



HAL
open science

Self-antigens, benign autoimmunity and type 1 diabetes: a beta-cell and T-cell perspective

Fatoumata Samassa, Roberto Mallone

► To cite this version:

Fatoumata Samassa, Roberto Mallone. Self-antigens, benign autoimmunity and type 1 diabetes: a beta-cell and T-cell perspective. *Current Opinion in Endocrinology, Diabetes and Obesity*, 2022, 29 (4), pp.370-378. 10.1097/MED.0000000000000735 . inserm-03834212

HAL Id: inserm-03834212

<https://inserm.hal.science/inserm-03834212>

Submitted on 29 Oct 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Self-antigens, benign autoimmunity and type 1 diabetes: a beta-cell and T-cell perspective

Fatoumata Samassa¹ and Roberto Mallone^{1,2}

¹Université de Paris, Institut Cochin, CNRS, INSERM, Paris, France.

²Assistance Publique Hôpitaux de Paris, Service de Diabétologie et Immunologie Clinique, Cochin Hospital, Paris, France.

Corresponding author: Roberto Mallone, MD PhD - INSERM U1016 Cochin Institute, G.H. Cochin-Port-Royal, Bâtiment Cassini - 123, boulevard de Port-Royal – F-75014 Paris, France.
Phone: +33-1-76.53.55.83 E-mail: roberto.mallone@inserm.fr.

Word count: 3,193

Figures: 4

Abstract

Purpose of review: recent work using immunopeptidomics and deconvolution of the antigenic reactivity of islet-infiltrating CD8⁺ T cells has expanded our knowledge about the autoimmune target epitopes of type 1 diabetes. The stem-like properties of autoimmune CD8⁺ T cells have also been described. We here propose a possible link between these findings.

Recent findings: weak major histocompatibility complex (MHC)-binding epitopes list among the major targets of human islet-infiltrating CD8⁺ T cells, likely resulting in low peptide-MHC presentation that delivers weak T-cell receptor (TCR) signals, especially in the face of low-affinity autoimmune TCRs. These weak TCR signals may favor the maintenance of the partially differentiated stem-like phenotype recently described for islet-reactive CD8⁺ T cells in the blood and pancreatic lymph nodes. These weak TCR signals may also be physiological, reflecting the need for self-peptide-MHC contacts to maintain homeostatic T-cell survival and proliferation. These features may underlie the universal state of benign autoimmunity that we recently described, which is characterized by islet-reactive, naïve-like CD8⁺ T cells circulating in all individuals.

Summary: these observations provide novel challenges and opportunities to develop circulating T-cell biomarkers for autoimmune staging. Therapeutic halting of islet autoimmunity may require targeting of stem-like T cells to blunt their self-regeneration.

Keywords: epitope, exhaustion, immune synapse, MHC, stem-like T cells.

Introduction

Increasing efforts are being invested to provide a comprehensive catalog of the self-antigens exposed by pancreatic beta cells that become autoimmune targets in type 1 diabetes (T1D). This catalog would provide critical information for detecting the corresponding T cells and auto-antibodies to improve disease staging, for developing antigen-specific immunotherapies, and for understanding T1D pathogenesis. Major gaps in knowledge exist, as there have been few systematic discovery studies to map this antigenic landscape. This is particularly true for T cells, which are the key actors in T1D pathogenesis and recognize peptides bound to and presented by HLA molecules (Class I for CD8+ T cells, Class II for CD4+ T cells). Typically, T-cell antigen discovery has been inherently biased by its reliance on prior knowledge of the same antigens being targeted by auto-antibodies, or by T cells found in islet infiltrates and pancreatic lymph nodes (PLNs) in the non-obese diabetic (NOD) mouse. Other efforts have been eminently hypothesis-driven, e.g. by looking at particular post-translational modifications (e.g. transglutamination, citrullination) or at processes such as defective ribosomal scanning that may generate neo-antigens. A unifying picture is missing. We will here discuss recent advances and some common emerging themes, and highlight controversial or understudied aspects that deserve further scrutiny.

What makes an antigenic epitope a solid one?

A recent meta-analysis [*1] of the T-cell epitopes identified to date has proposed a ranking based on the degree of available evidence. This classification considers 3 key features (Fig. 1):

- 1) The source of the T cells found to recognize a given epitope: humanized mice, e.g. transgenically expressing human HLA molecules, have been extensively used [2-5]. Most work has however focused on human tissues, with two different levels of evidence depending on whether

the studied T-cell source is peripheral (i.e. blood, spleen) or in the target organ (i.e. PLNs, pancreas). Arguably, the finding of T cells at the site of pathology adds an important argument for the relevance of the antigens identified.

2) The demonstration that the epitope is naturally processed and presented by target beta cells and/or antigen-presenting cells. This is important to avoid the pitfall of identifying antigens that are predicted *in silico* but may not exist in the real world, simply because they cannot be produced and exposed on the cell surface. Without this evidence, the odds of finding serendipitous, irrelevant T-cell reactivities are high, which reflect the broad cross-reactivity of T cells [6,7]. A teaching example of this cross-reactivity is provided by our recent work analyzing the recognition of HLA-A2-restricted epitopes in donors that express or not the HLA-A2 restriction element [8]. When looking at the recognition of self or viral epitopes recognized by naïve CD8⁺ T cells (reflecting lack of autoimmune disease or lack of exposure to the source viruses), the frequency of these T cells was similar in HLA-A2⁺ and negative donors. This exemplifies the permissiveness of the natural T-cell repertoire for recognition of disparate peptide-HLA (pHLA) complexes, even those that HLA-A2-negative donors will never encounter.

3) The isolation of T cells recognizing the epitope provides another strong evidence, as it allows more in-depth investigations to confirm antigen specificity.

Our approach to antigen discovery: strengths and limitations

We recently launched a systematic epitope discovery effort by focusing on the antigenic peptides that are naturally processed and presented by target beta cells [9]. With the exception of a similar study performed on murine beta-cell lines and primary islets from NOD mice [10], this information was not available from previous literature. This strategy is based on immunopeptidomics

techniques, which require the isolation of pHLA complexes from cell lysates by immunoaffinity purification, followed by peptide elution and identification by mass spectrometry. This work provided a first cartography of the peptides naturally processed and presented by beta cells, with some important messages. First, several known antigenic proteins and peptides were represented, particularly those derived from preproinsulin that have been more extensively studied. Second, some known antigens were marginally represented, with very few or no peptides identified, e.g. 1/each for GAD65 and ZnT8 and none for IGRP and IAPP. Third, secretory granules contributed one third of the total number of source proteins, in line with the functional specialization of beta cells to secrete insulin. Fourth, some novel granule antigens were identified, such as secretogranin-5, urocortin-3 and proconvertase-2. Fifth, some neo-antigens ([Fig. 2A](#)) derived from protein or mRNA splicing were found.

