Identification of children with Type 1 Diabetes Suitable for Antigen-specific Immunotherapy

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Background

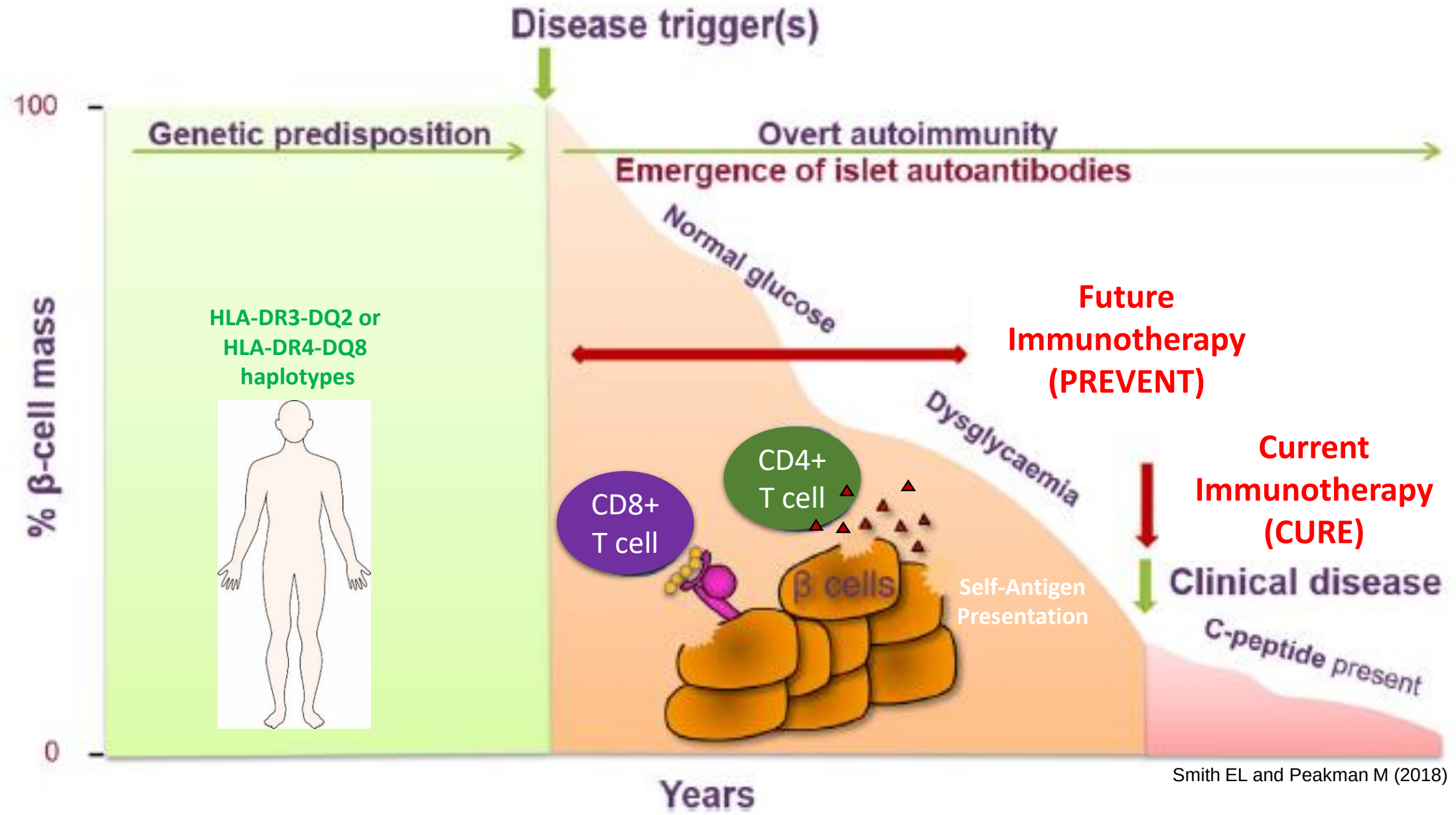
What we know about the cause and treatments for Type 1 Diabetes (T1D)

- Autoimmune disease
  - T cell-mediated pancreatic β-cell destruction
- Increasing incidence, Significant biopsychosocial burden
- Current (insulin replacement) therapies inadequate
- Need to stop the autoimmune process → preserve β-cells
  - Better metabolic control, less severe hypoglycaemia, fewer long term complications
  - Better quality of life

DCCT 1993, EDIC 2009

* β-cell = Insulin producing cells of the pancreas

Immunotherapy has the potential to cure and/or prevent T1D



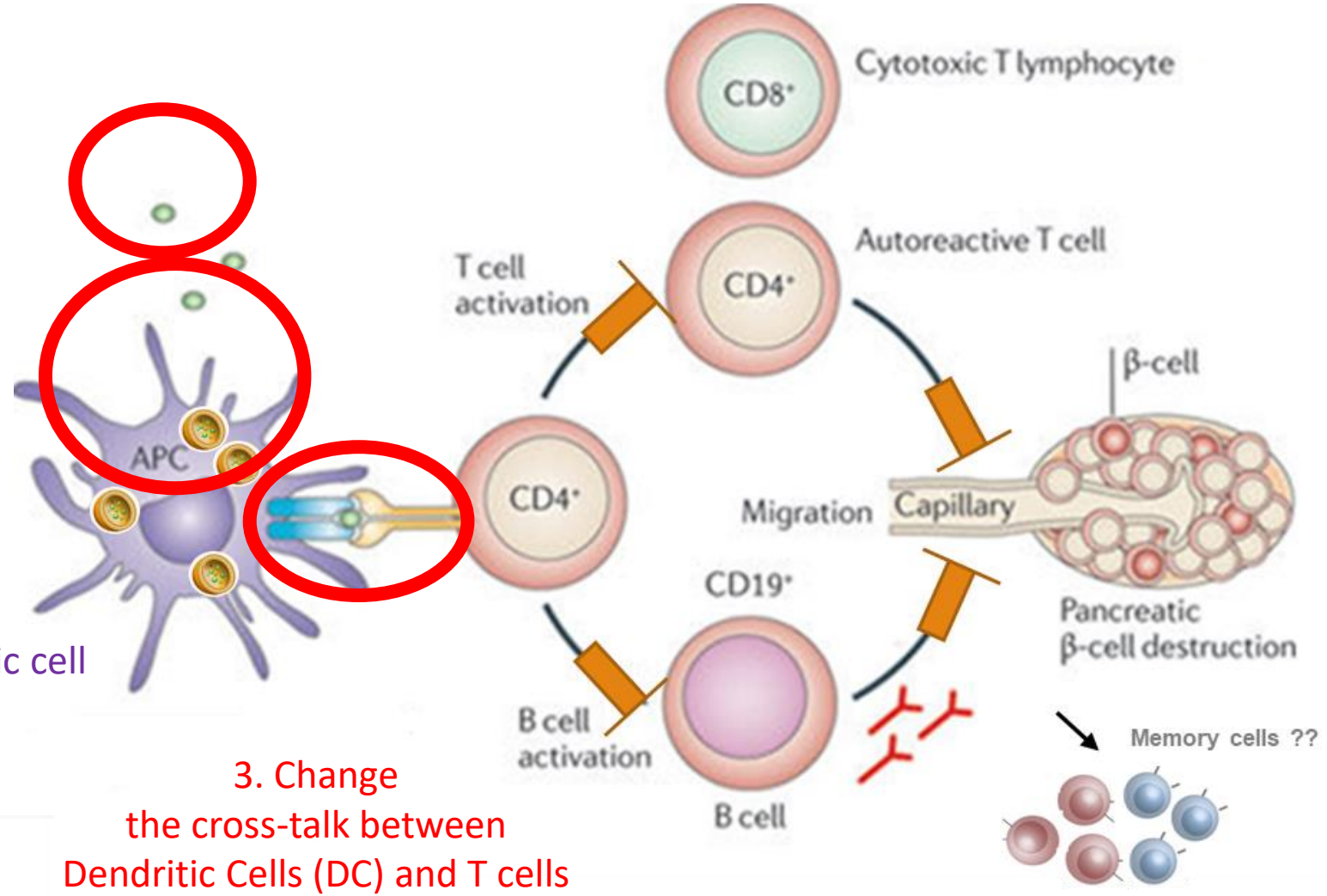
Smith EL and Peakman M (2018)

Developing Antigen Specific Immunotherapy

The 3 therapeutic targets

1. Identify the auto-antigen

2. Identify individuals in whom self-antigen recognition occurs



Dendritic cell

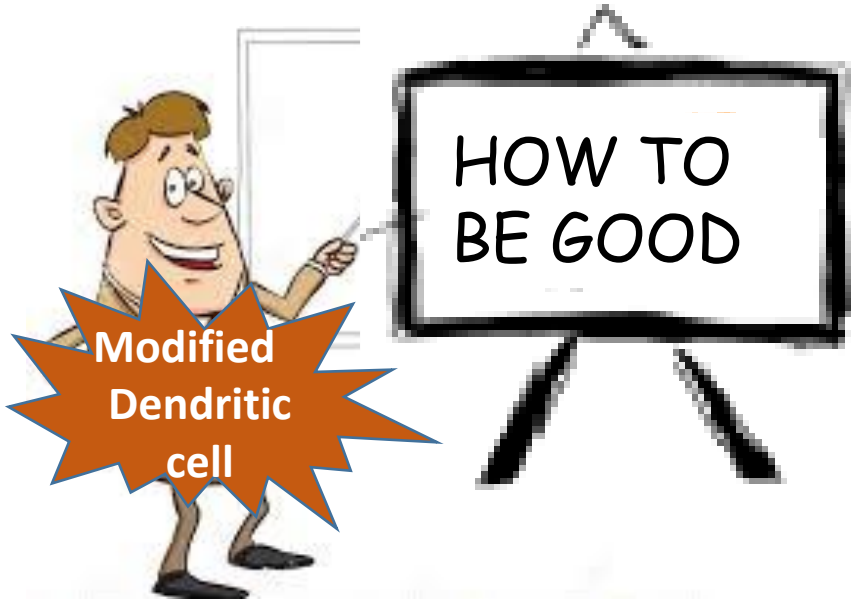
3. Change the cross-talk between Dendritic Cells (DC) and T cells (occurs via NF-κB activation)

Nature Reviews/ Immunology

Developing Antigen Specific Immunotherapy

Dendritic cells (DC) as therapeutic targets in T1D

Regulating the presentation of islet autoantigens by DCs to autoreactive T cells can restore self-tolerance.



