Tractament immunomodulador de la diabetis mellitus tipus 1

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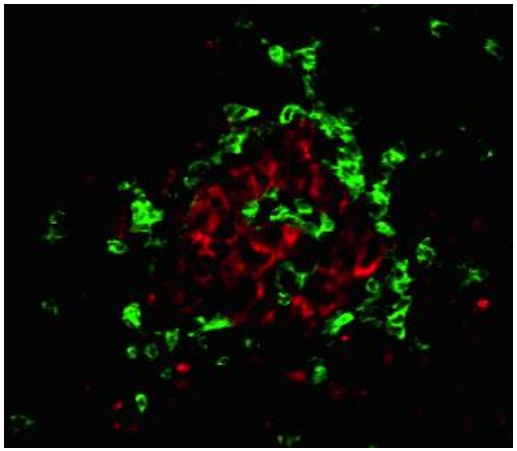
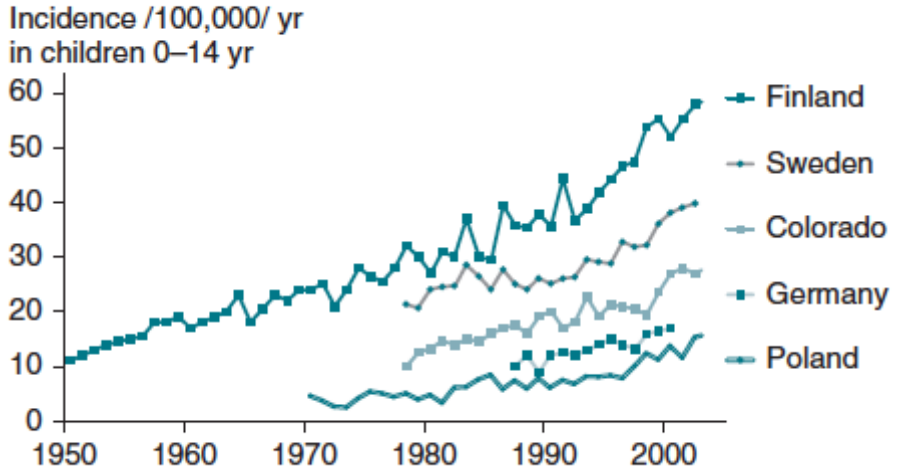
Type 1 diabetes is an autoimmune disease

Autoimmune disease characterized by β-cell destruction, resulting in a deficit in insulin secretion.

CD3 (T cells) / GAD (α-,β- cells)



Islet from a T1D patient (onset)
From Planas *et al.* Clin Exp Immunol, 2010

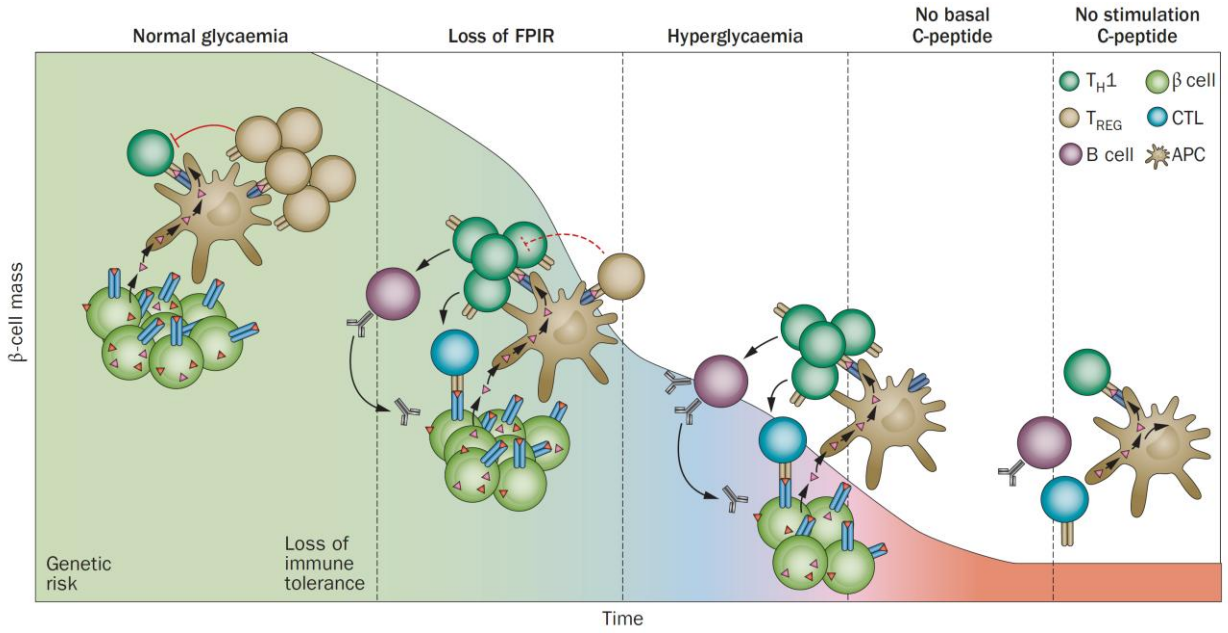


From International Textbook of Diabetes Mellitus, 4th Ed

- **No cure or prevention**
- **Lifelong insulin therapy**
- **Secondary complications**

- **Increasing incidence**
- **Peaking in ever-younger age groups**
- **Limited access to pancreatic tissue**

Immunopathogenesis of T1D



From Roep B & Tree T, 2014, Nat Rev Endocrinol

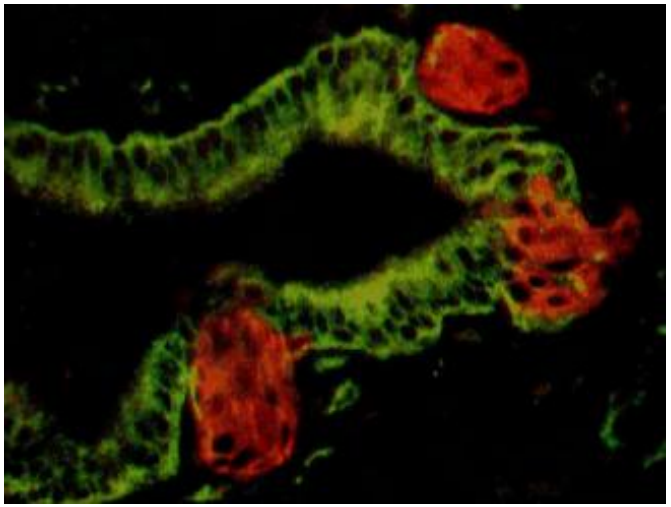
- **Unknown etiology: polygenic disease + environmental factors**
- **Lack of tolerance to β-cells**
- **Silent β-cell loss (prediabetes)**
- **Autoantibodies (biomarkers)**
- **Clinical onset**
- **Inflammation / Regulatory mechanisms**
- **Regeneration**

Immune balance - islet autoreactive T cells are kept in check by immune regulation. Unidentified factors – loss of tolerance and activation of APCs and autoreactive T cells.

