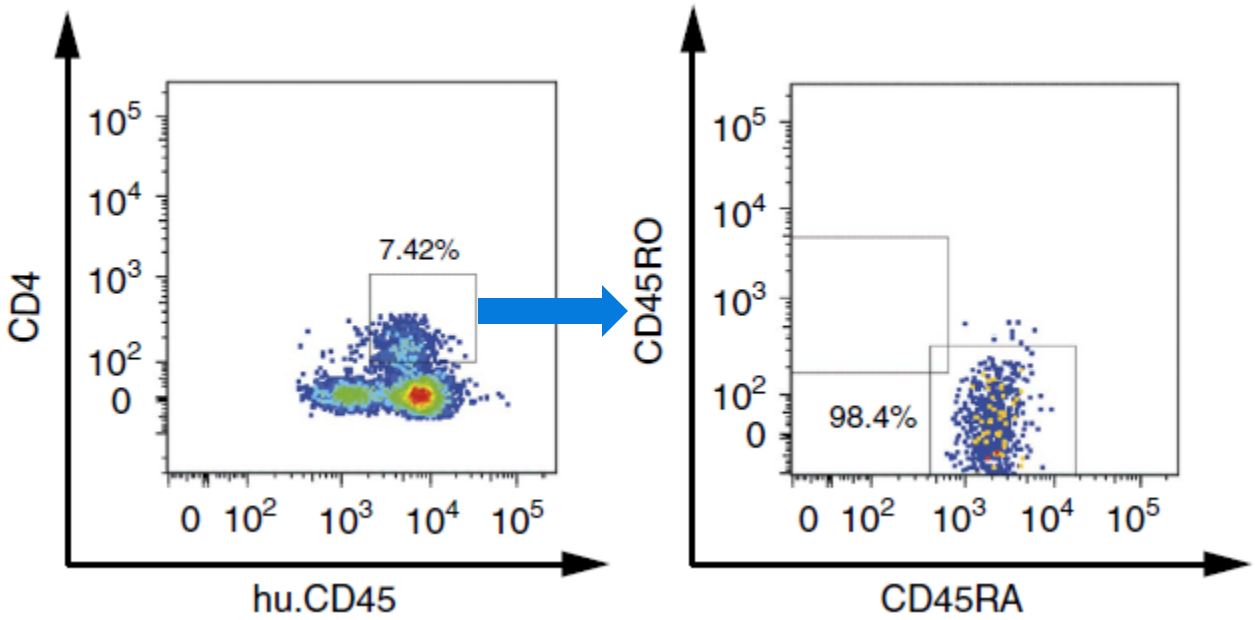




Serr et al., Nat Comm 2016

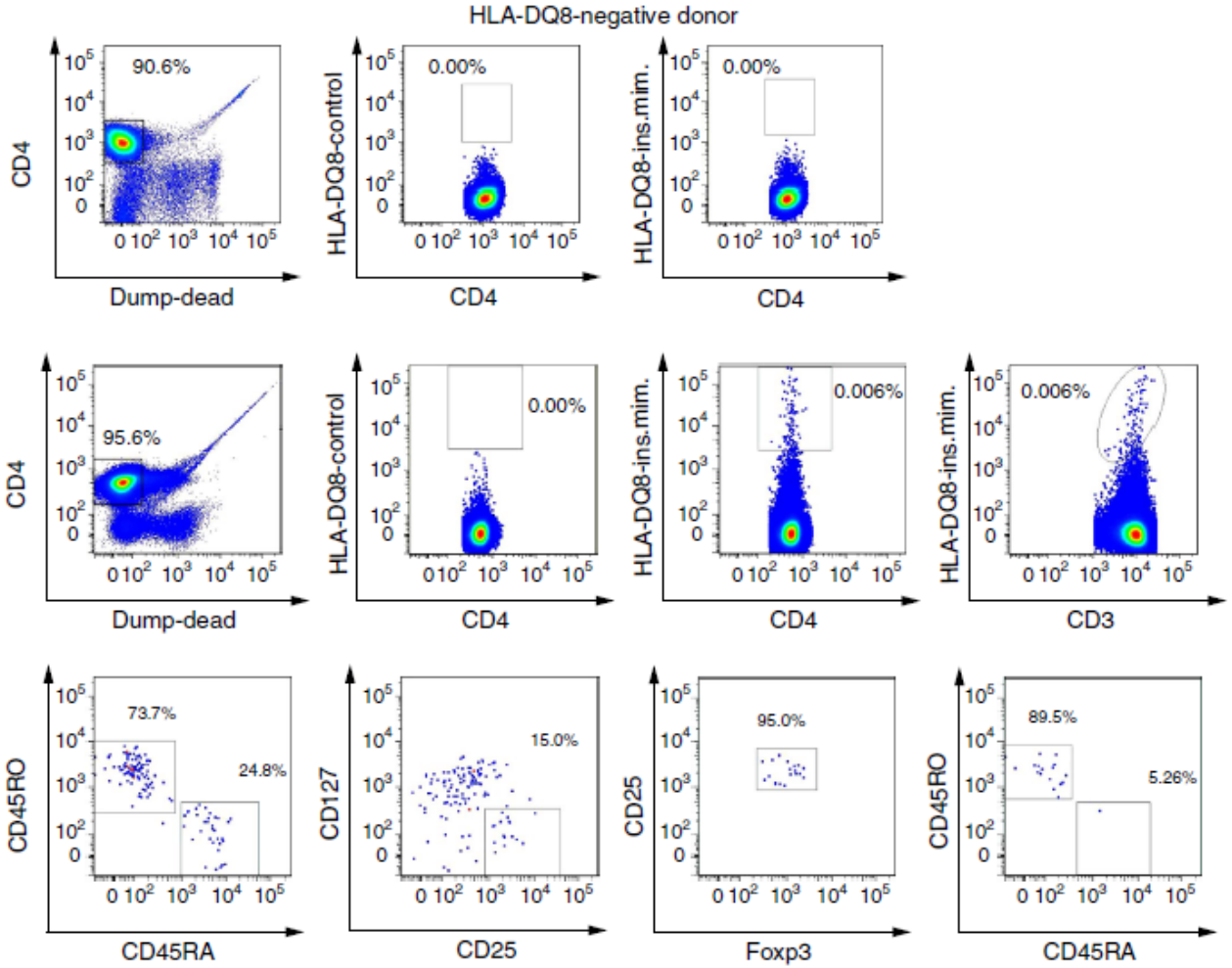
How to induce human Foxp3$^+$Tregs *in vivo*? Characterization of human CD4$^+$T cells