Slide Courtesy of Ranjeny Thomas

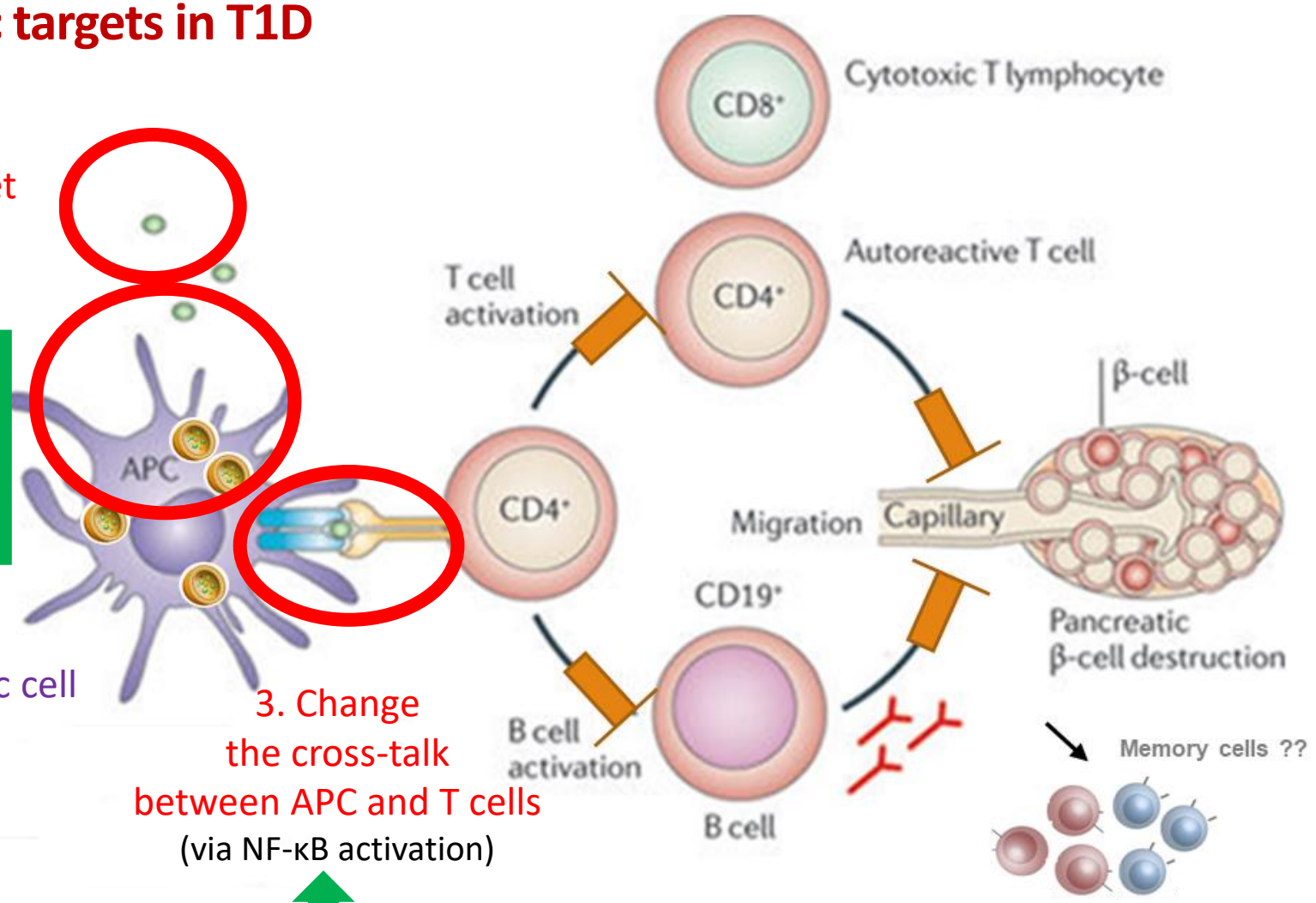
Developing Antigen Specific Immunotherapy

The 3 therapeutic targets in T1D

1. Identify the antigenic target

Proinsulin

2. Identify individuals in whom self- antigen recognition occurs



Dendritic cell

3. Change the cross-talk between APC and T cells (via NF-κB activation)

Calcitriol

(NF-κB inhibitor)

Nature Reviews/ Immunology

The T-cell response to Pro-insulin peptides

Hypothesis

$CD4^+$ T-cell responses in individuals with T1D will vary according to age, HLA*-type, disease duration, and C-peptide

Aims

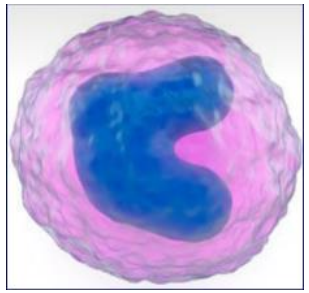
To (A) identify and (B) characterise individuals with T1D who have $CD4^+$ T-cell responses to established islet auto-antigens