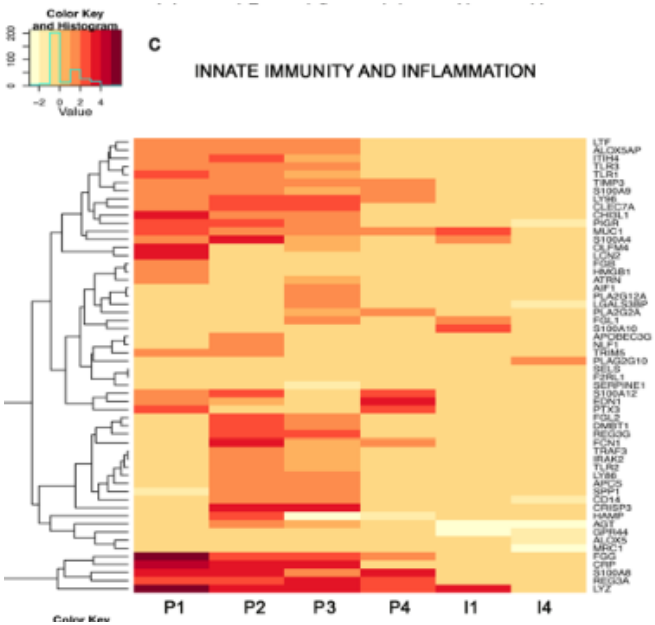
Activation of B cells (islet autoantibodies) and effector T cells (kill β-cells), and recruitment of inflammatory cells in the islets. Declining functional β-cell mass and impaired glucose response.

Immune regulation is outweighed by islet autoreactivity: clinical manifestation of T1D and hyperglycaemia.

T1D as a relapsing-remitting disease



From Somoza N et al, J Immunol 1994



From Planas R et al, Clin Exp Immunol 2010

- **β-cell mass and function is maintained before diagnosis, and declines rapidly at clinical onset**
  Rodriguez-Calvo T, Diabetes, 2017

- **β-cell regeneration in T1D patients**
  Somoza, J Immunol 1994

- **Chronicity of AI: β-cell function and ongoing autoimmunity in long-standing T1D**
  Planas, Clin Exp Immunol 2010 / Meier, Diabetologia 2005 / Williams GM, 2016, Diabetologia

- **T1D as a relapsing-remitting disease?**
  von Herrath, Nat Rev immunol 2007

Immunotherapies for T1D

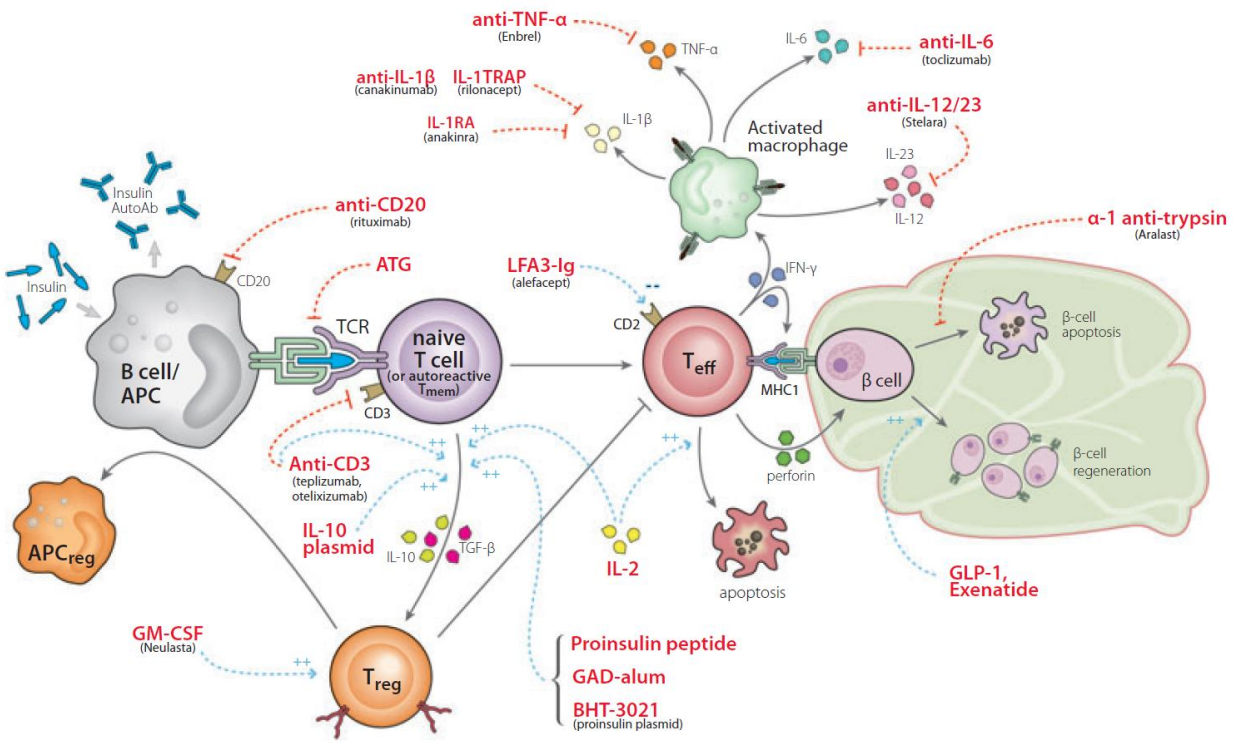
1920s — **Insulin**

1980s — **Immunosuppressors**

1990s — **Systemic immune modulators**

(Anti-CD3, ATG, Anti-CD20, CTLA4-Ig, LFA3-Ig, Anti-IL-1RA or Anti-IL-1β, TNFR, VitD)

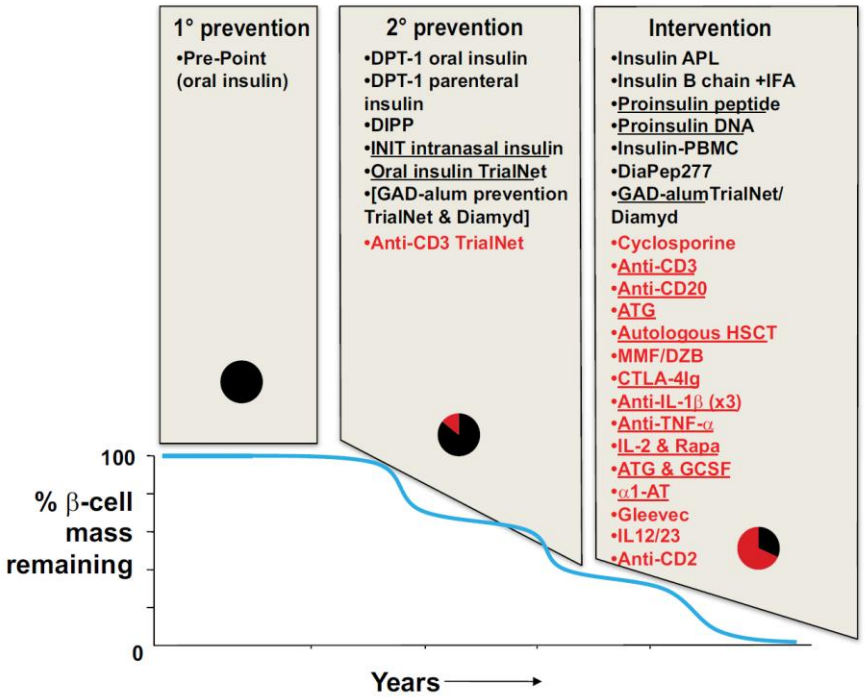
2000s — **Ag-specific** (INS, GAD, HSP60); **Cell-based** (MSCs, Tregs, TolDCs)