8 wks post reconstitution



Serr et al., Nat Comm 2016

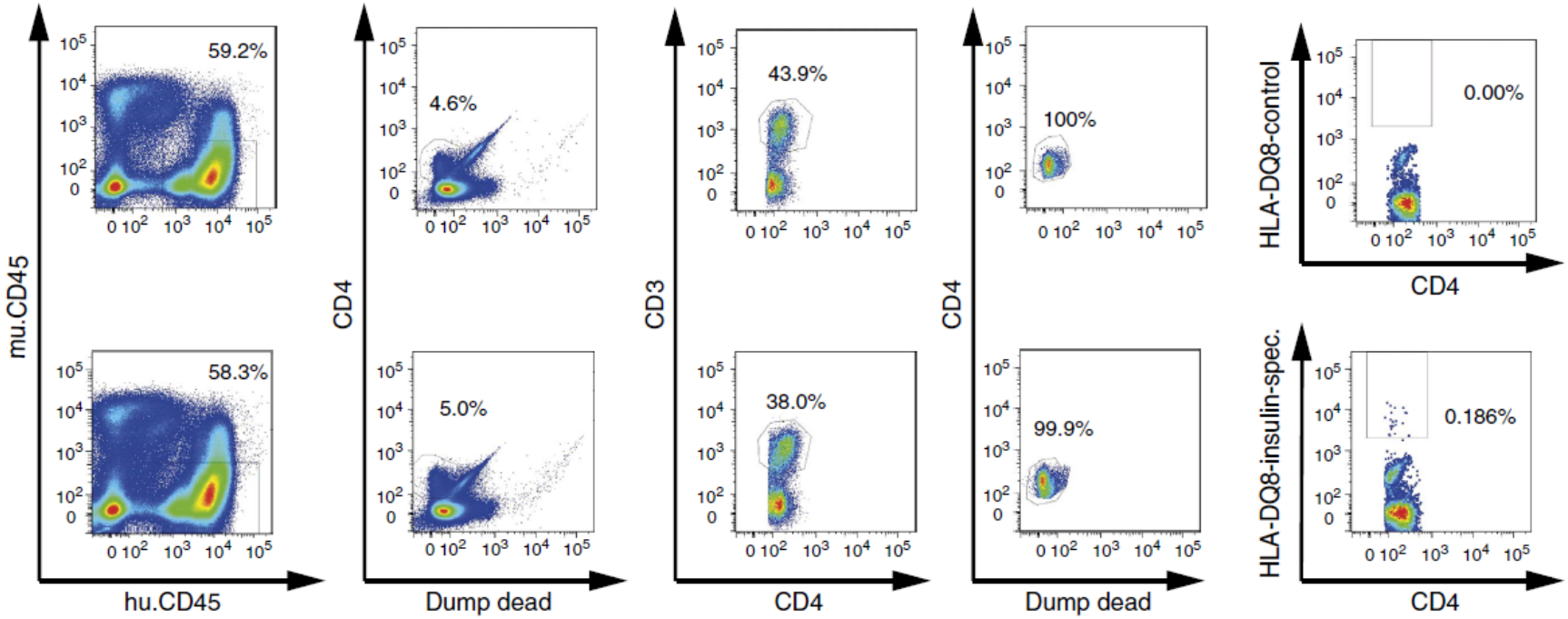
Ex vivo identification of HLA-DQ8 restricted insulin-specific CD4+T cells from human peripheral blood



Serr et al., Nat Comm 2016

How to induce human Foxp3$^+$Tregs *in vivo*? Characterization of human CD4$^+$T cells