* HLA = Human leucocyte antigen

Project overview and methodology

HLA typing

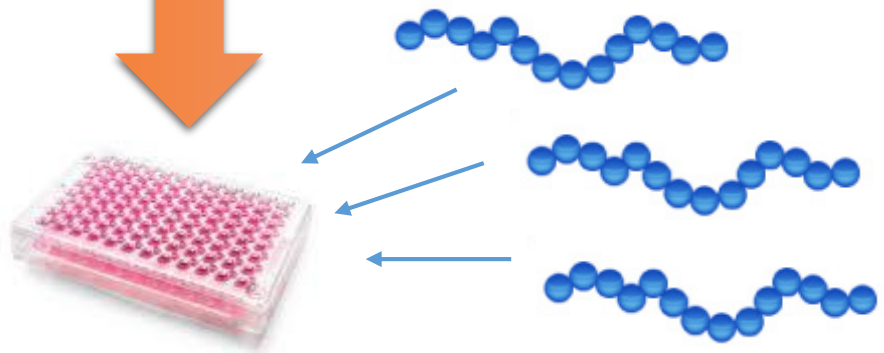
Isolate PBMC*



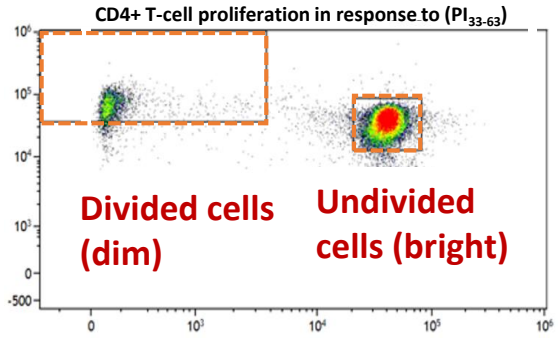
Label PBMC with Fluorescent dye CFSE

Incubate PBMC with islet autoantigens

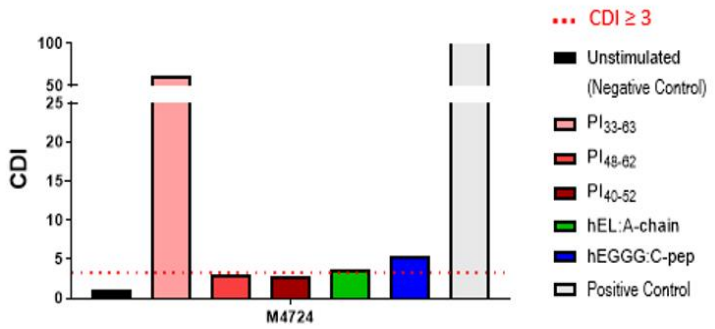
Proinsulin and Hybrid Insulin Peptides



Measure CD4+ T cell proliferation with Flow Cytometry



Generate CD4+ T cell readouts to different islet



*PBMC – Peripheral Blood mononuclear cells

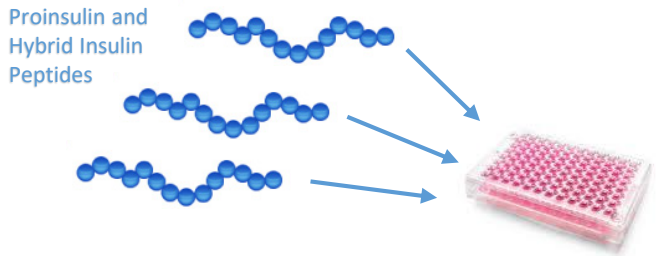
Procedures

The different *in vitro* stimulation conditions

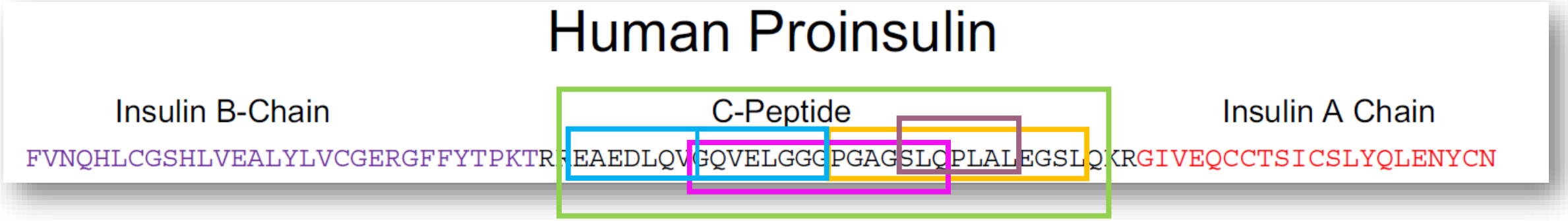
1. Negative control: No Antigen

2. Positive control: Human αCD3 / Tetanus Toxoid

3. Synthetic Islet Peptides
   - **Pro-insulin peptides**
     - Name: PI_{33-63}
       Sequence: EAEDLQVGQVELGGGPGAGSLQPLALEGSLQ
     - Name: PI_{48-62}
       Sequence: PGAGSLQPLALEGSL
     - Name: PI_{40-52}
       Sequence: GQVELGGGPGAGS



   - **Hybrid Insulin peptides (HIPs)**
     - Name: hEGGG:C-pep
       Sequence: GQVELGGGEAEDLQV
     - Name: hEGGG:IAPP2
       Sequence: GQVELGGGNAVEVLK
     - Name: hEL:A-chain
       Sequence: SLQPLALGIVEQCC

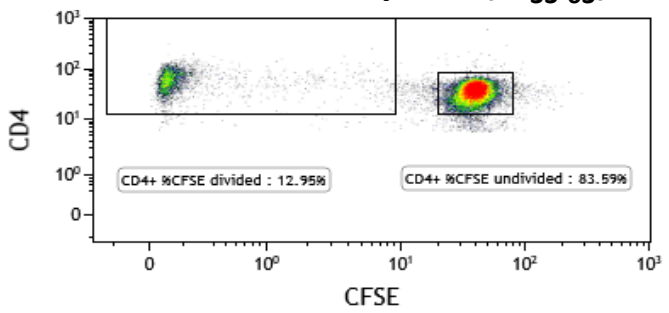


Calculating the Cell Division Index (CDI)

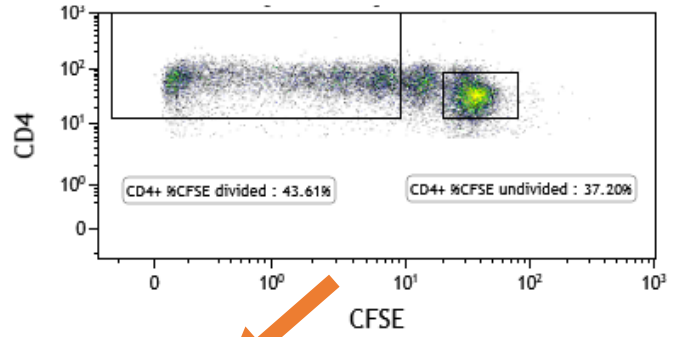
T cell proliferation in response to antigen stimulation is defined as the CDI

$$\text{CDI} = \frac{\text{number of divided CD4}^{+}\text{ cells per 5,000 CD4}^{+}\text{ CFSE}^{\text{undivided}}\text{ from “with antigen” group}}{\text{number of divided (CD4}^{+}\text{) from the “without antigen” group.}}$$

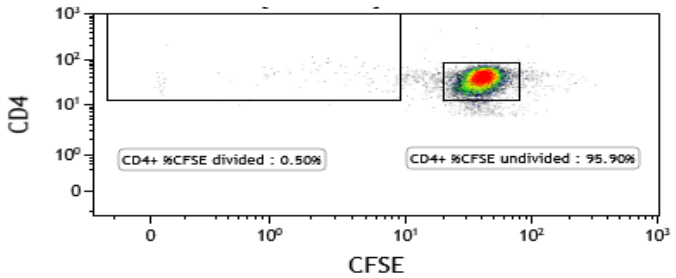
Proinsulin Peptide (PI_{33-63})

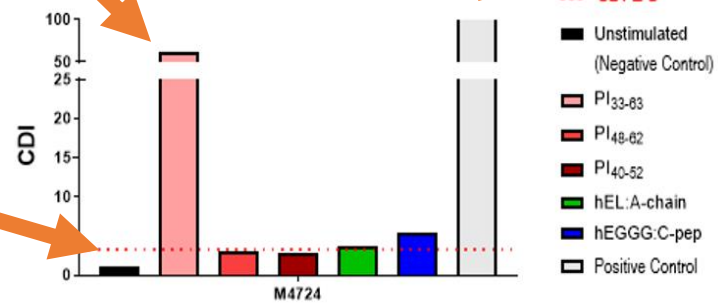


Positive control



Negative control (without antigen)





Results

Baseline Characteristics

	Healthy controls	All patients	T1D < 3 months (early-onset T1D)	T1D > 3 months
Number of subjects	10	48	16	32
Mean duration of diagnosis		1.44	0.05	25.63 **
Mean age (years; ± SD)	35.14 ± 10 *	10.1 ± 3.8	9.3 ± 4.1	10.3 ± 3.5
Mean Age at diagnosis (years)		8.8 ± 3.7	9.1 ± 4.3	8.6 ± 3.4
Gender (female:male)	7:3	21:27	7:9	14:18
Body Mass index (kg/m^2± SD)	25.1 ± 5.8 *	19.72 ± 4.5	17.9 ± 4.1	20.6 ± 4.5 **
Mean insulin dose adjusted glycated hemoglobin (% ± SD)		11.6 ± 3.4	10.9 ± 2.6	10.8 ± 2.5
Average total daily insulin dose (IU Kg−1 day−1; ±SD)		0.8 ±0.3	0.9 ± 0.2	0.7 ± 0.3
Estimated C-peptide[1]		0.4 ± 0.2	0.02 ± 0.3	0.5 ± 0.3 **

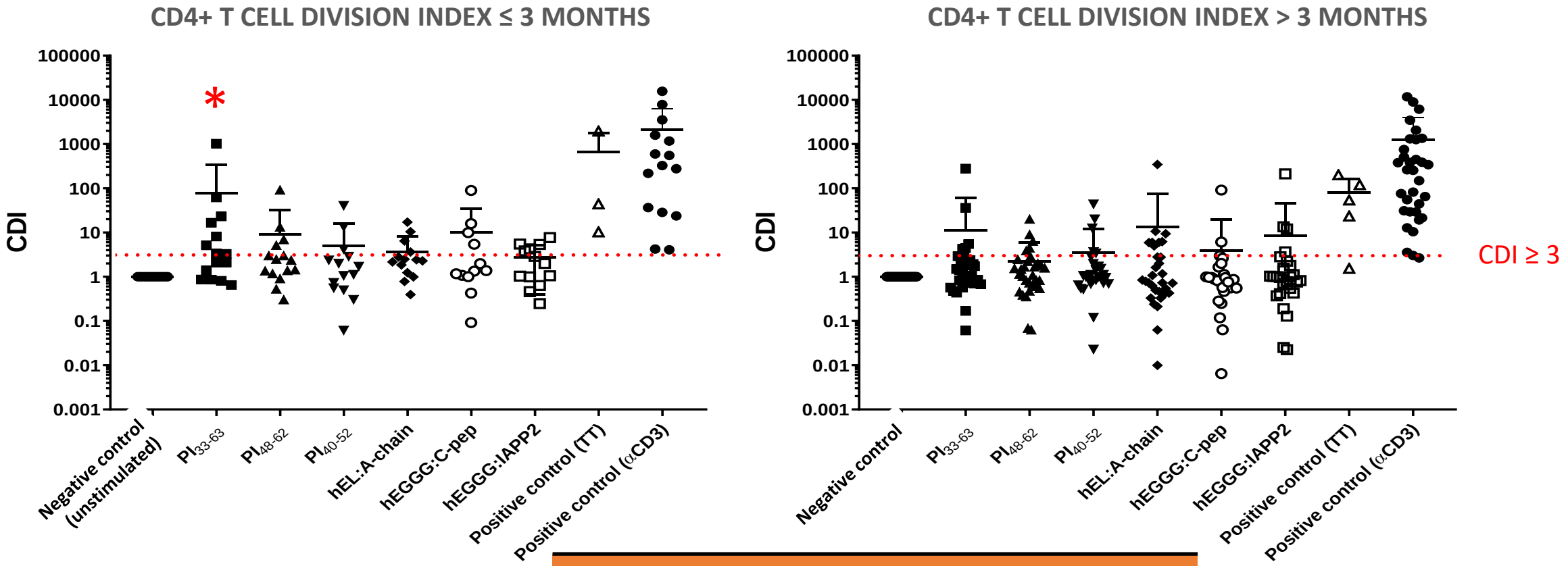
**p<0.05 value compares all T1D and HC*

***p value compares early-onset T1D and T1D > 3 months*

1. Buchanan, K, Mehdi, AM, Hughes, I, et al. An improved clinical model to predict stimulated C-peptide in children with recent-onset type 1 diabetes. *Pediatr Diabetes*. 2019; 20: 166– 171.