Adapted from Matthews JB, 2010, Clin Exp Immunol

Immunotherapies for T1D – Systemic

Temporary delay in β-cell function loss



From Peakman & von Herrath, 2010, Diabetes

Teplizumab, Otelixizumab*
*Fail to reach study end point - Phase III
Rituximab (Pescovitz et al. J Allergy Clin Immunol 2011)
Etanercept (Mastrandrea *et al*. Diab Care 2009)
IFN-α (Rother *et al*. Diab Care 2009)
Abatacept (Orban *et al*. Lancet 2011),
Alefacept (Rigby *et al*. J Clin Invest 2015)
BCG (Faustman et al. Plos One 2012)
ATG (Gitelman et al. Lancet Diabetes Endocrinol 2013)
Low IL-2 (Hartemann et al. Lanc Diab Immunol 2013)
Alpha-1 antitrypsin (Rachmiel et al. Ped Diab 2016)

- **Immune suppression rather than modulation**

e.g. **Rituximab** (Pescovitz *et al*. J Allergy Clin Immunol 2011)

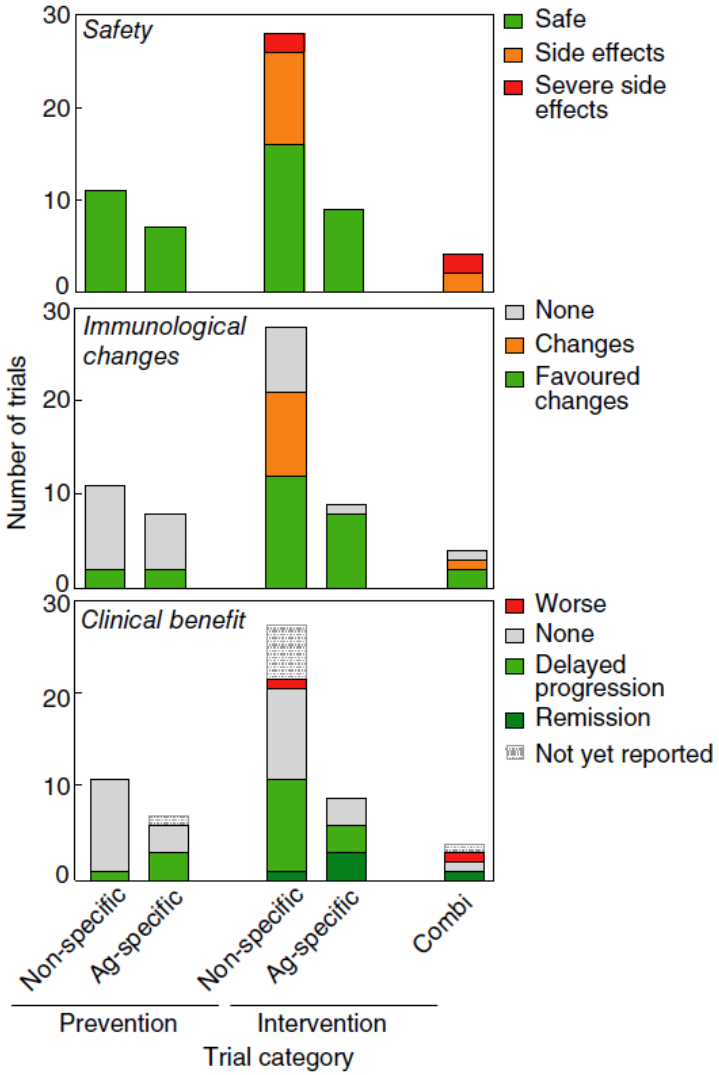
- **Preventing priming/spreading of the immune response *vs* affecting existing adaptive immunity**

e.g. **Abatacept** (Orban *et al*. Lancet 2011)

- **Interfering in effector cytokines?**

e.g. **TNF-α blockade** (Mastrandrea *et al*. Diabetes Care 2009); **IL-1 blockade** (Moran *et al*. Lancet 2013).

Immunotherapies for T1D – Antigen Specific



From von Herrath, Peakman M & Roep B, 2013, Clin Exp Immunol

- **Subcutaneous insulin** in prevention (Vandemeulebroucke *et al*. Diabetes Metab 2009) – No effect!
- **Nasal insulin** in prevention trials (Nanto-Salonen *et al*. Lancet 2008) – (Phase II) Fail!
- **Oral insulin** in prevention or new-onset T1D (Skyler *et al*. Diabetes Care 2005) – Fail!

* Repeated prevention trial in progress

- **GAD-alum** in new onset T1D - (Phase III) Fail!

*secondary prevention in progress

- **Diapep277** in new onset T1D - (Phase III) glucagon-stimulated test (GST) (Raz/Pozilli, et al. 2014) – retracted! (Diabetes Care 2015)
- **PI (C19-A3) peptide** – (Phase Ia) Safe in long-standing T1D (Thrower et al. Clin Exp Immunol 2008)

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Immunotherapies for T1D – Antigen Specific

Can antigen-based immunotherapies prevent progression of recent onset autoimmune diabetes?

Challenge	Discussion of issues
Setting for clinical trials	Traditionally, new therapies are trialled in the intervention setting (i.e. at disease onset). Disease reversal using antigen alone at this stage will most probably be difficult. Prevention studies are long duration and expensive; but without hints of efficacy as an intervention, will prevention studies be undertaken?
Dose	Both high- and low-dose immunological tolerance has been described, probably equating to predominantly deletional and regulatory mechanisms; which is better, and whether both effects could be harnessed, is not known, however
Regime	Frequent (daily) dosing has been the norm until now (e.g. for intranasal and oral insulin), but again this may favour deletion over regulation [27]
Adjuvants and enhancing combinations	A poorly explored area in general, despite encouraging data in preclinical models (e.g. anti-CD3 plus antigen; see Table 5)
Agent	It has yet to be determined whether whole antigens or fragments are superior; similarly, whether protein or DNA-based delivery is better; free peptide or complexed to peptide–human leucocyte antigen multimers or nanoparticles
Route of administration	Parenteral or oral/nasal routes predominate, but the relative advantages of either have not been explored head-to-head
Staging and stratification	Oral insulin appears effective in the subgroup of patients with high titres of insulin autoantibodies; is this a general principle for ASI?
Preclinical models	As a generalization, ASI works well if given early enough in disease models; but trialling the human antigens in humanized models is an under-developed area
Role of industry and biotech	Antigens face the dual challenges of being difficult to develop with robust intellectual property and having a clear route to market and have therefore been less favoured for commercial development than biologics and other immune modulators

From von Herrath, Peakman M & Roep B, 2013, Clin Exp Immunol

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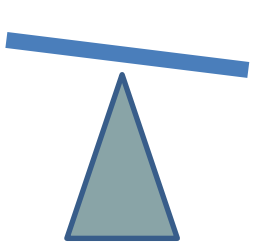
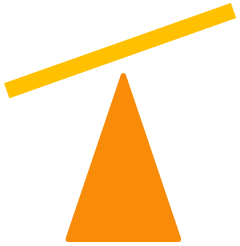
Immunotherapies for T1D