The exposure of these HLA-bound peptides by beta cells is a prerequisite, but does not warrant autoimmune recognition if the T-cell repertoire is not poised to do so. The second step of this work was therefore to test which of these peptides were recognized by circulating CD8+ T cells in T1D patients and age/sex-matched healthy controls, using peptide-loaded HLA Class I (HLA-A2 and -A3) multimers [9,*11]. Results were surprising: the frequency of these CD8+ T cells recognizing the peptides identified was similar irrespective of disease status – an observation that we previously reported for known islet antigens [8]. Moreover, a large fraction of these self-reactive CD8+ T cells displayed a naïve-like (CD45RA+CCR7+) phenotype. The overall frequency of these T cells was in line with this naïve status, as it fell in a predictable range (1-50/10⁶ CD8+ T cells, i.e. 0.0001-0.005%) previously reported for viral-reactive naïve CD8+ T cells [12]. CD8+ T cells recognizing these same peptides were instead enriched in the pancreas of T1D vs. healthy donors and displayed CD45RO expression compatible with an effector/memory phenotype,

suggesting their pathogenic relevance. Such relevance was directly demonstrated in the NOD mouse, where CD8⁺ T cells recognizing the murine orthologs of the novel granule antigens identified were found to infiltrate the islets of prediabetic animals, and to transfer disease in NOD/*scid* mice [*11].

Based on these results, we proposed the existence of a universal state of “benign” islet autoimmunity hardwired in all individuals [13,*14], which we and others traced back to a marginal deletion of autoreactive T cells in the thymus [8,15]. This benign autoimmune state is characterized by the circulation of naïve-like autoreactive CD8⁺ T cells, which become activated and sequestered in the pancreas only in T1D patients. The progression of this benign state to T1D may rely on two non-mutually exclusive mechanisms: related to beta cells, which become accessible, visible and vulnerable to CD8⁺ T cells under inflammatory conditions (i.e. loss of immune ignorance); and related to T cells, which escape from the control of immune regulatory mechanisms, both intrinsic (e.g. anergy, exhaustion) and extrinsic (e.g. Treg suppression).

This approach to antigen discovery carries strengths and limitations. Strengths include robust evidence of natural processing and presentation by beta cells, with enhanced antigen exposure under inflammatory conditions [9]; evidence of T-cell recognition in both blood and pancreas; and demonstration of diabetogenic properties in NOD mice for some of the novel antigens identified [*11]. A first limitation relates to the lack of exploration of the natural processing and presentation by antigen-presenting cells, which are the major source of T-cell priming in PLNs. This may explain the absent of marginal detection of some known antigens. A second limitation is the difficulty in providing stringent evidence for neo-epitopes derived from post-translational modifications (PTMs), which requires to differentiate biological PTMs from artifactual ones. Indeed, peptides synthesized in their native form are often found to carry these same PTMs,

suggesting that they may arise spontaneously (e.g. by oxidation) or may be introduced during sample preparation and mass spectrometry acquisition. Although this does not negate the existence of biological PTMs, it masks their actual prevalence. Other PTMs that may be particularly relevant in T1D (e.g. glycosylation/glycation) are challenging to study, still others have been elusive altogether. Citrullination provides a revealing example: despite its detection in inflamed cartilages and the diagnostic importance of anti-citrullinated protein auto-antibodies in rheumatoid arthritis, direct evidence of HLA-eluted citrullinated peptides is missing. Moreover, our recent study on citrullinated GRP78 peptides [16] documented that preferential T-cell recognition of post-translationally modified vs. native epitopes is not always the rule ([Fig. 2B](#)). First, this rule has been proposed based on the assumption that citrullinated peptides are not presented in the thymus, thus leading to the escape of their cognate T cells. Contrary to this assumption, citrullinated proteins were detected in the thymus and medullary epithelial cells, as was the case for the peptidyl-arginine deiminases catalyzing this PTM. Second, T-cell preference for some native rather than citrullinated GRP78 epitopes was driven by cross-recognition of bacterial mimotopes/homotopes.

Other limitations have been complemented by the work of other investigators and are discussed in the next section.

Lessons from human CD8⁺ T cells isolated from islet infiltrates: possible over-reliance on HLA binding affinity and functional implications.

Another limitation of our work is that we did not isolate T cells recognizing our antigens from the pancreas. M. Nakayama developed an elegant approach overcoming this limitation, based on the isolation of islet-infiltrating T cells and single-cell sequencing of their T-cell receptors (TCRs). Their antigen specificity is subsequently reconstructed by re-expressing these TCRs in a 5KC

fluorescent reporter cell system, in which a ZsGreen protein is expressed under the control of an NFAT-driven promoter activated upon TCR triggering [17]. Using this system, several TCRs were assigned to preproinsulin-derived epitopes [**18], including an epitope derived from a defective ribosomal product (DRiP) generated by mis-initiated translation using an alternative open-reading frame [19]. The large fraction of “orphan” TCRs with unassigned antigen specificities is a residual challenge that may yield additional information in the near future.

This seminal work [**18] provided two additional key messages. First, it was noted that several preproinsulin-reactive TCRs recognize their cognate antigens with low affinity, with EC50 mostly in the 0.1-100 μ M range. This is line with the lower affinity of self-reactive TCRs, and may reflect the outcome of thymic negative selection, which prunes high-affinity TCRs from the self-reactive T-cell repertoire [20].

A closer look reveals another striking feature (Fig. 3A): the predicted binding affinity for the identified HLA Class I restriction elements is extremely low for several of these peptides, with a percent rank in the NetMHCpan 4.1 algorithm $>2\%$, which is classically regarded as evidence for lack of binding altogether. The implications of these observations are two-fold. First, these peptides could be missed by immunopeptidomics strategies relying on pHLA purification. Second, this means that the TCR signal that these autoimmune T cells receive upon antigen encounter is extremely weak, as it results from the interaction between pHLA complexes that are poorly presented on the surface of beta cells, often in the face of a low-affinity TCR. An increasing number of reports documented the existence of epitopes that are highly immunogenic despite poor or undetectable HLA binding [21]. A recent elegant work [**22] described a mutated tumor neo-antigen that mediated tumor rejection by CD8⁺ T cells in a mouse model, despite undetectable

MHC Class I binding. While a strong HLA binding is a good predictor of immunogenicity for viral peptides, this seems less the case for tumor and self-epitopes [21].

How can this poor MHC presentation translate into T-cell recognition, even more so if low-affinity TCRs are involved? For CD4⁺ T cells, another elegant study demonstrated that both high- and low-affinity TCRs reactive to Ins B9-23 are diabetogenic [23]. We can speculate that the immune synapse (IS) formed by the interaction of TCRs (often of low affinity) and weak pHLA complexes may harbor some specific features (Fig. 3B). First, the local peptide concentration achieved in the constrained IS space may be sufficiently high to favor binding to HLA. Although unstable, this binding may result in intermittent, repeated “on-off” TCR signals. Conversely, it has been shown that a single pHLA complex can serially engage up to ~200 TCRs [24]. Second, the signal generated might be sufficient to trigger a transcriptional program that differs from the one triggered by high-affinity TCR/strong peptide-HLA complexes, but still lead to T-cell activation. In line with this, a self-reactive, low-affinity human CD4⁺ T-cell clone isolated from a T1D individual formed an IS where the usual central TCR cluster was replaced by micro-clusters, still able to trigger signaling, cytokine production and proliferation [25]. Third, compensatory interactions mediated by the CD4 and CD8 co-receptors, adhesion molecules and/or actin might increase the stability of the IS and subsequent strength of TCR signaling [26].