Results: Disease duration

CD4+ T cell responses were detected more frequently in early-onset T1D



	CDI ≥ 3 to any peptide	
All Patients (n = 48)	22 (46%)	
• Early onset (n= 16)	13 (81%)	**
• T1D > 3 months (n= 32)	10 (31%)	
Healthy Controls (n = 11)	4 (36%)	

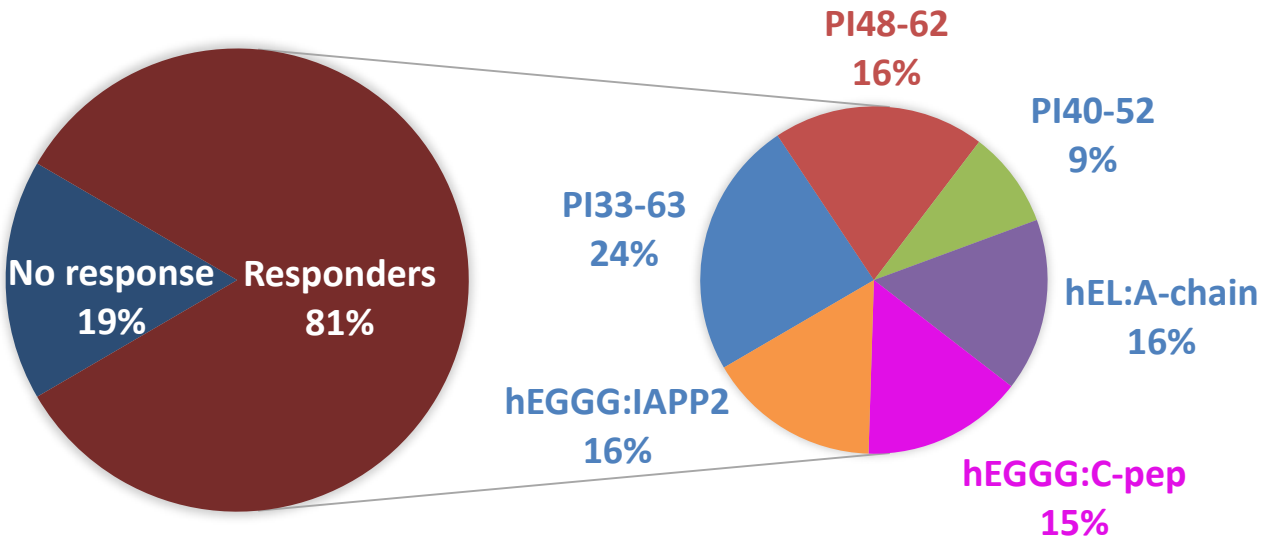
* *p =0.01, compares CDI PI_{33-63} in early-onset T1D and T1D > 3 months*

** *p =0.03, compares CDI for any peptide in early-onset and T1D > 3 months*

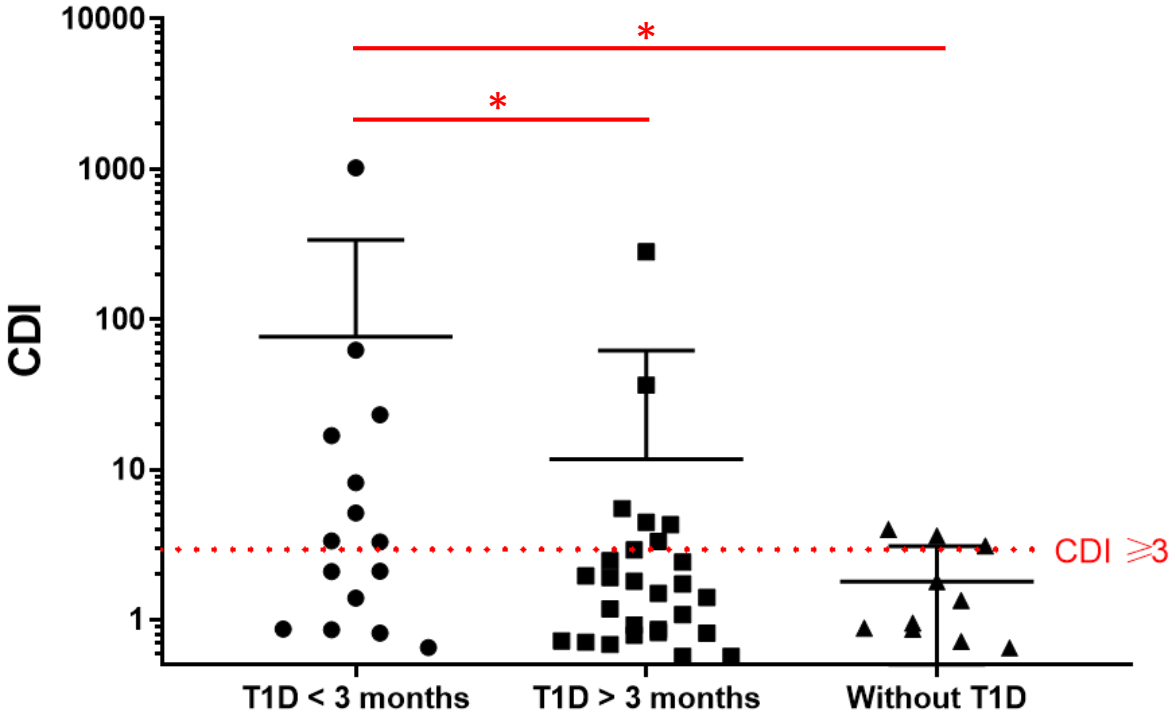
Results: Peptide specificity

CD4+ T cell responses to PI_{33-63} predominate

CD4+ T-CELL RESPONSES (CDI≥3) TO ISLET PEPTIDES IN EARLY-ONSET T1D



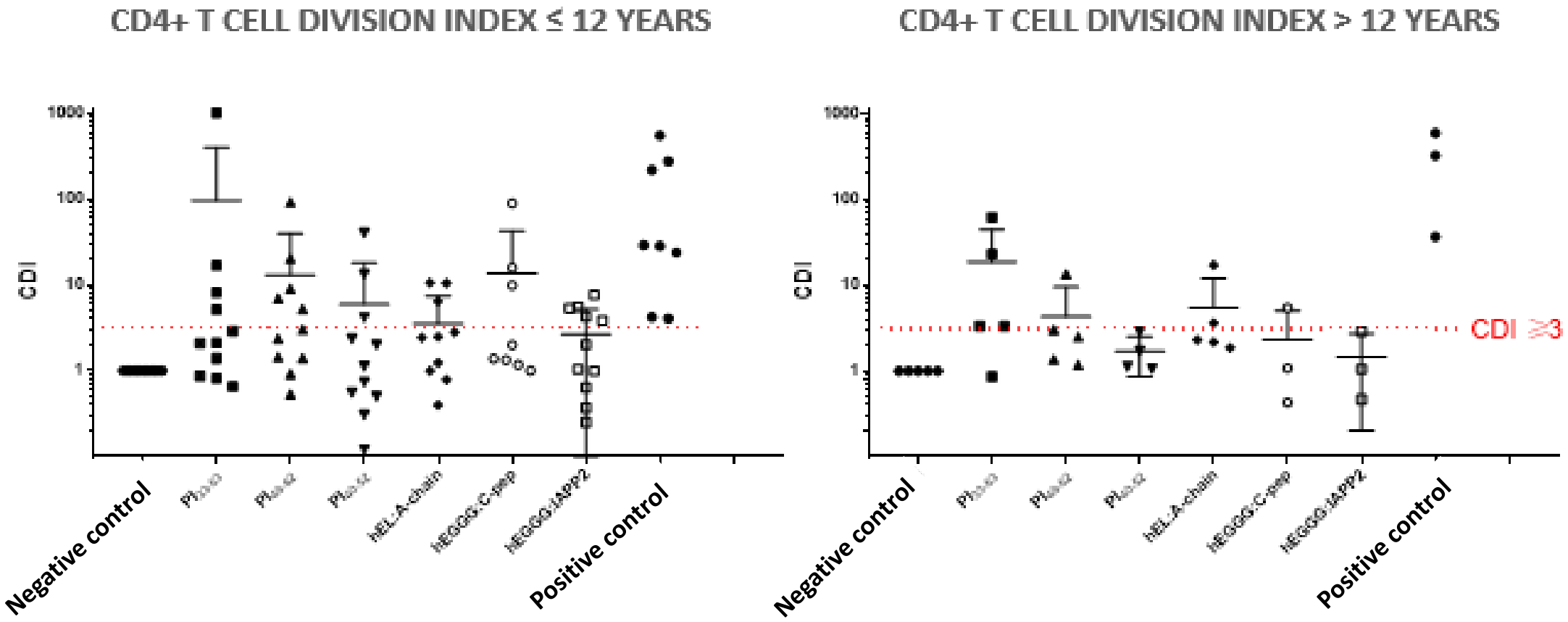
CD4+ T CELL DIVISION INDEX TO $PROINSULIN_{33-63}$