AIM: To stop autoimmune reaction in T1D



Healthy individuals



T1D patients

Imbalances between immune regulation and autoimmunity

Antigen-Specific Immunotherapies for T1D

- **Cell-based therapy**
- **Liposome-based therapy**
- **Peptide immunotherapy**

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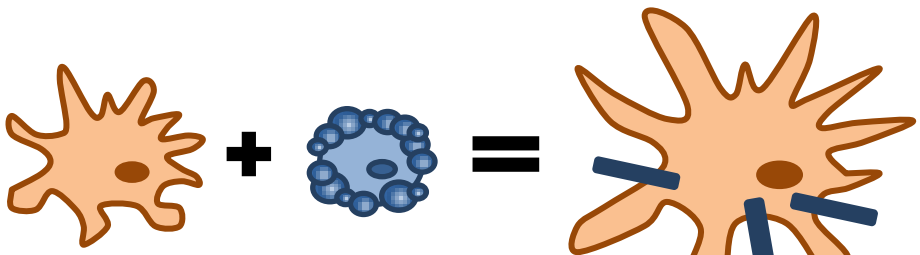
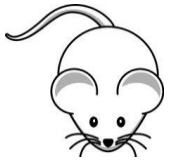
First approach: Dendritic cell-based immunotherapy



DCs

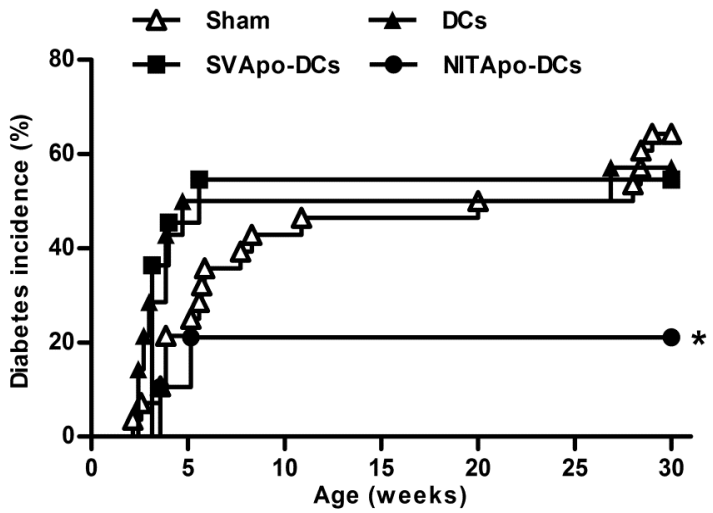
Apoptotic NIT-1 cells

TolDCs

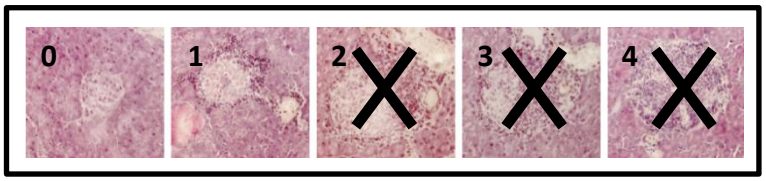


Non-Obese Diabetic (NOD) mouse

- ↓ Costimulation (CD40, CD86)
- ↓ Proinflammatory cytokines (IL-6, TNF-α)
- ↑ Prostaglandin E_2
- ✓ Stability
- ✓ Impaired autologous T cell proliferation
- ✓ Reduced Th1/Th17 polarization
- ✓ Suppressive ability



I. Pujol-Autonell, et al., PLOS ONE, 2012



S. Marín-Gallén, et al., Clin Exp Immunol, 2010

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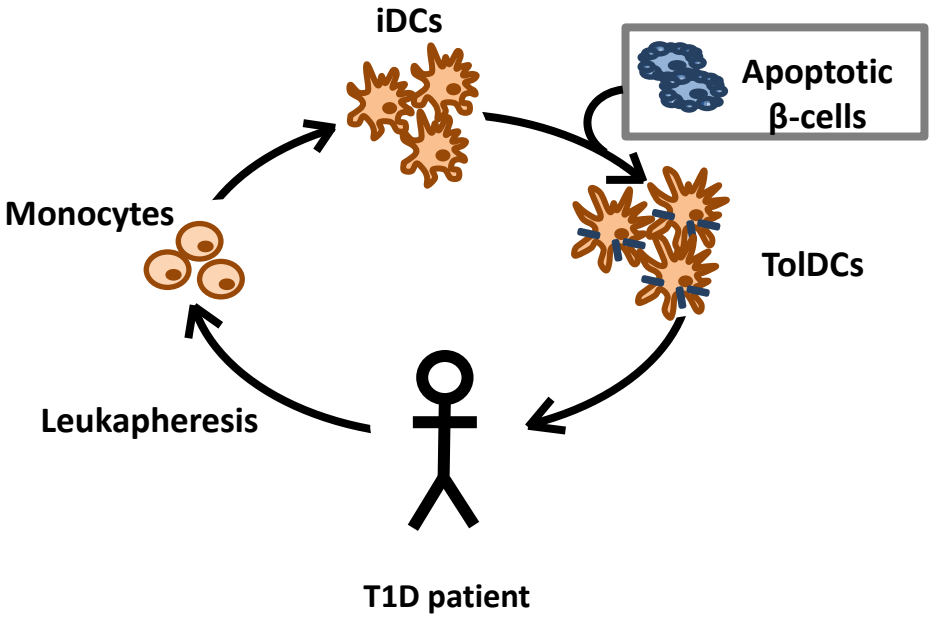
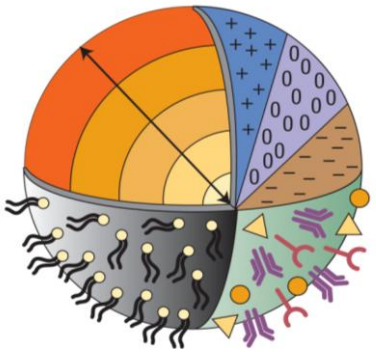
From dendritic cells to liposomes



How can we obtain apoptotic β-cells for the clinical use?