What could be the functional consequences of this weak TCR signals on the biology of autoimmune CD8⁺ T cells? The stem-like properties of circulating [**27] and/or PLN-residing [**28] autoimmune CD8⁺ T cells are increasingly recognized. An enrichment in the stem-cell memory fraction of circulating autoimmune CD8⁺ T cells from T1D patients compared to healthy donors was originally reported by the group of P. Monti [29]. This stem-like phenotype (mainly driven by the transcription factor TCF1) [30] is strikingly different from that of CD8⁺ T cells

recognizing viral epitopes in the context of chronic infection (Fig. 2C), which rather display an exhausted phenotype (mainly driven by the transcription factor TOX) [30], characterized by reduced effector functions due to prolonged antigen exposure [27,31,32]. In contrast, the stem-like properties of CD8⁺ T cells in T1D may be driven by these weak TCR signals that maintain T cells alive without differentiating them into a full effector/memory phenotype. This is in line with the notion that immunization with subdominant or low-affinity CD8⁺ T-cell epitopes can achieve superior viral and tumor clearance by limiting exhaustion and sparing T-cell stemness [33,34]. We can speculate that these stem-like CD8⁺ T cells may transiently recirculate in the bloodstream [27] prior to reaching the pancreas, where the re-encounter with their cognate antigen (possibly at higher density than in PLNs) drives their full differentiation into effector/memory cells with cytotoxic activity against beta cells. These so-called “autoimmune mediators” [28] display higher levels of apoptosis markers, suggesting that the antigen re-encounter in the pancreas may drive activation-induced cell death rather than exhaustion. The continual replenishing of the short-lived autoimmune mediator pool by stem-like CD8⁺ T cells from PLNs may prevent the exhaustion seen at the high antigen loads of chronic viral infection. This may explain the challenge of durably blunting islet autoimmunity, which, without proper targeting of stem-like T cells, may self-regenerate and mediate autoimmune progression or relapse. Conversely, it may also explain the autoimmune manifestations induced by therapeutic PD-1 blockade in cancer patients, which has been shown to target PD-1-expressing stem-like CD8⁺ T cells rather than their terminally differentiated, exhausted counterparts, which are also PD-1⁺ [35,36].

Benign islet autoimmunity: challenges and opportunities for translation into circulating T-cell biomarkers

The dynamics that may favor the maintenance of autoimmune T cells with stem-like properties may be physiological to a large extent. Prior to encounter with their cognate antigens, which is the most common situation outside some restricted time windows (e.g. outside viral infection episodes), tonic, low-grade TCR signals triggered by self-p/MHC complexes ensure homeostatic T-cell survival and proliferation [37-39]. In the absence of MHC contact, the half-life of peripheral CD8+ and CD4+ T cells is reduced, even more so for CD8+ T cells [40-42].

Moreover, most (~90%) of the energy required for pMHC/TCR interaction is peptide-independent and is given solely by the MHC molecule [43]. The cognate peptide turns this initial association into stable binding, thus imparting specificity and eliciting T-cell activation by modulating the duration of the contact. These features may underlie the benign autoimmunity phenomenon that we recently described. It is also possible that the naïve-like status described in our work may overlap, to a certain extent, with the epigenetically imprinted stem-like phenotype, intermediate between naïve and effector/memory, described by B. Youngblood and co-workers [**27].

How could the benign CD8+ T-cell autoimmunity be missed by previous investigations? A first key problem relates to the sampling size employed by several studies, in terms of number of peripheral blood mononuclear cells (PBMCs) processed. The frequency of these self-peptide-reactive CD8+ T cells falls in a relatively narrow range, between 1 and 50/10⁶ total CD8+ T cells [8,9,*11]. As CD8+ T cells account for ~20% of PBMCs, this means that at least 20x10⁶ PBMCs (~20 ml of blood) are needed for appropriate sampling, i.e. to be able to count and phenotype 4-200 HLA multimer+ CD8+ T cells recognizing a given epitope. Several previous studies have analyzed a much more limited number of cells. A second problem may be a “streetlight effect” [44], i.e. our bias of over-emphasizing experimental results in line with prior assumptions and de-emphasizing discordant ones. Indeed, a closer scrutiny of previous works reveals that the

frequency differences in circulating islet-reactive CD8⁺ T cells (detected by multimer staining) between T1D and healthy donors have been often marginal, and that major naïve fractions within this autoimmune repertoire have been neglected altogether [19,45,46].

Does the universal presence of circulating islet-reactive CD8⁺ T cells put a gravestone on our ambition to develop circulating T-cell biomarkers for autoimmune staging? These recent observations pose additional challenges, but also offer unprecedented opportunities to better understand autoimmune pathology and ultimately improve our disease staging capabilities. Indeed, previous T-cell assays using functional readouts, e.g. interferon- γ ELISpot, have repeatedly documented the possibility to discriminate between T1D and healthy donors [2,4,47,48]. This may reflect the fact that these assays are performed using unfractionated PBMCs, which may maintain the immune regulatory networks at play under healthy conditions. On one hand, it will be important to broaden our knowledge of target islet antigens, to be able to measure the overall autoimmune T-cell burden of a given individual as comprehensively as possible. This is similar to staging strategies using islet auto-antibodies, where the presence of multiple autoantibodies rather than a single one marks a higher risk of disease progression [49]. Similarly, we may expect that analyzing autoreactive T cells recognizing more antigens rather than just a few may provide better prognostic stratification. On the other, it will be critically to “grade” the pathogenic potential of these autoreactive T cells by identifying their surface or epigenetic markers associated with disease progression.

Conclusion

Besides providing a more comprehensive description of the autoimmune T-cell repertoire, islet antigen discovery is advancing our understanding of T1D pathophysiology. The distinctive

features of the self-peptides presented by HLA molecules and of the TCRs that recognize them may help explaining the peculiar stem-like properties of their cognate autoreactive T cells and their benign vs. pathogenic profile. The complementary perspectives of beta cells (HLA-driven) and T cells (TCR-driven) on the antigens that they eventually select for presentation and recognition (Fig. 4) trace the path to pursue these integrated investigations. Such investigations are embedded into a more holistic view of islet autoimmunity as a disease of T cells, beta cells and beyond [13,*14].

Key points

- A combined beta-cell and T-cell perspective is needed to maximize our understanding of T1D pathogenesis through islet antigen discovery.
- Recent data suggest that weak MHC-binding epitopes list among the major targets of human islet infiltrating CD8+ T cells, likely resulting in low peptide-MHC presentation that delivers weak TCR signals, especially in the face of low-affinity autoimmune TCRs.
- These weak TCR signals may favor the maintenance of the partially differentiated stem-like phenotype recently described for islet-reactive CD8+ T cells in the blood and PLNs.
- These weak TCR signals may also be physiological, reflecting the need for self-peptide MHC contacts to maintain homeostatic T-cell survival and proliferation.
- These features may underlie the universal state of benign autoimmunity that we recently described.

Acknowledgements. Figures 1, 2 and 3 were created with elements from Servier Medical Art (smart.servier.com).