Error bars display the mean ± SD. ** p =0.01*

Results: The influence of age

CD4+ T cell responses occur equally across age brackets in early onset T1D

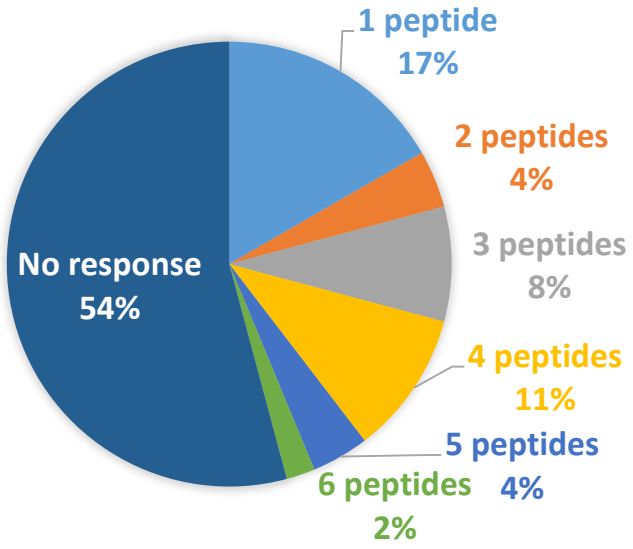


Error bars display the mean ± SD.

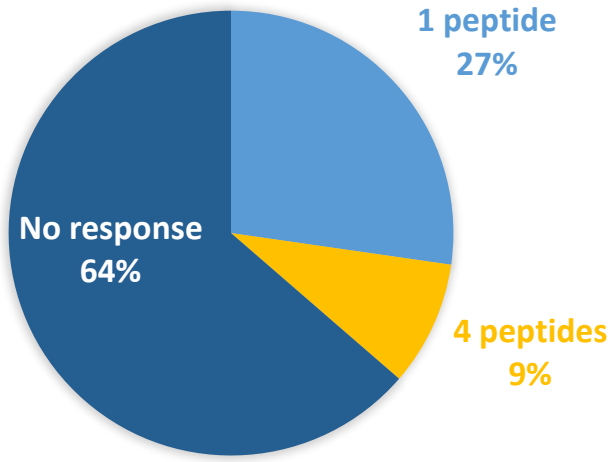
Results: Multiple peptides

CD4+ T cell responses to multiple peptides were detected more frequently in early-onset T1D

ALL T1D PATIENTS



HEALTHY CONTROLS



	CDI ≥ 3 to multiple peptides	
All Patients (n = 48)	14 (29%)	
• Early onset (n= 16)	8 (50%)	** p =0.03*
• T1D > 3 months (n= 32)	6 (19%)	
Healthy Controls (n = 11)	1 (9%)	

**p value* compares early onset T1D against T1D > 3 months and healthy controls

Results: The influence of glycaemia

CD4+ T cell responses correlated negatively with Estimated C-peptide

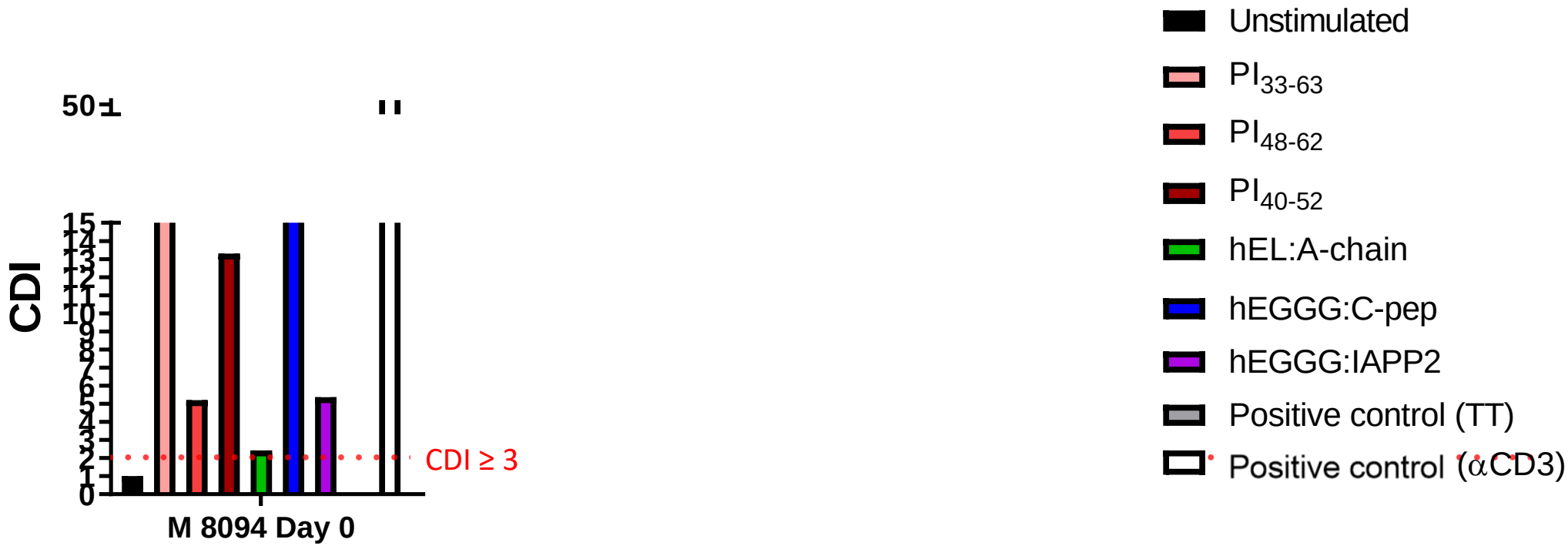
	CDI ≥ 3
Estimated C peptide*	r = -0.47 to -0.32 **
Insulin dose adjusted HbA1c	r = -0.18 to 0.36

*Clinical model incorporating age, gender, BMI-Z score, HbA1c, time since diagnosis and insulin, correlates significantly with 90-minute stimulated C-peptide measurements (adjusted R2 = 0.62, P <0.0001). *Buchanan et al 2019.*

** *P < 0.05* using spearman's test, range provided for different peptides

Results: Longitudinal CD4+ T cell responses

CD4+ T cell responses diminish with time



*CDI – Cell Division Index

Results: Cytokine responses

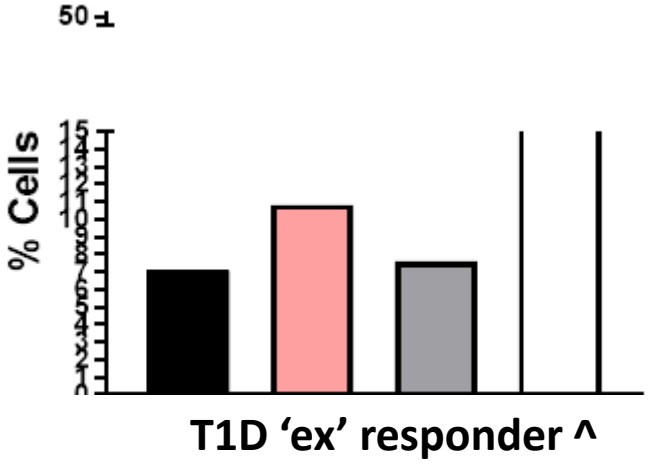
CD4+ T cell 'non' responders may demonstrate cytokine responses

Negative control (unstimulated)

PI_{33-63}

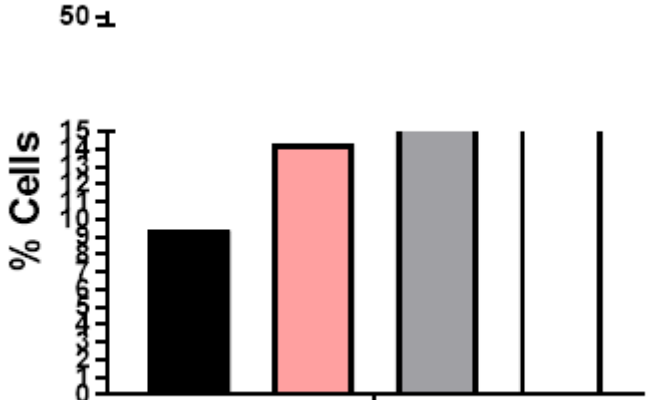
TetTox, αCD3 — Positive control

% CD4+ T cells with IFNγ*



T1D 'ex' responder ^

% CD4+ T cells TGF-β (LAP)*



T1D 'ex' responder ^

* IFN-γ = Interferon-gamma, LAP = latency-associated peptide, TGF-β = transforming growth factor beta