20 wks post reconstitution

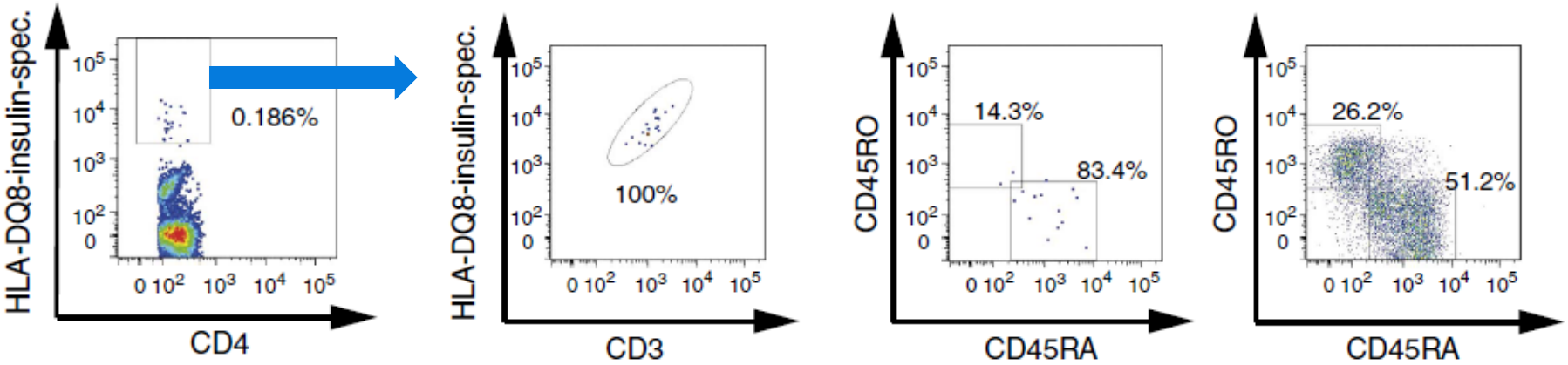


Serr et al., Nat Comm 2016

How to induce human Foxp3$^+$Tregs *in vivo*? Identification of human insulin-specific CD4$^+$T cells

20 wks post reconstitution

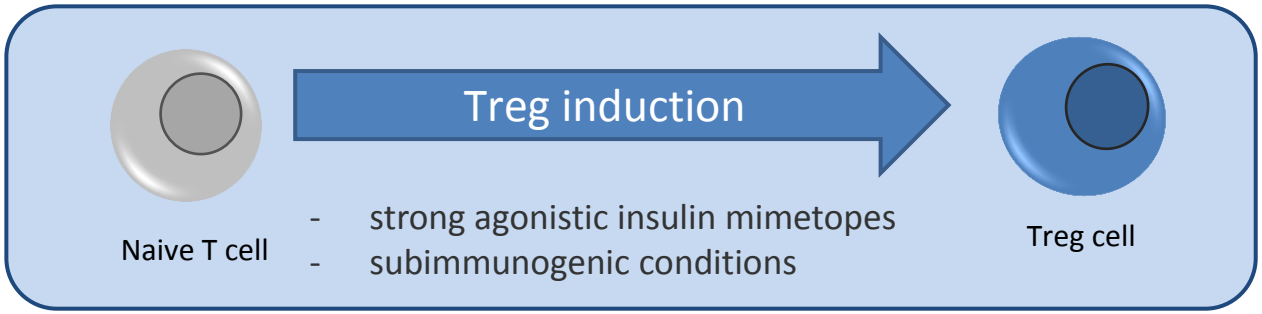
HLA-DQ8 restricted insulin-specific CD4$^+$T cells **polyclonal CD4$^+$T cells**

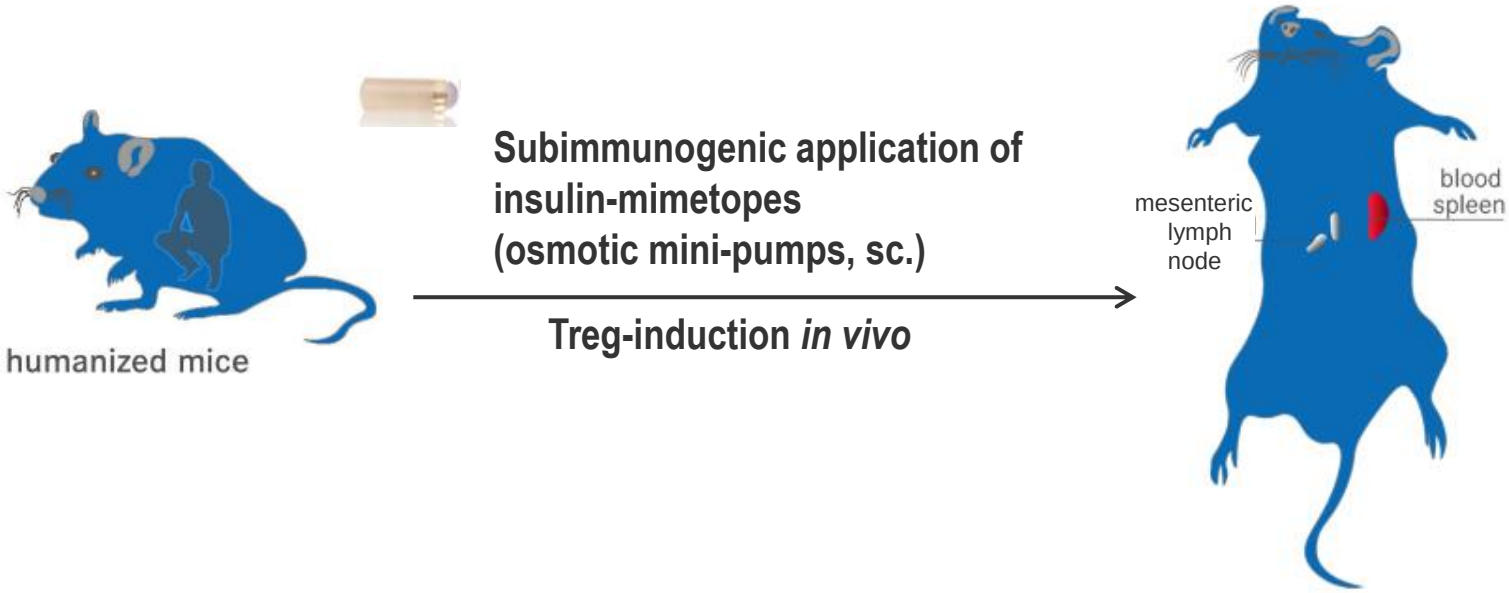


Identification of HLA-DQ8 restricted insulin-specific CD4$^+$T cells in humanized HLA-DQ8 Tg NSG mice.