Financial support and sponsorship. Work performed in the Laboratory of R.M. is supported by The Leona M. and Harry B. Helmsley Charitable Trust (Helmsley #1901-03689), the *Agence Nationale de la Recherche* (ANR-19-CE15-0014-01) and the *Fondation pour la Recherche Medicale* (EQU20193007831); and by the Innovative Medicines Initiative 2 Joint Undertaking under grant agreements 115797 and 945268 (INNODIA and INNODIA HARVEST), which receive support from the EU Horizon 2020 program, the European Federation of Pharmaceutical Industries and Associations, JDRF, and The Leona M. & Harry B. Helmsley Charitable Trust.

Conflicts of interest. None.

References

- *1. James EA, Mallone R, Kent SC, DiLorenzo TP. T-Cell Epitopes and Neo-epitopes in Type 1 Diabetes: A Comprehensive Update and Reappraisal. *Diabetes* 2020, 69:1311-1335.
A comprehensive meta-analysis of T-cell epitopes in type 1 diabetes proposing a grading by level of evidence.
2. Blancou P, Mallone R, Martinuzzi E et al. Immunization of HLA class I transgenic mice identifies autoantigenic epitopes eliciting dominant responses in type 1 diabetes patients. *J Immunol* 2007, 178:7458-7466.
3. Jarchum I, Baker JC, Yamada T et al. In vivo cytotoxicity of insulin-specific CD8+ T-cells in HLA-A*0201 transgenic NOD mice. *Diabetes* 2007, 56:2551-2560.
4. Scotto M, Afonso G, Larger E et al. Zinc transporter (ZnT)8(186-194) is an immunodominant CD8+ T cell epitope in HLA-A2+ type 1 diabetic patients. *Diabetologia* 2012, 55:2026-2031.
5. Scotto M, Afonso G, Osterbye T et al. HLA-B7-restricted islet epitopes are differentially recognized in type 1 diabetic children and adults and form weak peptide-HLA complexes. *Diabetes* 2012, 61:2546-2555.
6. Sewell AK. Why must T cells be cross-reactive? *Nat Rev Immunol* 2012, 12:669-677.
7. Quiniou V, Barennes P, Martina F et al. Human thymopoiesis selects unconventional CD8+ α/β T cells that respond to multiple viruses. *bioRxiv* 2020:2020.2007.2027.223354.
8. Culina S, Lalanne AI, Afonso G et al. Islet-reactive CD8+ T cell frequencies in the pancreas, but not in blood, distinguish type 1 diabetic patients from healthy donors. *Sci Immunol* 2018, 3:eaa04013.
9. Gonzalez-Duque S, Azoury ME, Colli ML et al. Conventional and Neo-antigenic Peptides Presented by beta Cells Are Targeted by Circulating Naive CD8+ T Cells in Type 1 Diabetic and Healthy Donors. *Cell Metab* 2018, 28:946-960 e946.
10. Dudek NL, Tan CT, Gorasia DG et al. Constitutive and inflammatory immunopeptidome of pancreatic beta-cells. *Diabetes* 2012, 61:3018-3025.
- *11. Azoury ME, Tarayrah M, Afonso G et al. Peptides Derived From Insulin Granule Proteins are Targeted by CD8+ T Cells Across MHC Class I Restrictions in Humans and NOD Mice. *Diabetes* 2020, 69:2678-2690.
This paper builds on previous ref. 9 and describes novel pathogenic antigens derived from insulin granules.
12. Alanio C, Lemaitre F, Law HK et al. Enumeration of human antigen-specific naive CD8+ T cells reveals conserved precursor frequencies. *Blood* 2010, 115:3718-3725.
13. Mallone R, Eizirik DL. Presumption of innocence for beta cells: why are they vulnerable autoimmune targets in type 1 diabetes? *Diabetologia* 2020, 63:1999-2006.
- *14. Carré A, Richardson SJ, Larger E, Mallone R. Presumption of guilt for T cells in type 1 diabetes: lead culprits or partners in crime depending on age of onset? *Diabetologia* 2021, 64:15-25.
This work complements previous ref. 13 by reviewing the role of autoimmune T cells in different T1D 'endotypes'.
15. Davis MM. Not-So-Negative Selection. *Immunity* 2015, 43:833-835.
16. Azoury ME, Samassa F, Buitinga M et al. CD8+ T cells variably recognize native versus citrullinated GRP78 epitopes in type 1 diabetes. *Diabetes* 2021, 70:2879-2891.
17. Mann SE, Zhou Z, Landry LG et al. Multiplex T Cell Stimulation Assay Utilizing a T Cell Activation Reporter-Based Detection System. *Front Immunol* 2020, 11:633.

- **18. Anderson AM, Landry LG, Alkanani AA et al. Human islet T cells are highly reactive to preproinsulin in type 1 diabetes. *Proc Natl Acad Sci U S A* 2021, 118:e2107208118.
A milestone description of the preproinsulin epitopes recognized by the islet-infiltrating CD8+ T cells of T1D patients.
19. Kracht MJ, van Lummel M, Nikolic T et al. Autoimmunity against a defective ribosomal insulin gene product in type 1 diabetes. *Nat Med* 2017, 23:501-507.
20. Yu W, Jiang N, Ebert PJ et al. Clonal deletion prunes but does not eliminate self-specific alphabeta CD8(+) T lymphocytes. *Immunity* 2015, 42:929-941.
21. Brennick CA, George MM, Srivastava PK, Karandikar SH. Prediction of cancer neoepitopes needs new rules. *Semin Immunol* 2020, 47:101387.
- **22. Ebrahimi-Nik H, Moussa M, Englander RP et al. Reversion analysis reveals the in vivo immunogenicity of a poorly MHC I-binding cancer neoepitope. *Nat Commun* 2021, 12:6423.
This work demonstrates that epitopes with weak HLA binding can nonetheless be strongly immunogenic and mediate tumor rejection.
23. Bettini M, Blanchfield L, Castellaw A et al. TCR affinity and tolerance mechanisms converge to shape T cell diabetogenic potential. *J Immunol* 2014, 193:571-579.
24. Valitutti S, Muller S, Cella M et al. Serial triggering of many T-cell receptors by a few peptide-MHC complexes. *Nature* 1995, 375:148-151.
25. Schubert DA, Gordo S, Sabatino JJ, Jr. et al. Self-reactive human CD4 T cell clones form unusual immunological synapses. *J Exp Med* 2012, 209:335-352.
26. Laugel B, van den Berg HA, Gostick E et al. Different T cell receptor affinity thresholds and CD8 coreceptor dependence govern cytotoxic T lymphocyte activation and tetramer binding properties. *J Biol Chem* 2007, 282:23799-23810.
- **27. Abdelsamed HA, Zebley CC, Nguyen H et al. Beta cell-specific CD8(+) T cells maintain stem cell memory-associated epigenetic programs during type 1 diabetes. *Nat Immunol* 2020, 21:578-587.
A first description of the stem-like properties of islet-reactive CD8+ T cells and their epigenetic imprinting.
- **28. Gearty SV, Dundar F, Zumbo P et al. An autoimmune stem-like CD8 T cell population drives type 1 diabetes. *Nature* 2022, 602:156-161.
This work describes stem-like islet-reactive CD8+ T cells in the PLNs of NOD mice that replenish effectors migrating to the pancreas target organ.
29. Vignali D, Cantarelli E, Bordignon C et al. Detection and Characterization of CD8(+) Autoreactive Memory Stem T Cells in Patients With Type 1 Diabetes. *Diabetes* 2018, 67:936-945.
30. Gonzalez NM, Zou D, Gu A, Chen W. Schrodinger's T Cells: Molecular Insights Into Stemness and Exhaustion. *Front Immunol* 2021, 12:725618.
31. Zajac AJ, Blattman JN, Murali-Krishna K et al. Viral immune evasion due to persistence of activated T cells without effector function. *J.Exp.Med.* 1998, 188:2205-2213.
32. Gallimore A, Glithero A, Godkin A et al. Induction and exhaustion of lymphocytic choriomeningitis virus-specific cytotoxic T lymphocytes visualized using soluble tetrameric major histocompatibility complex class I-peptide complexes. *J Exp Med* 1998, 187:1383-1393.
33. Holst PJ, Jensen BA, Ragonnaud E et al. Targeting of non-dominant antigens as a vaccine strategy to broaden T-cell responses during chronic viral infection. *PLoS One* 2015, 10:e0117242.
34. Gross DA, Graff-Dubois S, Opolon P et al. High vaccination efficiency of low-affinity epitopes in antitumor immunotherapy. *J Clin Invest* 2004, 113:425-433.