^ T1D patient with CD4+ T cell proliferative response at day 0 (diagnosis) but not at day 150 or 330

Double Antibody positive euglycaemic individual

Summary of preliminary results

- Peptide*-specific CD4+ T-cells can be detected in peripheral blood of most children early-onset T1D, half of whom show responses to multiple islet peptides.
  - CD4+ T cells proliferative responses may diminish with time
  - CD4+ T cells may continue to produce cytokine responses
  - Further evaluation of clinical variables and cytokine profiles is warranted

- Of the peptides tested, CD4+ T-cell responses to $Proinsulin_{33\text{-}63}$ may be an attractive candidate for a T-cell based biomarker

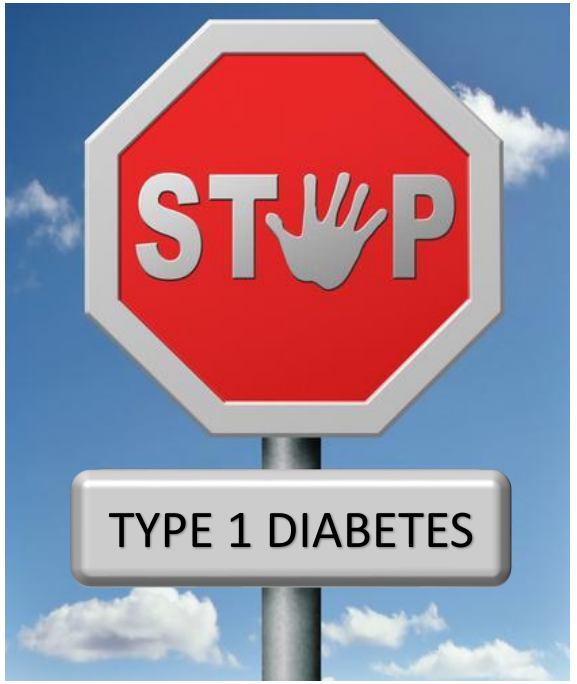
* Natural Proinsulin peptides or Hybrid Insulin Peptides

Significance of our findings

- Phase Ib Clinical trial of antigen-specific immunotherapy (ASI) in Rheumatoid Arthritis

Thomas Group

- Findings from this study can support the development of **ASI in T1D** by identifying:
  - the best candidate peptides to incorporate into ASI
  - the patients who are most likely to respond to ASI



Acknowledgements

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New onset T1D team (Queensland Children's Hospital)

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RACP Foundation

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Dr Hendrick Nel, Nathan Stone

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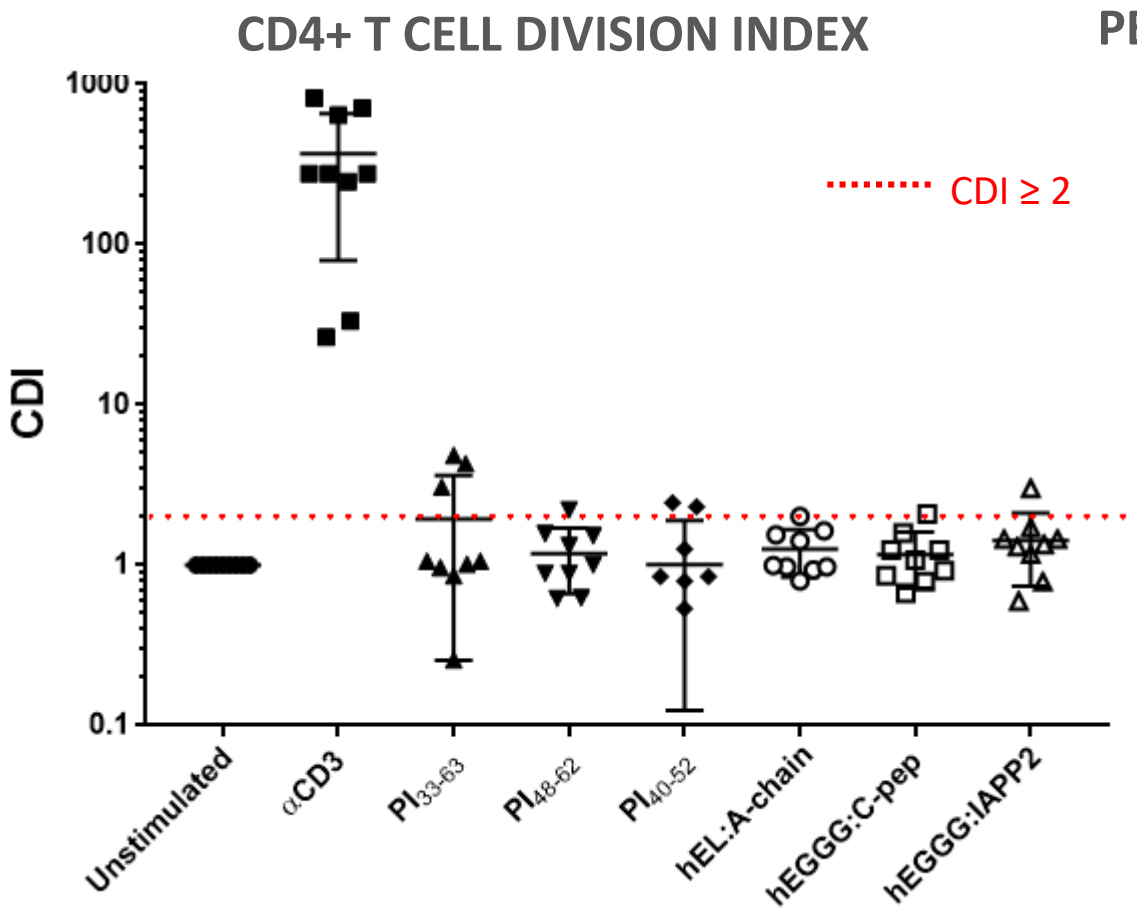
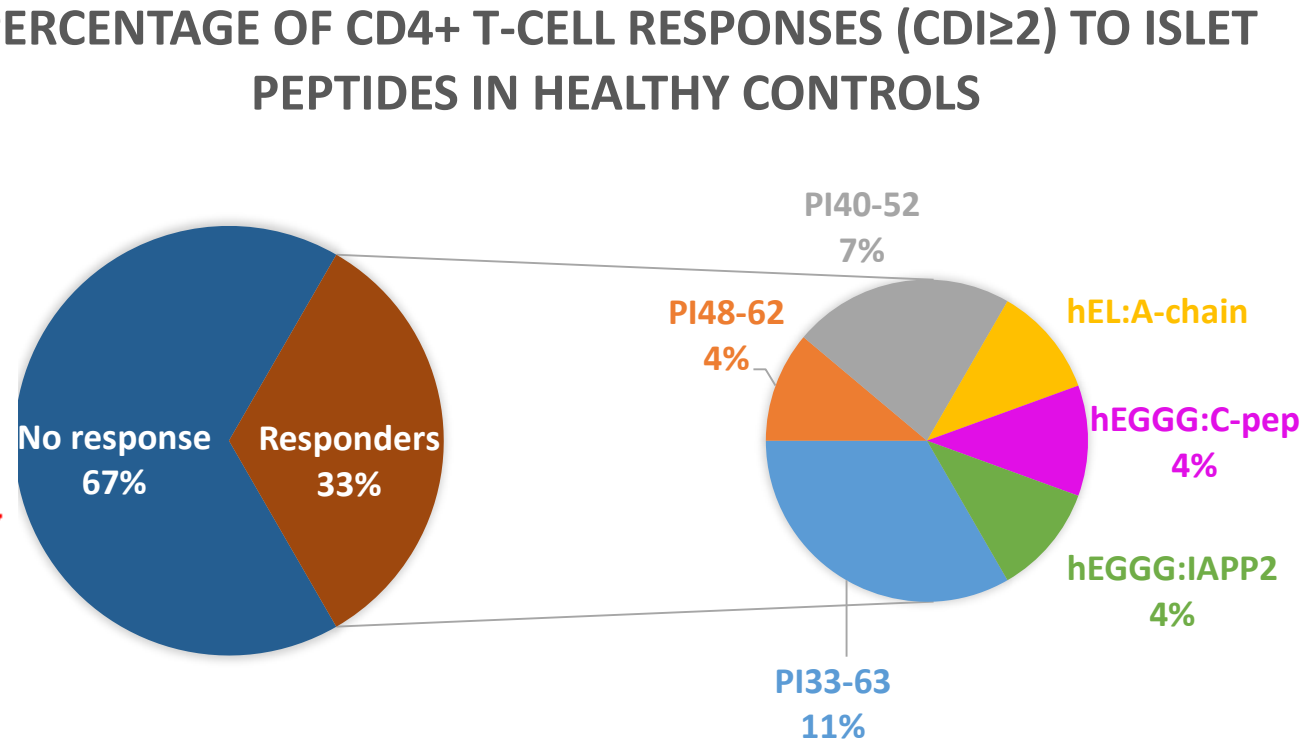
Butta Clinician researcher Bursary
RACP Foundation NZ Development Scholarship
Children's Health Foundation PhD Top up Scholarship