-Synthetic approach: liposomes ✓



Charge

Size

Hydrophobicity

Targeting

Adapted from Dobrovolskaia M, 2007, Nat Nanotechnol

Emerging Treatments and Technologies
ORIGINAL ARTICLE

Phase I (Safety) Study of Autologous Tolerogenic Dendritic Cells in Type 1 Diabetic Patients

Giannoukakis *et al*. Diabetes Care 2011

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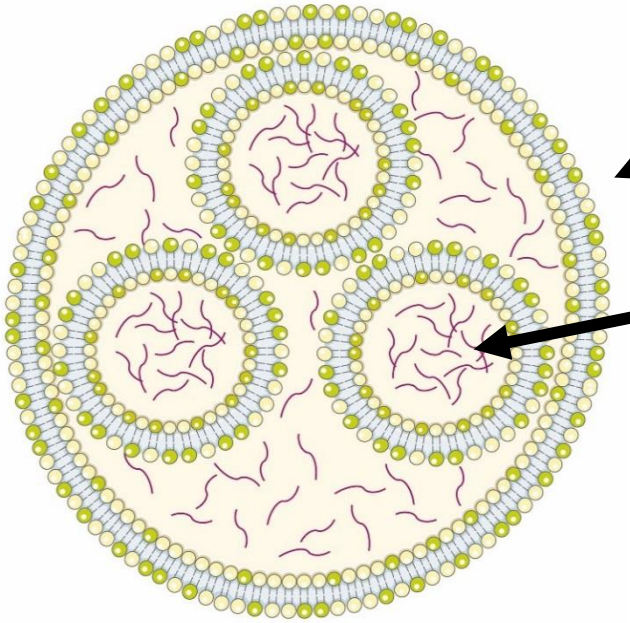
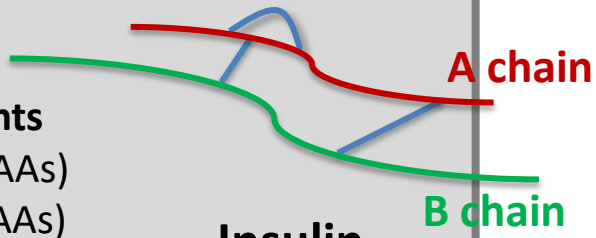
Phosphatidylserine-containing liposomes

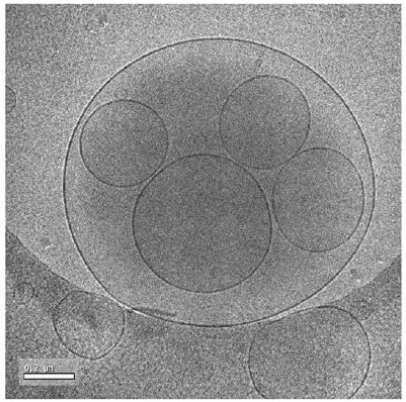


Membrane composition
PS:PC:CH

Autoantigenic contents
- **Insulin A chain** (21 AAs)
- **Insulin B chain** (30 AAs)



ICN2 Institut Català de Nanociència i Nanotecnologia



	Particle size (nm)	Polydispersity index (PdI)	Zeta potential (mV)	Encapsulation efficiency (%)
PS-liposomes	996.71 ± 89.42	0.31 ± 0.05	-29.26 ± 2.82	-
PSA-liposomes	1051.43 ± 45.15	0.31 ± 0.06	-30.79 ± 2.35	41.07 ± 23.58
PSB-liposomes	968.57 ± 86.32	0.27 ± 0.08	-29.44 ± 1.48	87.44 ± 4.54

Data are expressed as mean ± SD.

I. Pujol-Autonell, et al., PLOS ONE, 2015

PS-liposomes display MVV (multivesicular vesicles) morphology.

European patent filed

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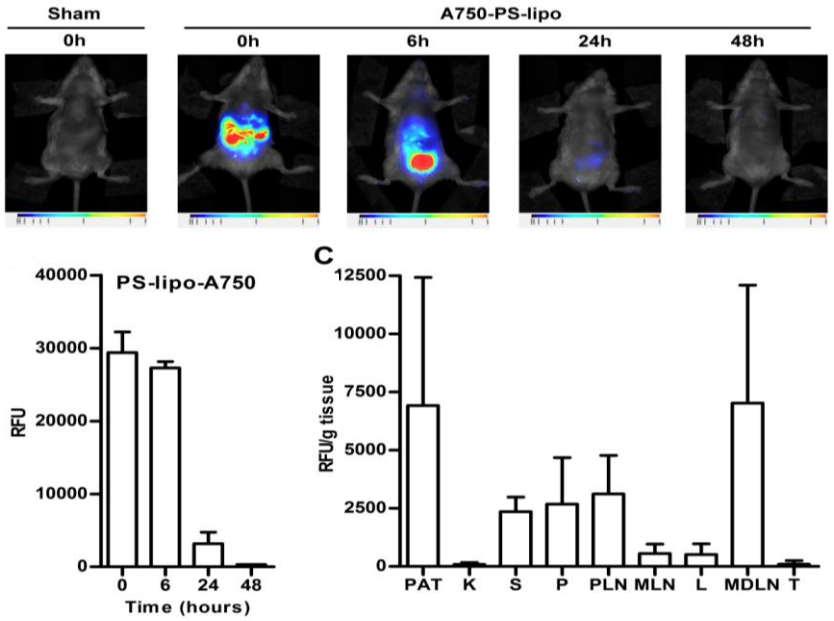
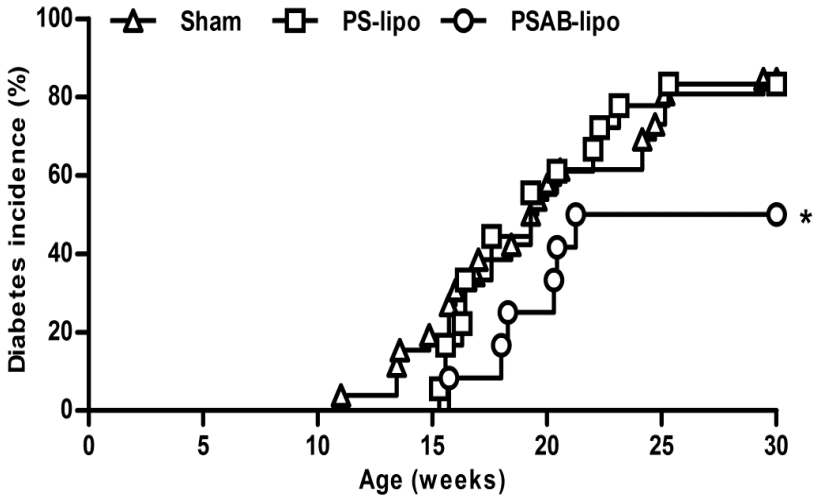
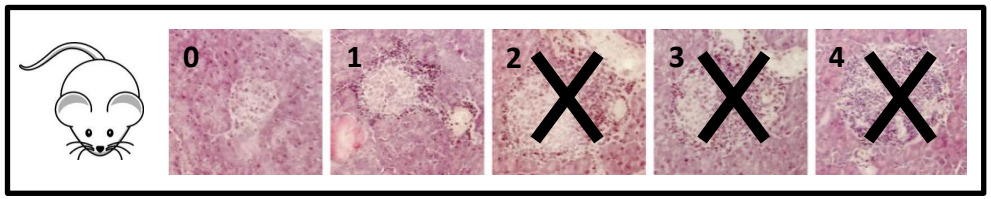
Autoantigen-loaded PS-liposomes prevent T1D