35. Im SJ, Hashimoto M, Gerner MY et al. Defining CD8+ T cells that provide the proliferative burst after PD-1 therapy. *Nature* 2016, 537:417-421.
36. Miller BC, Sen DR, Al Abosy R et al. Subsets of exhausted CD8(+) T cells differentially mediate tumor control and respond to checkpoint blockade. *Nat Immunol* 2019, 20:326-336.
37. Takeda S, Rodewald HR, Arakawa H et al. MHC class II molecules are not required for survival of newly generated CD4+ T cells, but affect their long-term life span. *Immunity* 1996, 5:217-228.
38. Tanchot C, Lemonnier FA, Perarnau B et al. Differential requirements for survival and proliferation of CD8 naive or memory T cells. *Science* 1997, 276:2057-2062.
39. Dorfman JR, Stefanova I, Yasutomo K, Germain RN. CD4+ T cell survival is not directly linked to self-MHC-induced TCR signaling. *Nat Immunol* 2000, 1:329-335.
40. Polic B, Kunkel D, Scheffold A, Rajewsky K. How alpha beta T cells deal with induced TCR alpha ablation. *Proc Natl Acad Sci U S A* 2001, 98:8744-8749.
41. Labrecque N, Whitfield LS, Obst R et al. How much TCR does a T cell need? *Immunity* 2001, 15:71-82.
42. Seddon B, Zamoyska R. TCR and IL-7 receptor signals can operate independently or synergize to promote lymphopenia-induced expansion of naive T cells. *J Immunol* 2002, 169:3752-3759.
43. Wu LC, Tuot DS, Lyons DS et al. Two-step binding mechanism for T-cell receptor recognition of peptide MHC. *Nature* 2002, 418:552-556.
44. Battaglia M, Atkinson MA. The streetlight effect in type 1 diabetes. *Diabetes* 2015, 64:1081-1090.
45. Luce S, Lemonnier F, Briand JP et al. Single insulin-specific CD8+ T cells show characteristic gene expression profiles in human type 1 diabetes. *Diabetes* 2011, 60:3289-3299.
46. Skowera A, Ladell K, McLaren JE et al. Beta-cell-specific CD8 T cell phenotype in type 1 diabetes reflects chronic autoantigen exposure. *Diabetes* 2015, 64:916-925.
47. Mallone R, Martinuzzi E, Blancou P et al. CD8+ T-cell responses identify beta-cell autoimmunity in human type 1 diabetes. *Diabetes* 2007, 56:613-621.
48. Skowera A, Ellis RJ, Varela-Calvino R et al. CTLs are targeted to kill beta cells in patients with type 1 diabetes through recognition of a glucose-regulated preproinsulin epitope. *J Clin Invest* 2008, 118:3390-3402.
49. Insel RA, Dunne JL, Atkinson MA et al. Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the Endocrine Society, and the American Diabetes Association. *Diabetes Care* 2015, 38:1964-1974.

Figure legends

Figure 1. Levels of evidence for islet-derived T-cell epitopes. The epitope scoring criteria proposed in a recent meta-analysis [*1] include the source of T cells used to define the epitope (humanized HLA-transgenic mice, human peripheral tissues (blood, spleen) or target organ tissues (PLNs, pancreas), evidence of natural processing/presentation, and the isolation of T cells with confirmed recognition of the corresponding epitope.

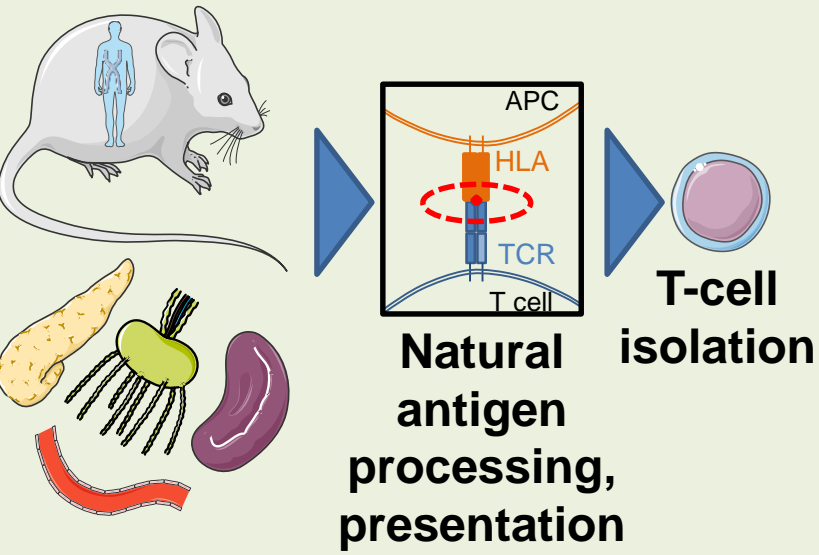
Figure 2. Generation and recognition of neo-antigens in beta cells. A. Mechanisms of neo-antigen generation. 1) Alternative mRNA splicing can translate into amino acid neo-sequences when exons are either added or skipped compared with the canonical mRNA. 2) Defective ribosomal products (DRiPs) can derive, among others, from mis-initiated mRNA translation at alternative start sites. 3) Peptide-spliced epitopes result from the fusion of two non-contiguous fragments from the same protein (*cis*-splicing) or from different ones (*trans*-splicing), in the proteasome or in the insulin granule. 4) Post-translational modifications are formed by biochemical changes introduced after protein translation. All these processes can be upregulated under inflammatory conditions and can result in presentation of neo-peptides on surface HLA Class I molecules and subsequent recognition by CD8⁺ T cells. **B.** The case of citrullinated GRP78 peptides [16] demonstrates that preferential T-cell recognition of post-translationally modified vs. native epitopes is not always the rule. On one hand, the citrullinating peptidyl-arginine deiminase enzymes are expressed in the thymus and citrullinated proteins are detected, suggesting that post-translationally modified peptides do not necessarily lead to thymic escape of their cognate T cells. On the other, the cross-reactivity of native GRP78 epitopes with bacterial mimotopes/homotopes shapes the T-cell preference for some native epitope versions.