Results

CD4+ T cell responses in Healthy controls





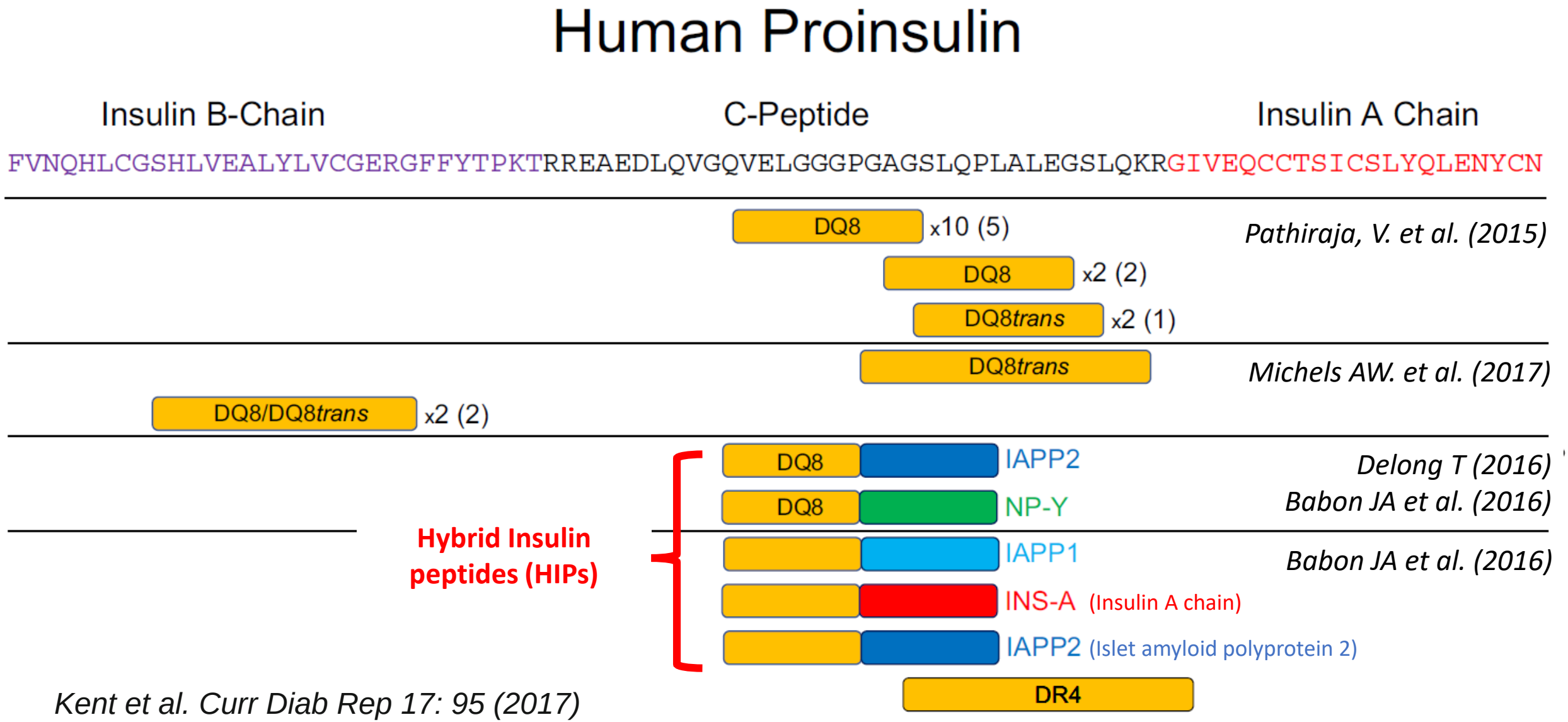
CD4+ T-cell responses were seen in 3/9 of HC (33%)

Finding the 'right' self antigen to generate a T-cell response

- Proinsulin
  - Major autoantigen in NOD mice — *Nature 2005; 435: 220–223*
  - Humans
    - Insulin gene locus is a T1D susceptibility gene — *Bennett 1995;Pugliese 1997*
    - Insulin –specific antibodies are first marker of pre-diabetes — *Ziegler 2013*
    - Insulitis only seen in islets with insulin positive cells — *Campbell-Thompson 2016*
    - Insulin-specific CD4+ T cells isolated from blood of recent onset T1D, and from islets and pancreatic lymph nodes of donors — *Mannering 2010, Kent 2005*
    - Same epitopes found from islets of different patients

Babon 2016, Nakayama 2017, Pathiraja/Mannering 2015

Proinsulin-derived epitopes recognized by human islet-infiltrating CD4+ T cells

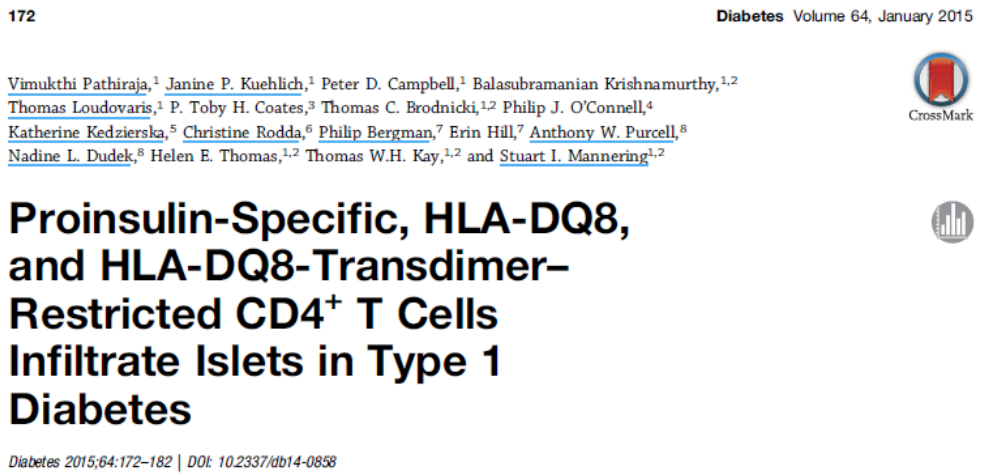


Kent et al. Curr Diab Rep 17: 95 (2017)

Preliminary Human T1D Data

Proinsulin specific CD4+ T cells in islets of people with T1D

- CD4+ T cell clones isolated from the islets of a deceased T1D donor
- These clones recognised epitopes from proinsulin, insulin's precursor.
- All the pro-insulin specific clones were restricted by HLA-DQ8, or HLA-DQ8 *trans*dimer that only forms with HLA-DQ2/DQ8 APCs



172 Diabetes Volume 64, January 2015

Vimukthi Pathiraja,[1] Janine P. Kuehlich,[1] Peter D. Campbell,[1] Balasubramanian Krishnamurthy,[1,2] Thomas Loudovaris,[1] P. Toby H. Coates,[3] Thomas C. Brodnicki,[1,2] Philip J. O'Connell,[4] Katherine Kedzierska,[5] Christine Rodda,[6] Philip Bergman,[7] Erin Hill,[7] Anthony W. Purcell,[8] Nadine L. Dudek,[8] Helen E. Thomas,[1,2] Thomas W.H. Kay,[1,2] and Stuart I. Mannering[1,2]

Proinsulin-Specific, HLA-DQ8, and HLA-DQ8-Transdimer–Restricted CD4$^+$ T Cells Infiltrate Islets in Type 1 Diabetes

Diabetes 2015;64:172–182 | DOI: 10.2337/db14-0858

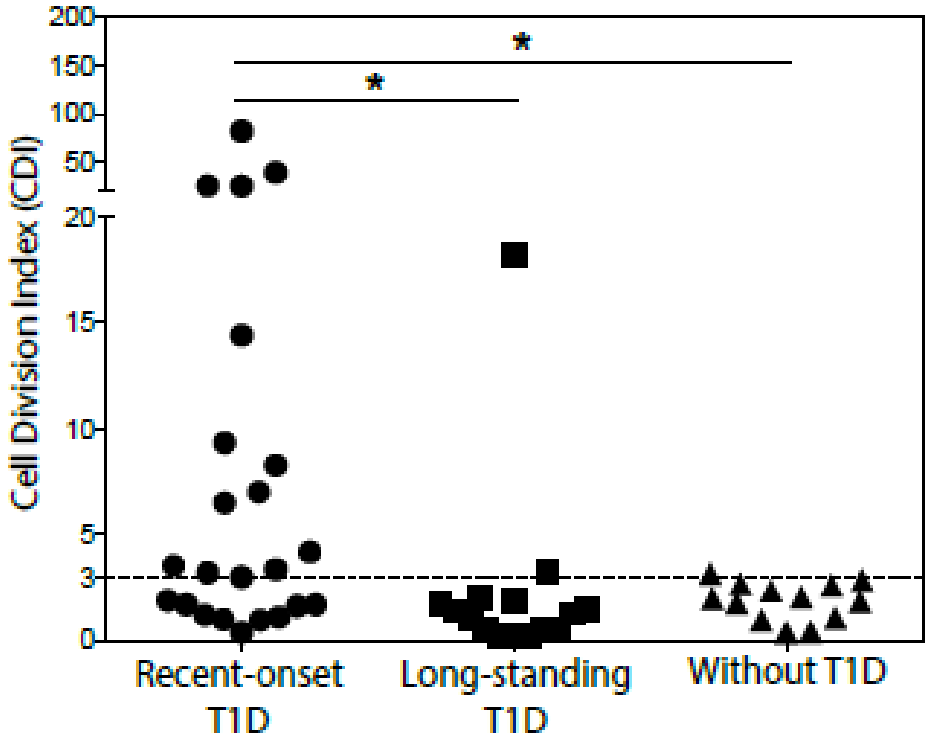
*Pathiraja, V. et al. Diabetes **64**, 172-82 (2015).*