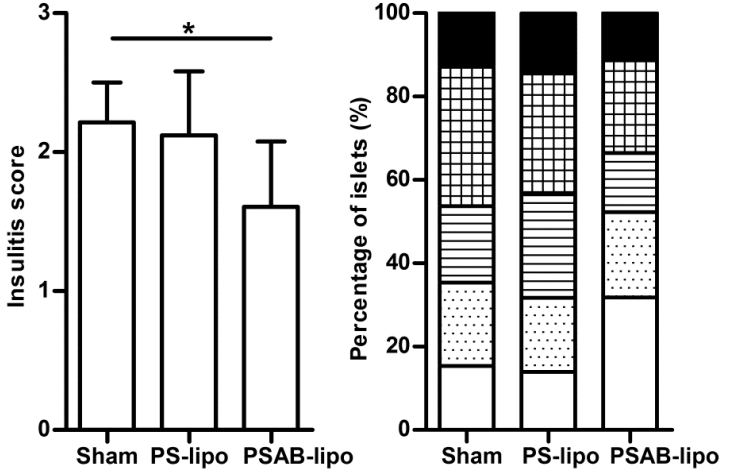
n=3, mean+SD



n= 26 (Sham), 18 (PS-lipo), 12 (PSAB-lipo)



I. Pujol-Autonell, et al., PLOS ONE, 2015



n=3-6 mice, mean+SD

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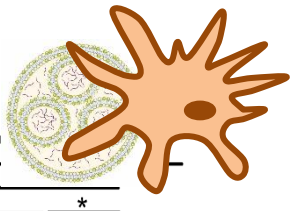
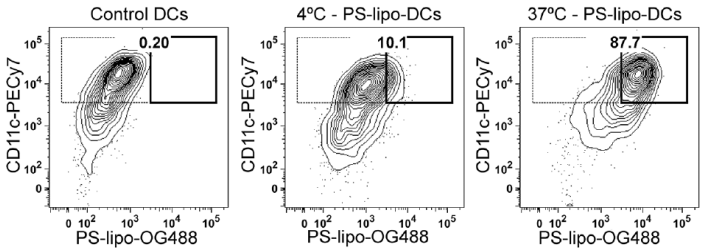
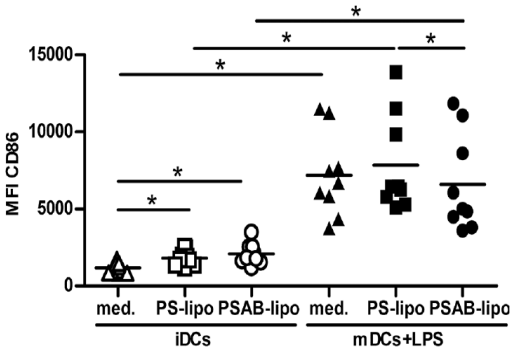
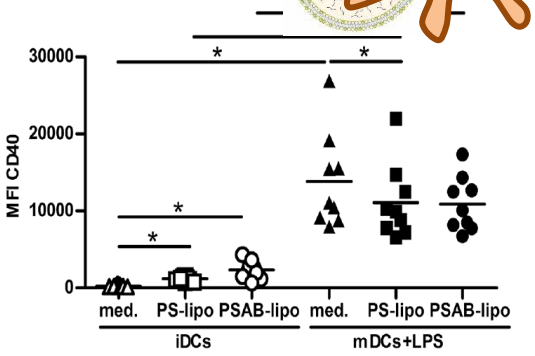
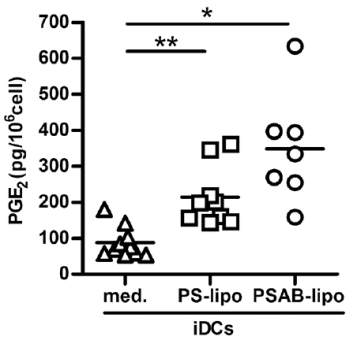
PS-liposomes induce tolDCs



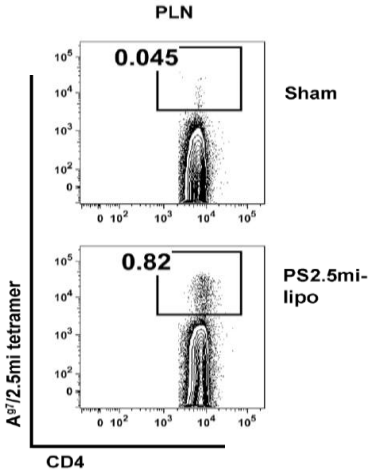


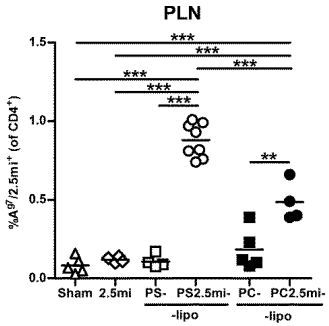


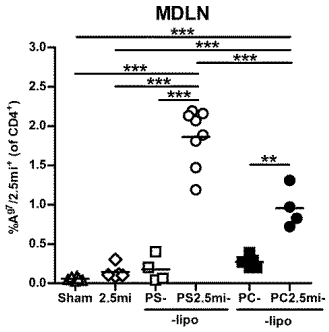


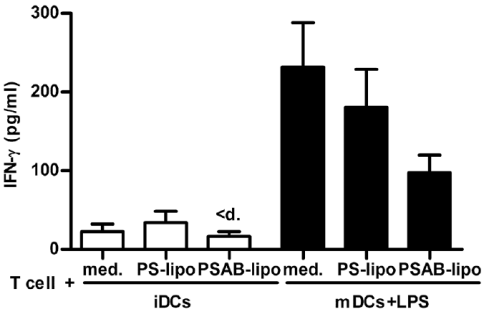


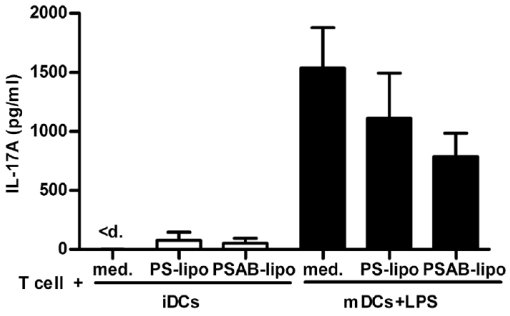
✓ **Efferocytosis promotes suppressive effects in DCs involving prostaglandin E_2 production**











✗ **Th1/Th17**

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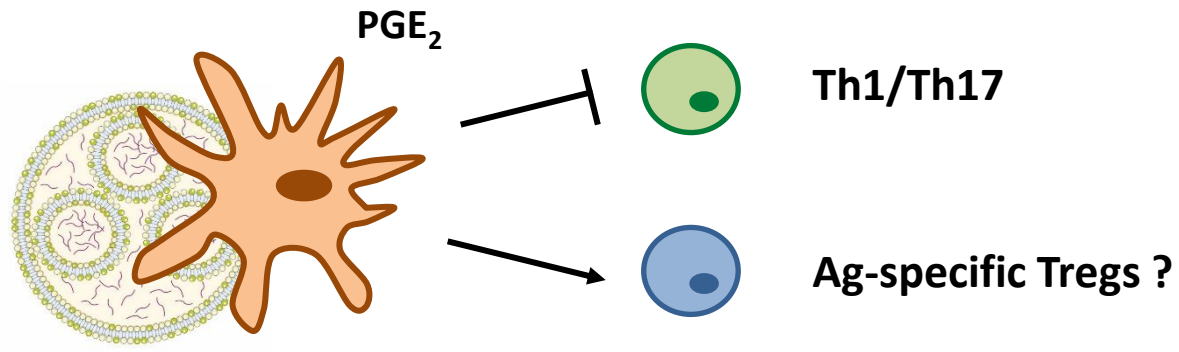
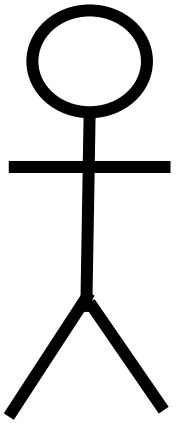
I. Pujol-Autonell, et al., PLOS ONE, 2015

Autoantigen-loaded PS-liposomes arrest autoimmunity in T1D



DCs can be programmed to induce specific **immune tolerance** using **nanovesicles** mimicking apoptotic bodies from β-cells (phosphatidylserine-liposomes loaded with insulin peptides) resulting in the re-education of autoreactive T cells and the arrest of the autoimmune aggression.