Figure 3. Weak HLA-binding peptides: possible mechanisms of T-cell recognition and functional outcomes. **A.** Predicted HLA-binding affinity of the preproinsulin peptides targeted by the islet-infiltrating CD8⁺ T cells of T1D donors characterized by M. Nakayama and co-workers [**18]. Predictions were made using the NetMHCpan 4.1 algorithm. The horizontal dotted lines indicated the customary percent rank cut-off values used to define strong binders (rank <0.5%), weak binders (<2%) and ultra-weak binders/non-binders (>2%). The color code indicates the different regions of preproinsulin, with the insulin DRiP peptide (MLYQHLLPL) recognized in the frame of either HLA-A2 or -B8 indicated in black. **B.** Three non-mutually exclusive hypotheses that may explain how weak HLA-binding peptides presented by antigen-presenting cells (APCs) may activate T cells. The canonical immune synapse (IS) displays concentrically organized clusters called supramolecular activation clusters (SMACs). The central SMAC (cSMAC, in green) is enriched in pMHC/TCR and the CD4/CD8 co-receptors. The distal SMAC (dSMAC, in blue) is enriched in adhesion molecules such as LFA-1 and ICAM-1. The peripheral SMAC (pSMAC, in pink) is enriched in actin. *Top right:* the local peptide concentration in the IS might be sufficiently high to allow binding to the HLA. *Middle right:* the IS may be organized into multiple micro-clusters enriched in pMHC/TCR and LFA-1/ICAM-1, still able to trigger signaling. *Bottom right:* compensatory interactions mediated by the CD4 and CD8 co-receptors, adhesion molecules and/or actin might increase the strength of TCR signaling. **C.** Possible functional outcomes of T-cell activation under conditions of high vs. low pMHC availability. *Top:* high antigen availability is usually present in chronic viral infections. In the lymph node (LN), APCs interact with naive T cells, triggering a strong TCR signal that leads to full effector/memory differentiation. These T cells subsequently recirculate toward the infection site, where antigen

availability may be even higher and eventually trigger exhaustion. *Bottom:* antigen availability may be lower in T1D, where several weak HLA-binding peptides are presented. Together with the frequently low affinity of autoimmune TCRs, this may trigger a weak TCR signal that leads to partial effector/memory differentiation, thus maintaining T-cell stemness that continuously replenish fully differentiated, cytotoxic T cells in the pancreas. These stem-like T cells transit through the blood to recirculate toward the pancreas to reach their beta-cell targets, and may provide circulating biomarkers of pathogenic islet autoimmunity. In the pancreas, activation-induced apoptosis rather than exhaustion seems to be triggered, possibly favored by a higher antigen availability.

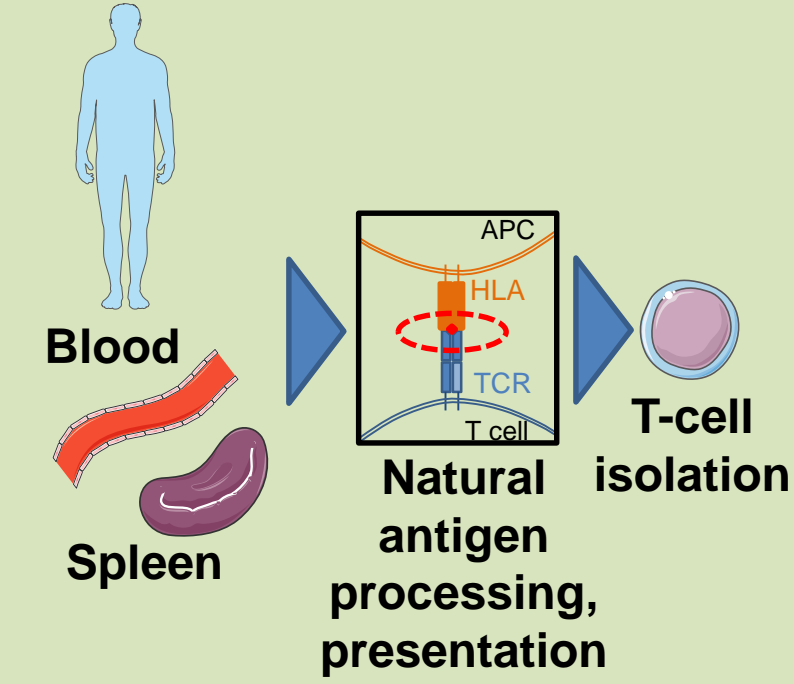
Figure 4. The complementary beta-cell and T-cell perspective on HLA-driven antigen presentation and TCR-driven antigen recognition. Both perspectives need to be integrated to advance our understanding of islet autoimmunity.

Humanized mouse (HLA-transgenic)



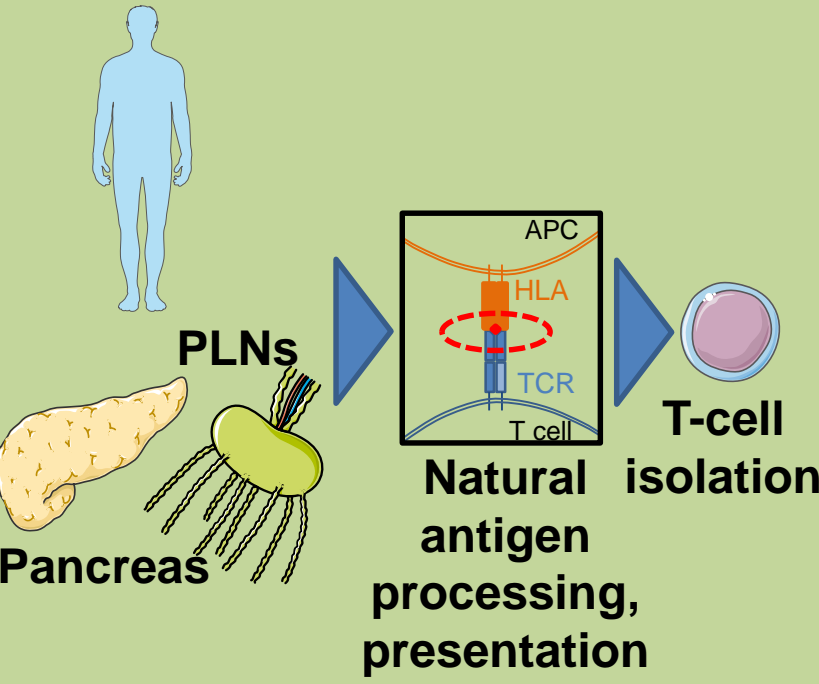
Source: any tissue/organ

Human (T1D, healthy)