Serr et al., Nat Comm 2016

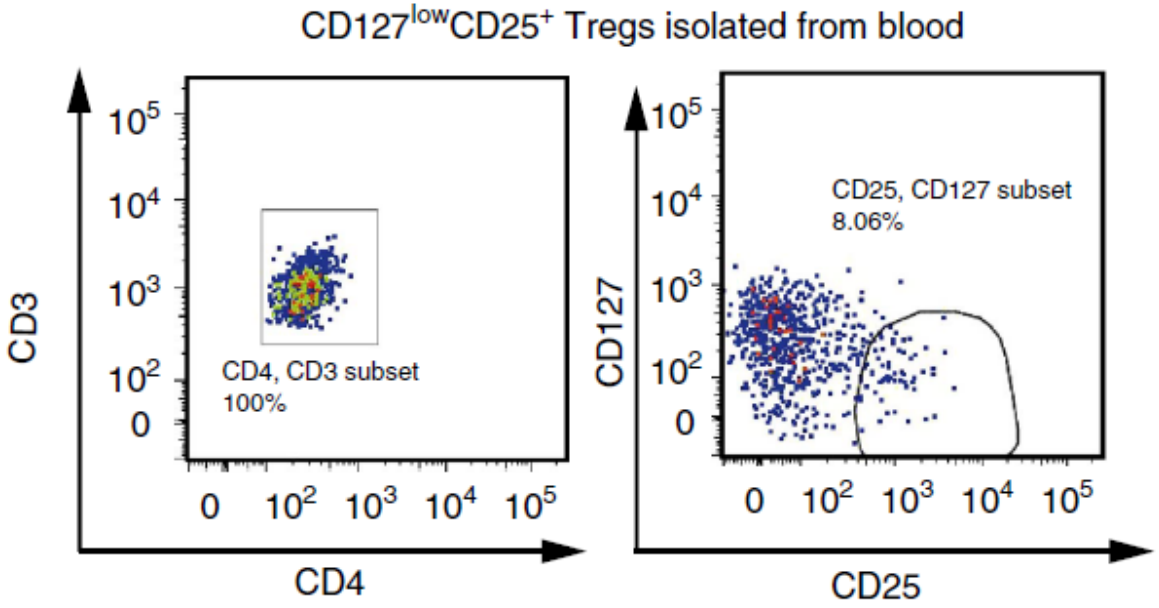
How to induce human Foxp3^{+}Tregs *in vivo*?
Insulin-specific Treg induction *in vivo*





Serr et al., Nat Comm 2016

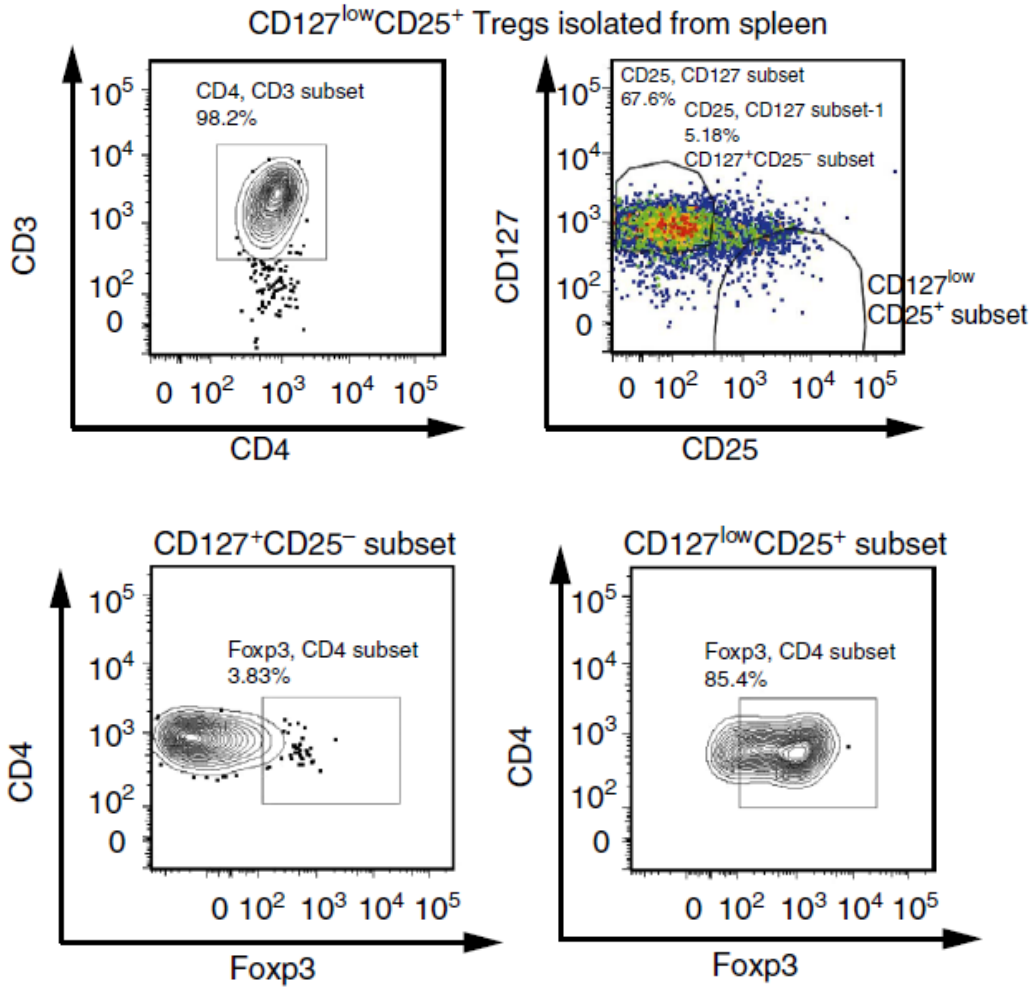
Analysis and functional characterization of human Tregs in humanized mice