These liposomes can offer a solution to the complexity of cell-based therapies with many benefits, such as being low-cost and easy to standardize, large-scale production and customization.



- Tolerogenic potential validated in another AI disease (induced MS in mice) (Pujol-Autonell I *et al*. Nanomedicine, 2017, *under review*)

- Validation in human cells *in vitro* in progress ……

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Peptide Immunotherapy

Clinical & Experimental Immunology
The Journal of Translational Immunology
British Society for immunology

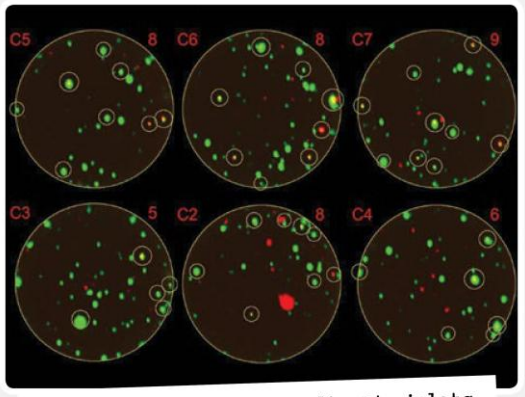
Clinical and Experimental Immunology | ORIGINAL ARTICLE | doi:10.1111/j.1365-2249.2008.03814.x

Proinsulin peptide immunotherapy in type 1 diabetes: report of a first-in-man Phase I safety study

S. L. Thrower,* L. James,† W. Hall,†
K. M. Green,‡ S. Arif,† J. S. Allen,†
C. Van-Krinks,† B. Lozanoska-Ochser,†
L. Marquesini,* S. Brown,§
F. S. Wong,§ C. M. Dayan* and
M. Peakman†¶

Safety *and* ***mechanistic*** *outcomes during* ***first-in-man intradermal*** *administration of a human leucocyte antigen-DR4 (HLA-DR4)-restricted peptide epitope of* ***proinsulin (C19-A3)*** *in patients with* ***long-standing T1D****.*

- ✗ **Systemic hypersensitivity**
- ✗ **Ag-specific proinflammatory response**
- ✓ **Antigen-specific IL-10 response in low dose group**



Helper T cells responding to islets

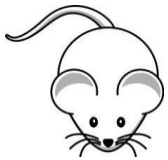
MonoPepT1De (2012 – 2015)

Safety study to assess whether proinsulin peptide injections can slow or stop the body damaging its own insulin-making cells in the pancreas in patients newly diagnosed with T1D.

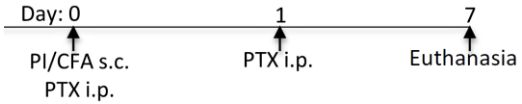
MultiPepT1De (2015 – 2017)

Safety and tolerability study of the administration of multiple islet peptide administration in patients with type 1 diabetes.

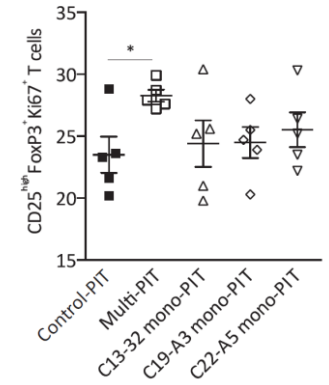
Peptide Immunotherapy

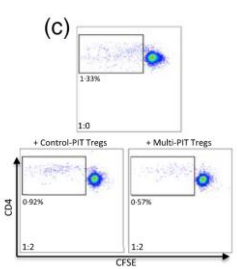


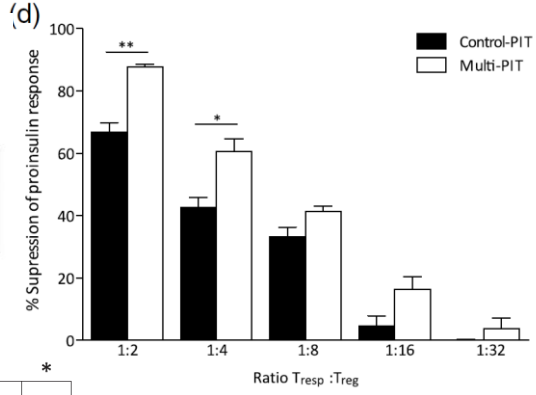
humanized HLADRB1*
0401 transgenic HLA-DR4 Tg mouse

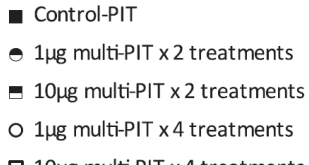


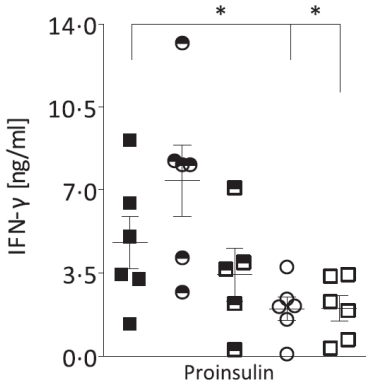
- PI-sp T cell Pr
- IFN-γ
- Auto-Ab

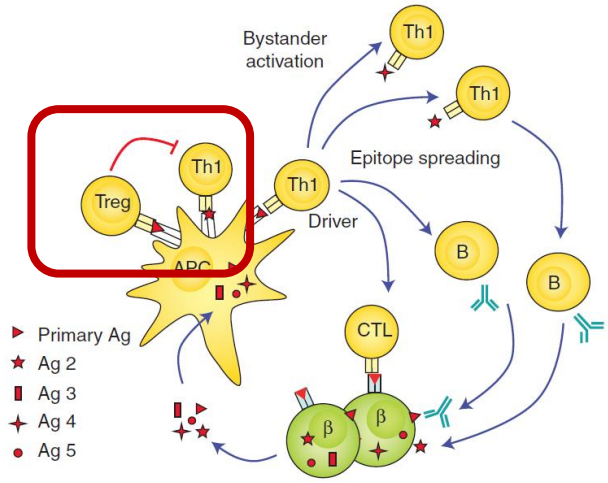












From Roep B & Peakman M, 2012, Cold Spring Harb Persect Med

- Intradermal proinsulin-peptide injection controls autoimmunity in association with enhanced proliferation of regulatory FoxP3^{+}CD25^{high}CD4 T cells.