Source: peripheral

Human (T1D, healthy)



Source: target organ

Level of evidence

Figure 1

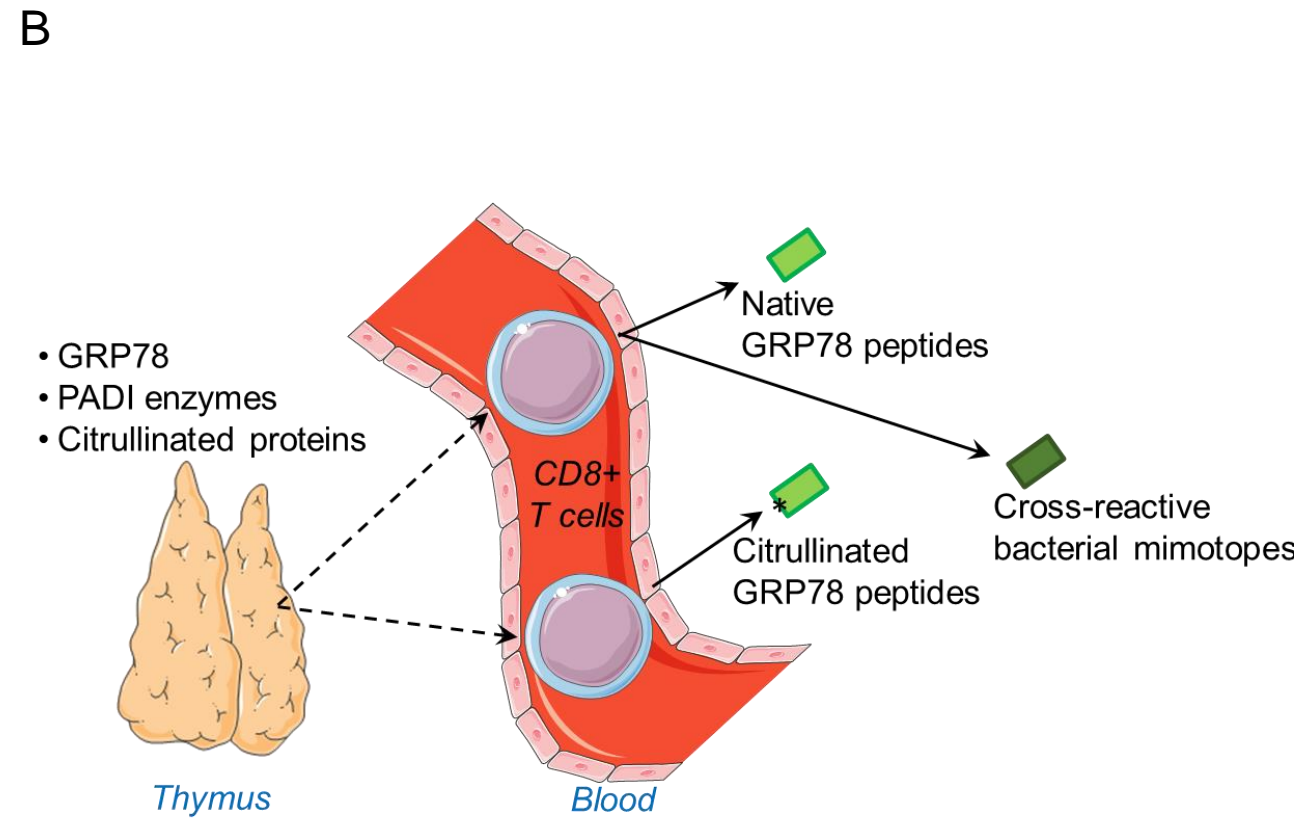
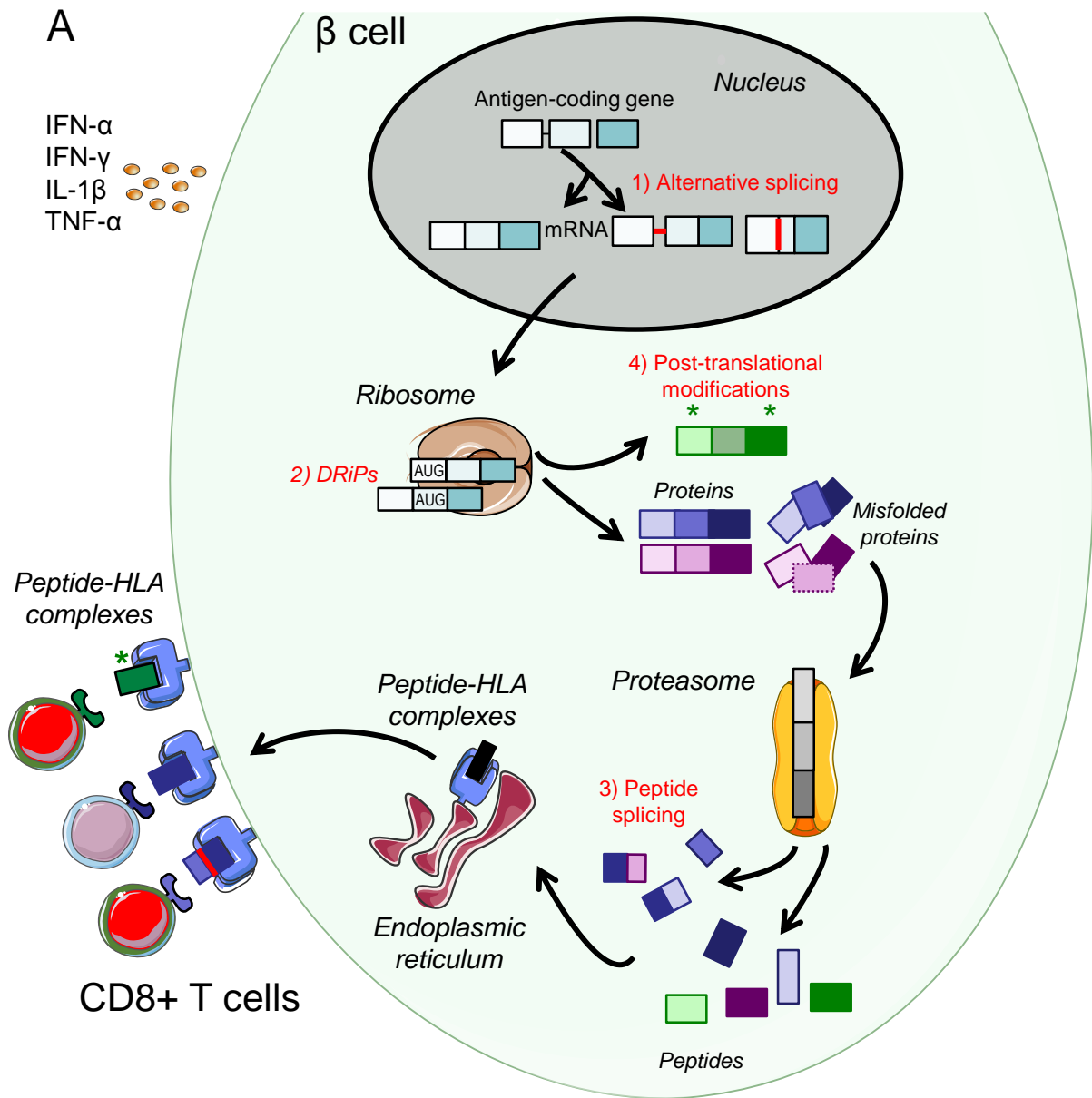