Serr et al., Nat Comm 2016

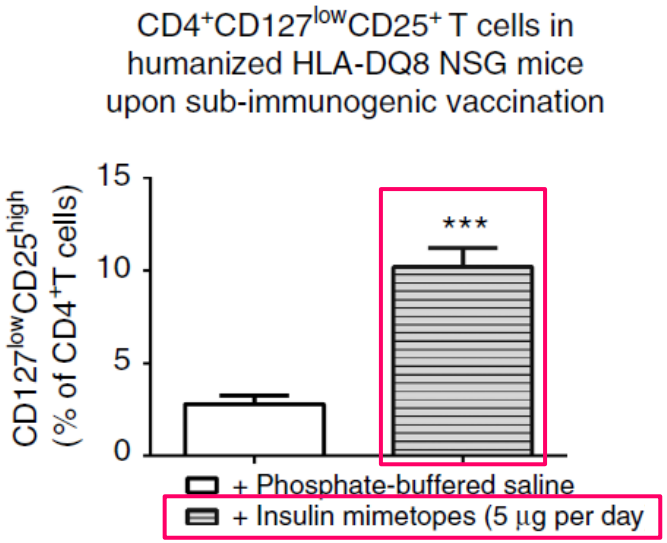
HelmholtzZentrum münchen
German Research Center for Environmental Health
HELMHOLTZ ASSOCIATION

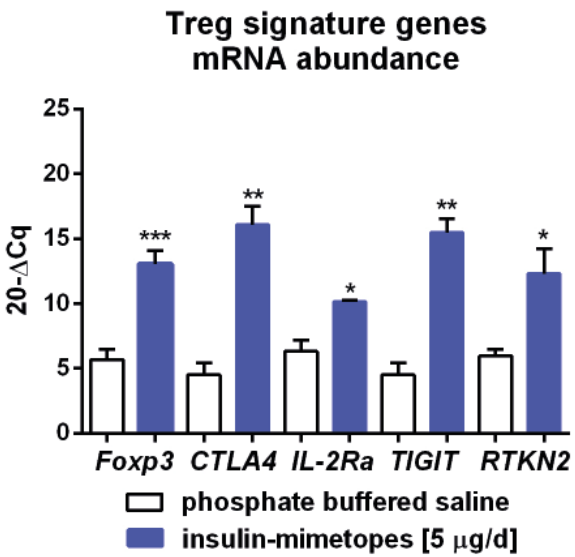
Analysis and functional characterization of human Tregs in humanized mice