- The success of multi-peptide immunotherapy depends upon the number of peptides used and frequency of dosing

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Peptide Immunotherapy – Immunological Biomarkers

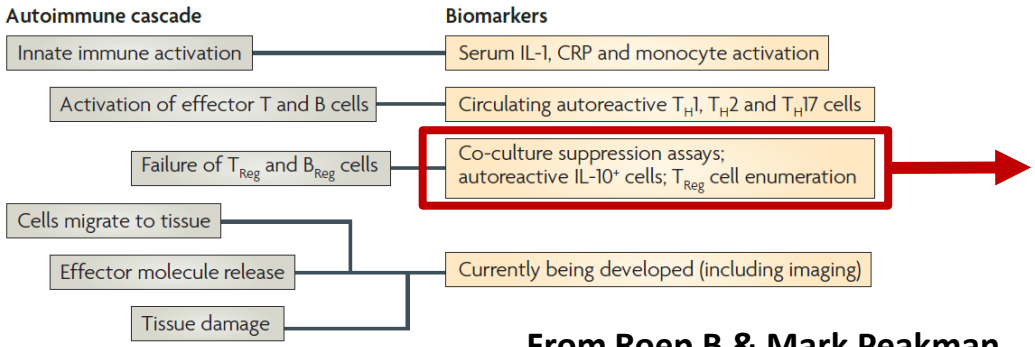
Is the therapy safe or is there a risk of adverse events?

Is there a noticeable effect of the drug on the immune system?

Does the effect represent immunological efficacy?

Does this reflect therapeutic efficacy?

UTILIZING IMMUNE RESPONSES AS DISEASE BIOMARKERS



From Roep B & Mark Peakman, 2010, Nat Reviews

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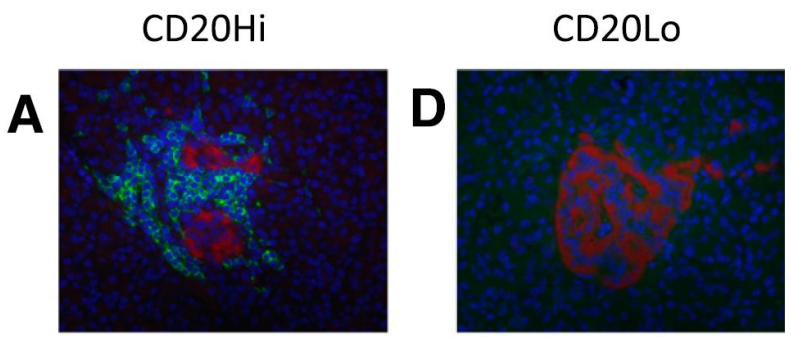
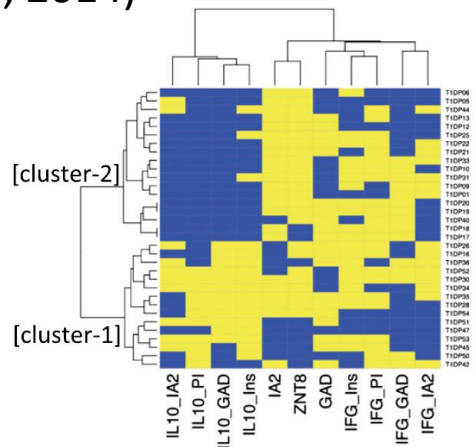
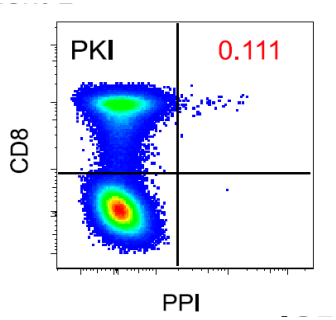
Characterizing the autoreactive T cells

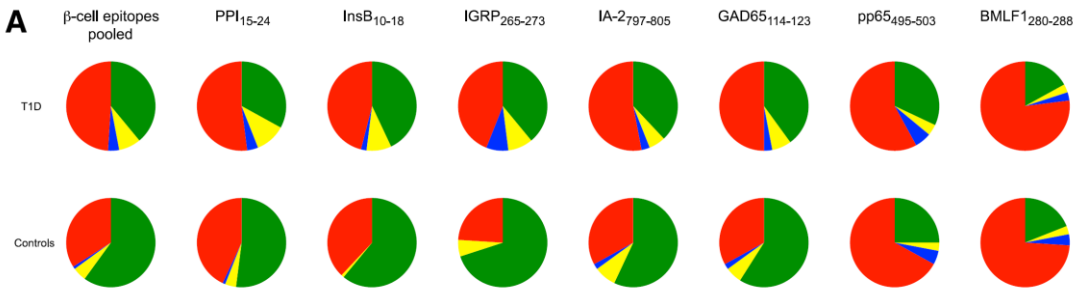
- **Regulatory phenotype in autoreactive T cell responses in health** (Arif *et al.*, 2004)
- **IL-17 signature in T1D, with promotes β-cell death** (Arif *et al.*, 2011)
- **Immunological heterogeneity in T1D** (Arif *et al.*, 2014)

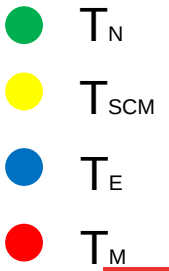




- **More Ag-experienced in T1D** (Skowera *et al.*, 2015)







- **Stronger pro-inflammatory bias in the young** (Arif *et al.*, 2017)

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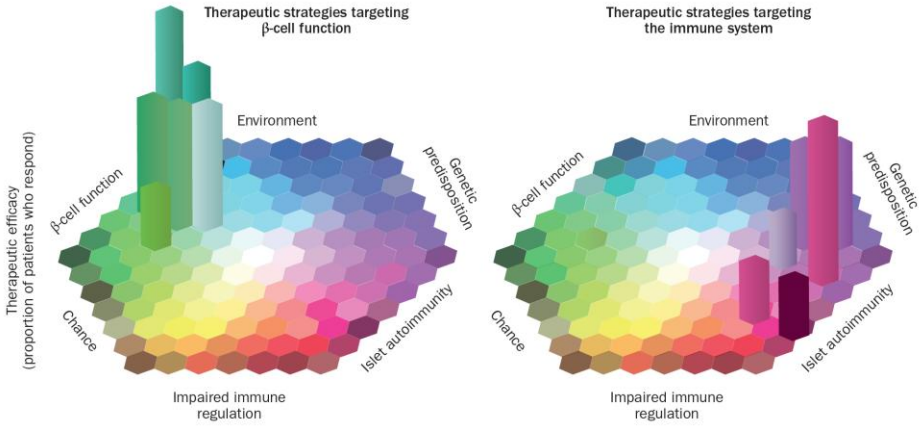
Trial designs for type 1 diabetes

DISEASE HETEROGENEITY

* Rituximab - greater response in children/adolescents (Pescovitz *et a*l. N Engl J Med 2009)
* Abatacept - appeared to worsen clinical outcome in African American subjects (Orban T *et al*. Lancet 2011)
* Teplizumab (Phase II) - metabolic and immunologic features at baseline identify a subgroup of responders (Herold *et al*. Diabetes 2013)

- **Better patient stratification for given treatment (include immunological parameters?)**
- **Need for immunological biomarkers (possible study end-points?)**
- **Combination with β-cell regeneration?**



From Roep B & Tree T, 2014, Nat Rev Endocrinol

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Thank you!

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