Preliminary Human T1D Data

Proinsulin specific CD4+ T cells in peripheral blood of people with T1D

- CFSE labelled CD4+ T cell proliferation
  - Recent onset: < 100 days T1D diagnosis
  - Long standing: > 100 days T1D diagnosis
  - Healthy control: HLA matched
  - CDI (Cell division index)
    - = ratio of number of cells that have proliferated in response to antigen: the number of cells that have proliferated in absence of antigen.
  - CDI ≥2 traditionally considered a significant response;
  - CDI ≥3 was considered to improve the specificity of the results

Proinsulin response in HLA-DQ8^{+} patients



Michelle So, Stuart Mannering

Methods

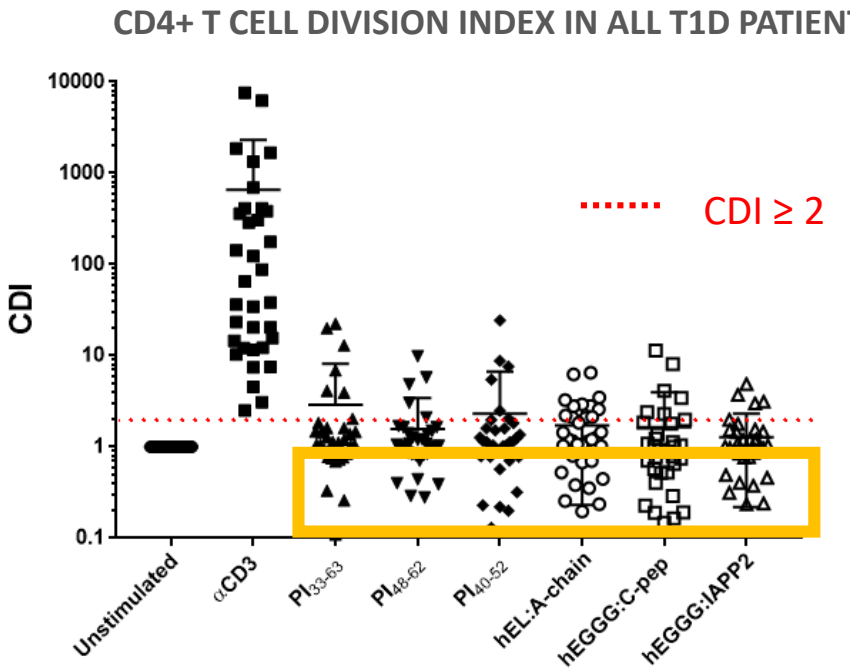
Subjects and Samples

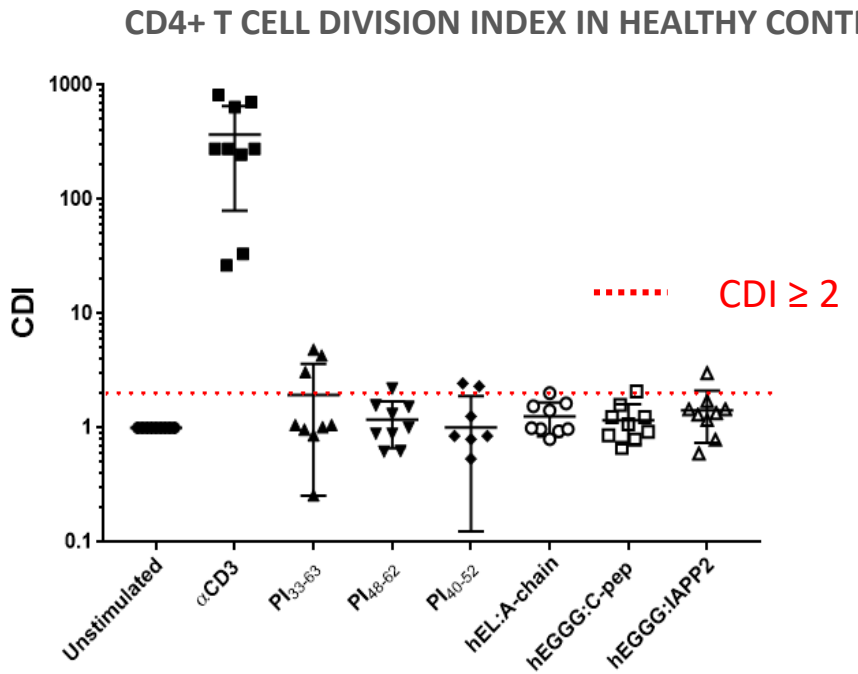
- Subjects
  - 200 children/adolescents from dedicated New Diagnosis TID clinic
  - Inclusion criteria:
    - Age 2-16y (male or female) at varying stages following T1D diagnosis
  - Exclusion criteria:
    - Auto-immune disease (except treated thyroid and coeliac disease)
- Participant Samples
  - 5-15 mls blood
    - HLA-typing
    - preparation of peripheral blood mononuclear cells (PBMC) for fresh CFSE labelled CD4+ T cell readouts in response to islet peptides
    - ± Cryopreservation

Future directions

Measuring cytokine responses

- In addition to cell proliferation, CD4+ T cells may produce cytokine responses to proinsulin
  - Particularly relevant to assessing longitudinal responses
  - ? Biomarker for use with frozen samples





Future directions

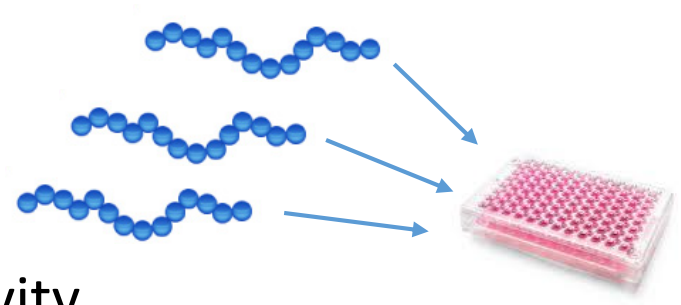
Measuring cytokine responses

Cytokine	Relevance in Type 1 Diabetes
IFN-γ*	Pro inflammatory • Promotes CD4+ T cell effector function • cytotoxic, cytostatic (inhibits insulin synthesis and secretion), or cytocidal actions to pancreatic islets
TNF-α*	
IL* 17a	Pro inflammatory • Enhances IL-1b, IFN-γ, and TNF-α-induced apoptosis in human islets
TGF-β (LAP*) and IL*-10	Anti inflammatory • Suppress T cell proliferation and DCs*, Inhibit effector T cell responses

* IFN-γ = Interferon-gamma, TNF-α = Tumour necrosis factor alpha, IL = interleukin, LAP = latency-associated peptide, TGF-β = transforming growth factor beta, DC = dendritic cell

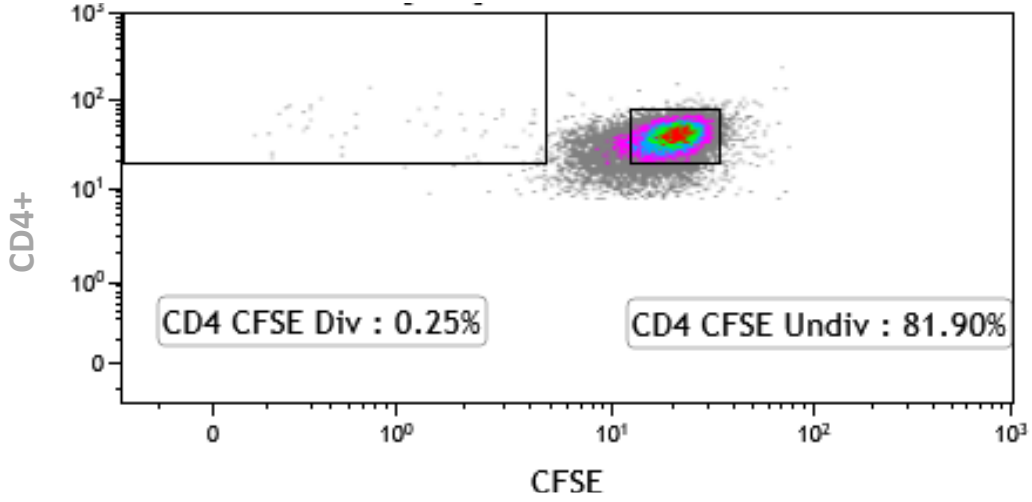
Procedures

Incubation Period with antigenic conditions and controls



- Optimal duration of 7 days
  - Shorter periods insufficient proliferation, reduced sensitivity
  - Longer periods increased background proliferation

CD4+ T CELL DIVISION AFTER 7 DAYS INCUBATION – UNSTIMULATED SAMPLE



CD4+ T CELL DIVISION AFTER 9 DAYS INCUBATION – UNSTIMULATED SAMPLE