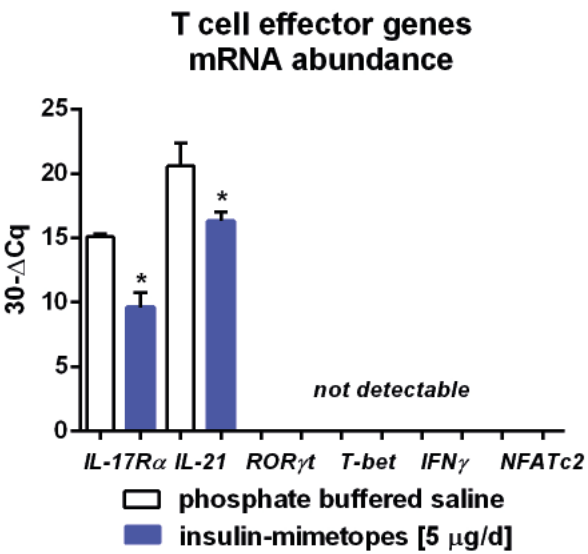


Serr et al., Nat Comm 2016

Analysis and functional characterization of human Tregs in humanized mice



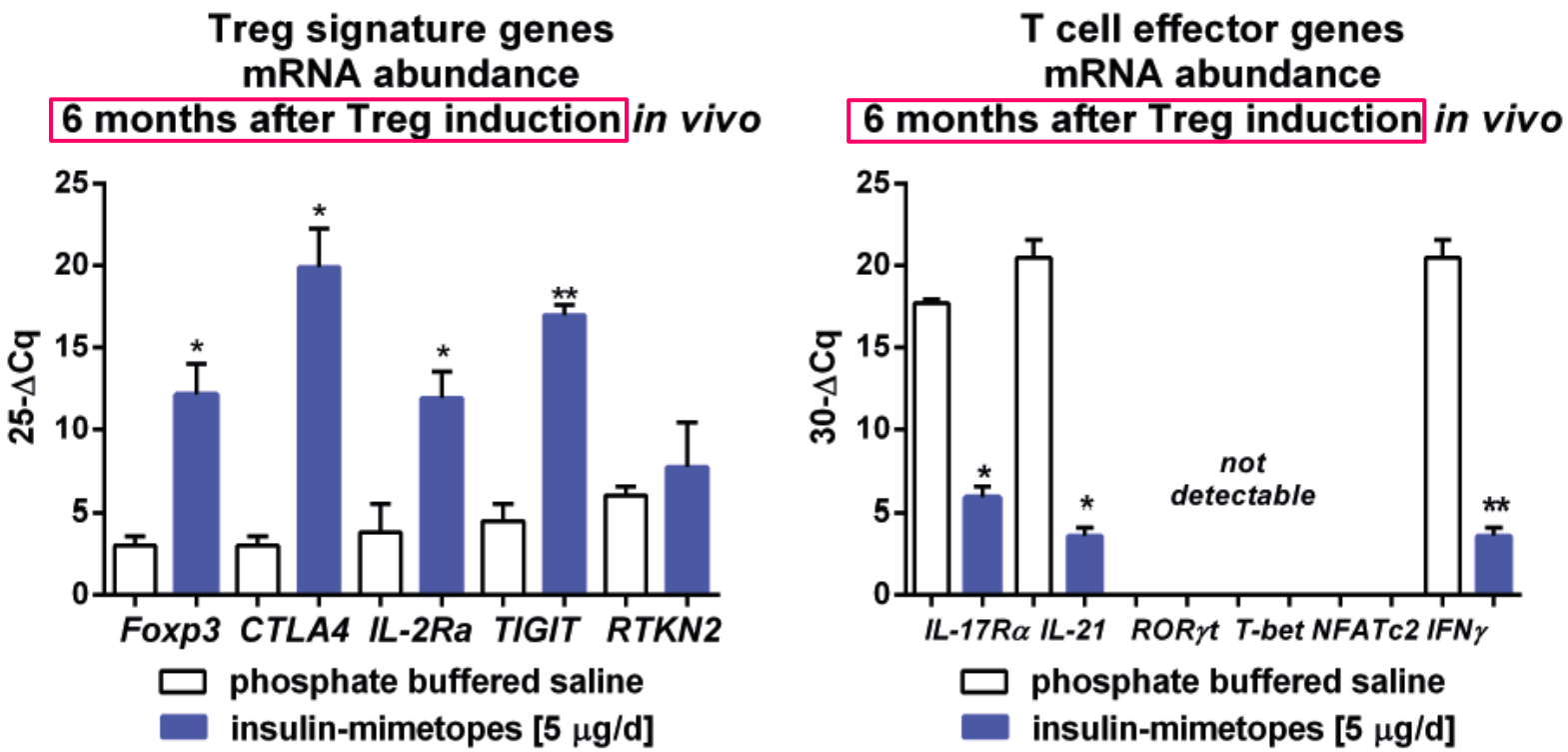




Increased frequencies of human Tregs upon Treg induction with insulin-mimetopes in humanized HLA-DQ8 Tg NSG mice.

Serr et al., Nat Comm 2016

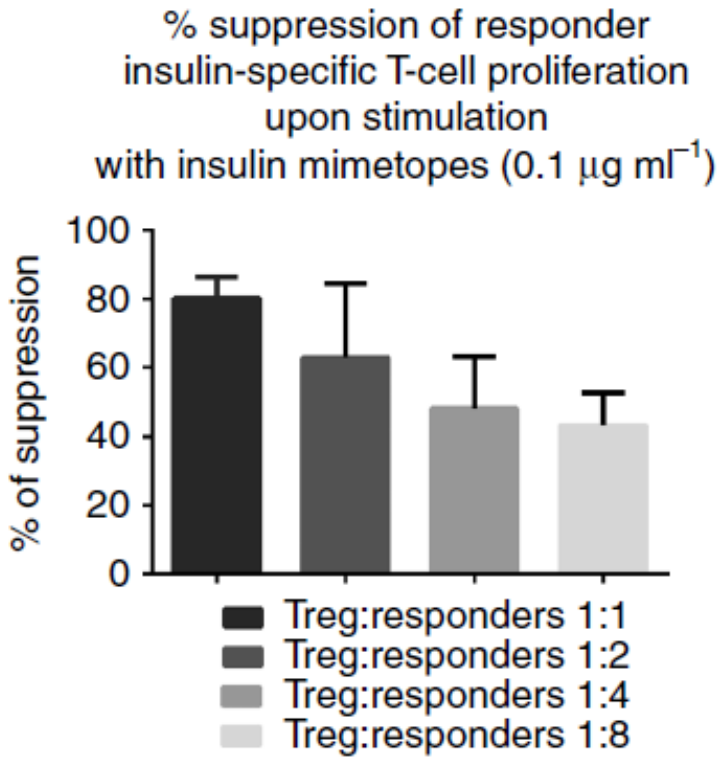
Efficient human insulin-specific Treg induction in humanized mice



Phenotypes of induced human Tregs in humanized HLA-DQ8 Tg NSG mice are stable.

Serr et al., Nat Comm 2016

Analysis and functional characterization of human Tregs in humanized HLA-DQ8 Tg NSG mice

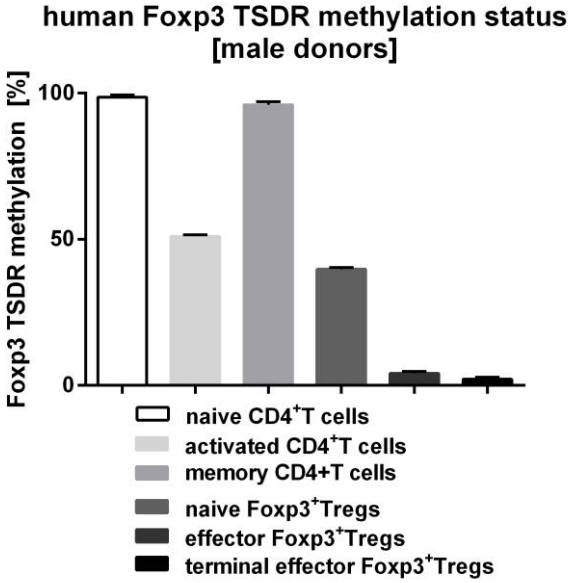


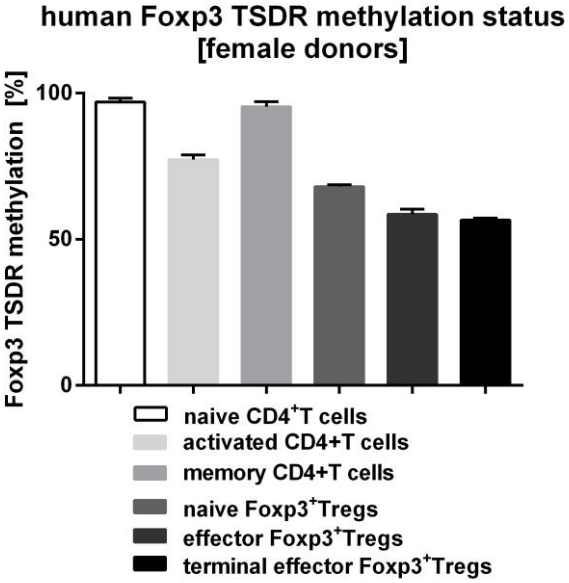
Induced human Tregs from humanized mice are functional and suppressive.

Serr et al., Nat Comm 2016

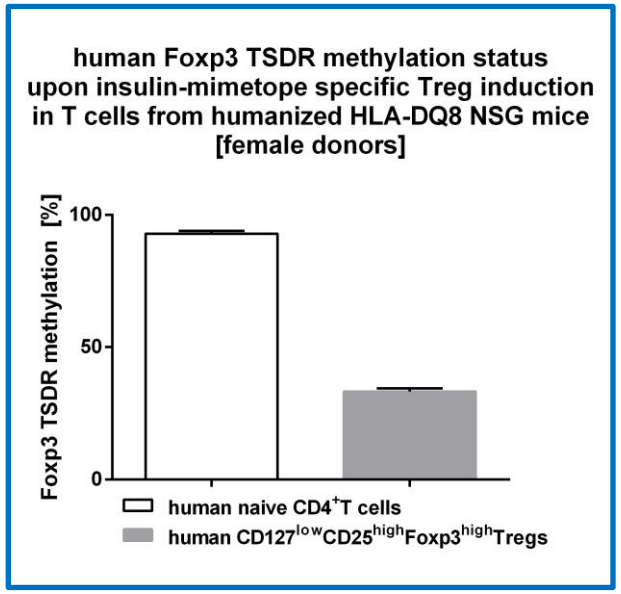
Methylation analyses of Foxp3 Treg-specific demethylated region (TSDR) from humanized mice

Ex vivo **human CD4⁺T cells from peripheral blood**





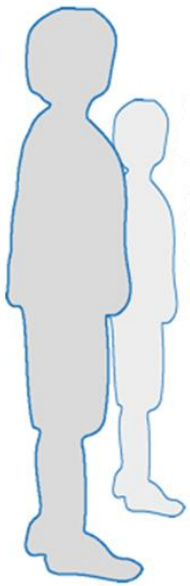
Ex vivo **human CD4⁺T cells from humanized mice**



Induced human Tregs from humanized mice are functional and stable.

Serr et al., Nat Comm 2016

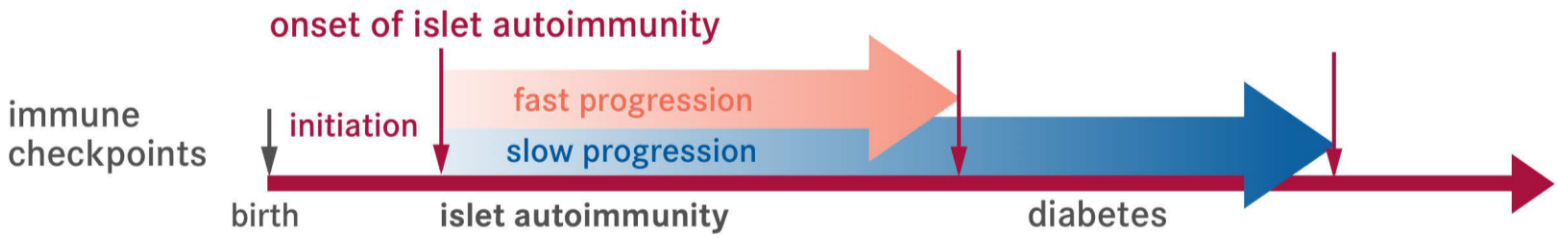
Relevance for childhood Type 1 diabetes



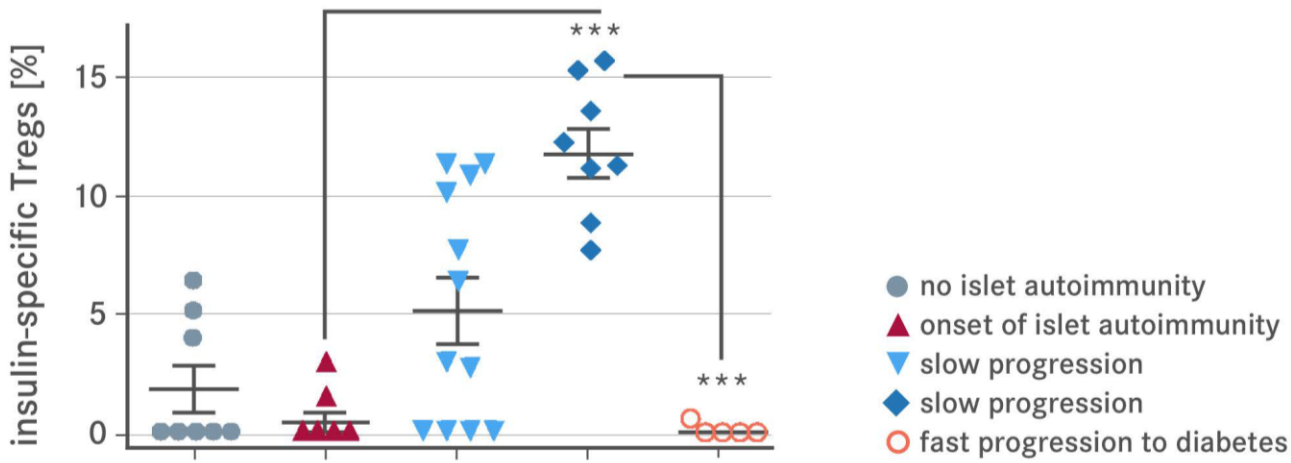
Is there evidence in children with islet autoimmunity that insulin-specific Treg cells can impact the progression to Type 1 diabetes?

Serr et al., Nat Comm 2016

Analysis of insulin-specific Treg cells in peripheral blood of children with different duration of islet autoimmunity



HLA-DQ8-restricted insulin-specific Tregs



High frequencies of insulin-specific Foxp3⁺Tregs do associate with slow progression to Type 1 diabetes.

Serr et al., Nat Comm 2016

Summary

Human insulin mimetopes induce insulin-specific Treg cells in a pre-clinical setting of humanized mice

⇨ Induced human Tregs in humanized HLA-DQ8 Tg NSG mice are functional and suppressive.

In children, high frequencies of insulin-specific Tregs or low frequencies of insulin-specific TFH precursor cells do associate with slow progression to Type 1 diabetes

⇨ Critical next steps:

Understand identified T tolerance defects mechanistically

Dissect T tolerance defects and mechanisms *in vivo*

→ Modelling of islet autoimmunity in humanized mice

Study insulin-specific Treg induction using combination strategies to interfere with autoimmune activation

Acknowledgements, cooperations and network

- **Institute of Diabetes Research**
  - **Anette-G. Ziegler**
  - All members of IDF1 lab
    - Rainer Fürst
- **Research Group**
  - **Isabelle Serr**
  - **Verena Ott**
  - **Martin Scherm**
  - Victoria Flynn
  - Maike Becker
- **The Jackson Laboratory**
  - Lenny Shultz
- **TUM/IAS – University of Pennsylvania**
  - Klaus H. Kaestner
- **Institute of Diabetes and Obesity**
  - **Matthias Tschöp**
  - **Stefanie Kälin**
  - **Sini Susan Joseph**
  - Martin Jastroch
  - Siegfried Ussar
- **University of Leipzig**
  - Ingo Bechmann
  - Matthias Blüher

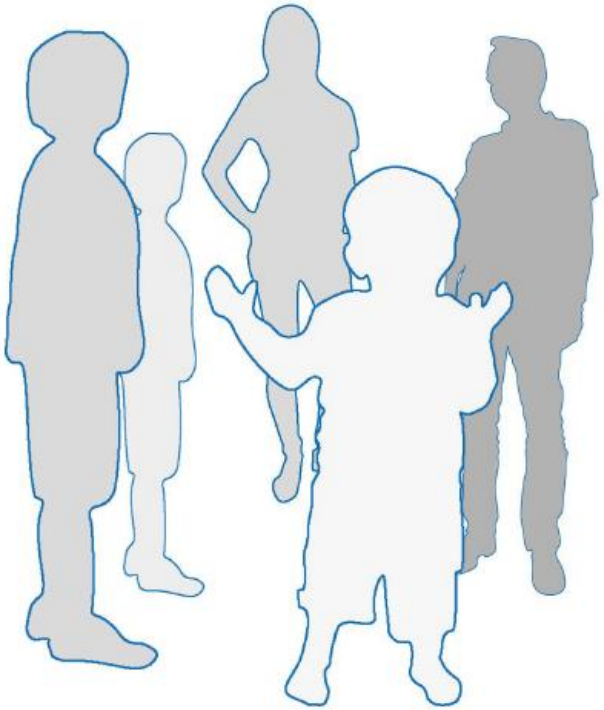
JDRF IMPROVING LIVES. CURING TYPE 1 DIABETES.

GeneCore

SFB 1054 Control and Plasticity of Cell-Fate Decisions in the Immune System

Helmholtz Diabetes Center

HelmholtzZentrum münchen
Deutsches Forschungszentrum für Gesundheit und Umwelt



THANK YOU

All study participants, families and their children

BABYDIAB INIT DiMelli MUNICH DIABETES BIORESOURCE Frida Pre-POINT early

carolin.daniel@helmholtz-muenchen.de

HelmholtzZentrum münchen
German Research Center for Environmental Health

HELMHOLTZ ASSOCIATION