Figure 2

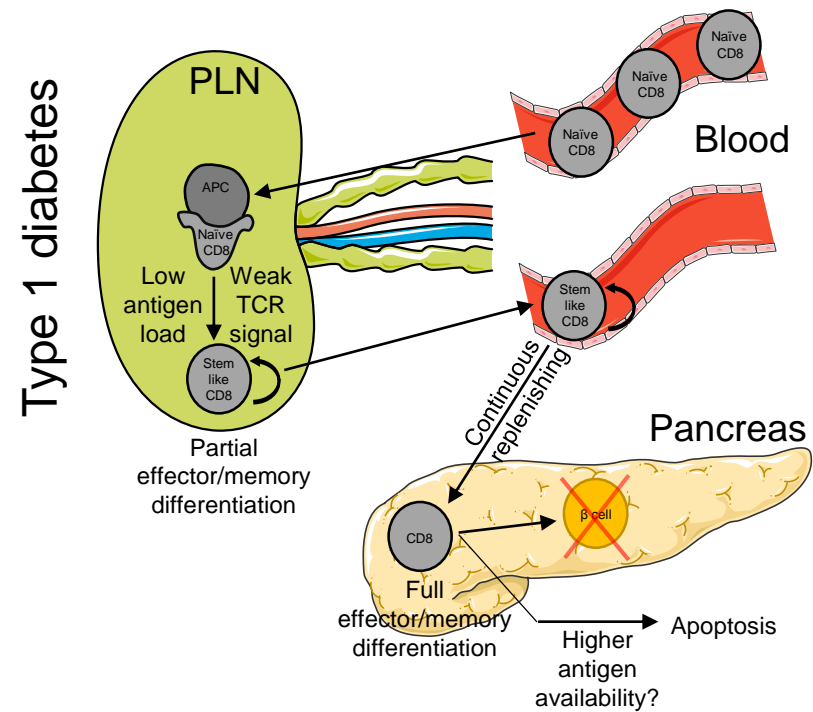
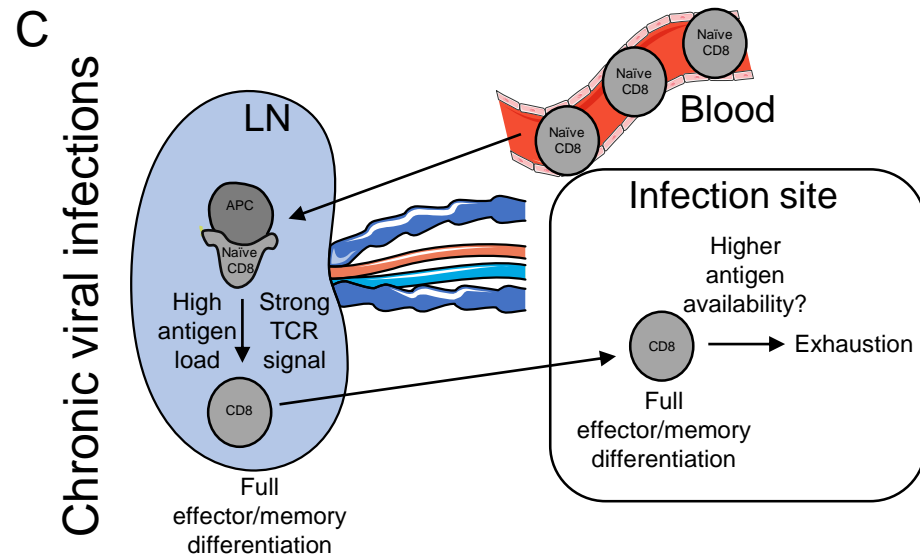
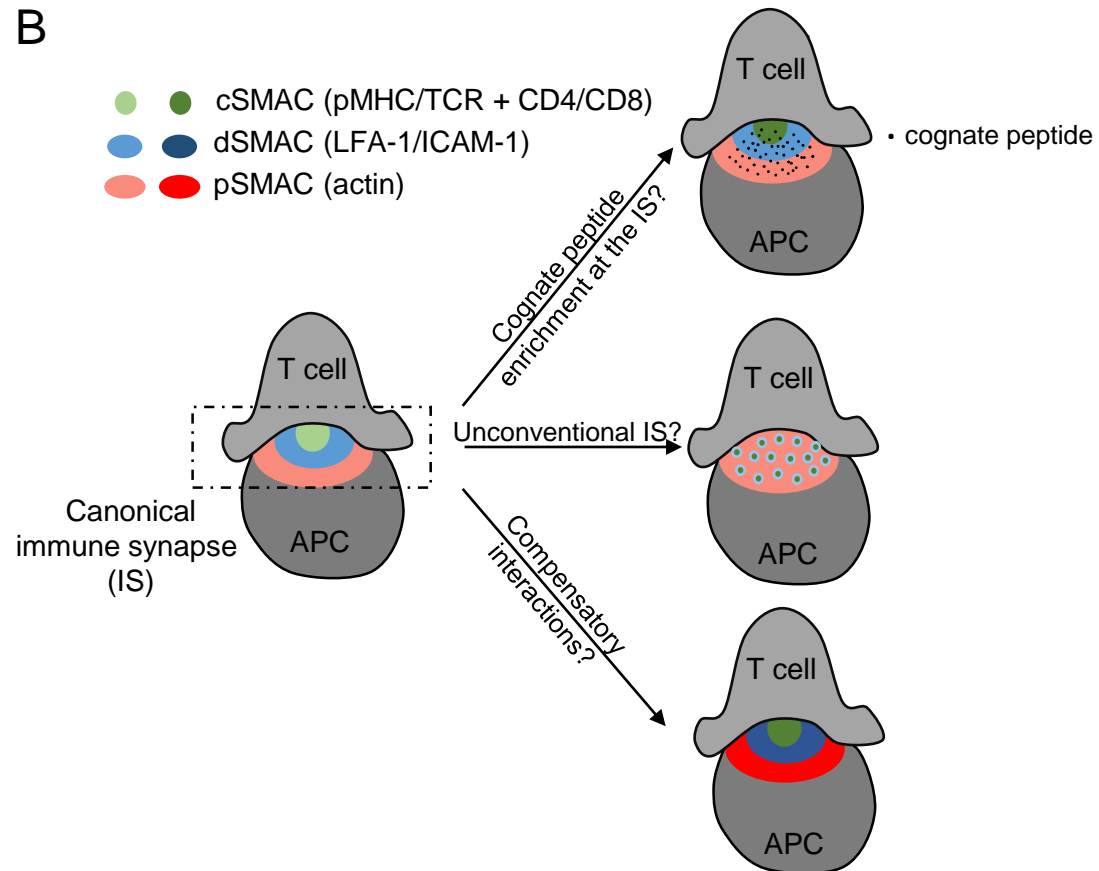
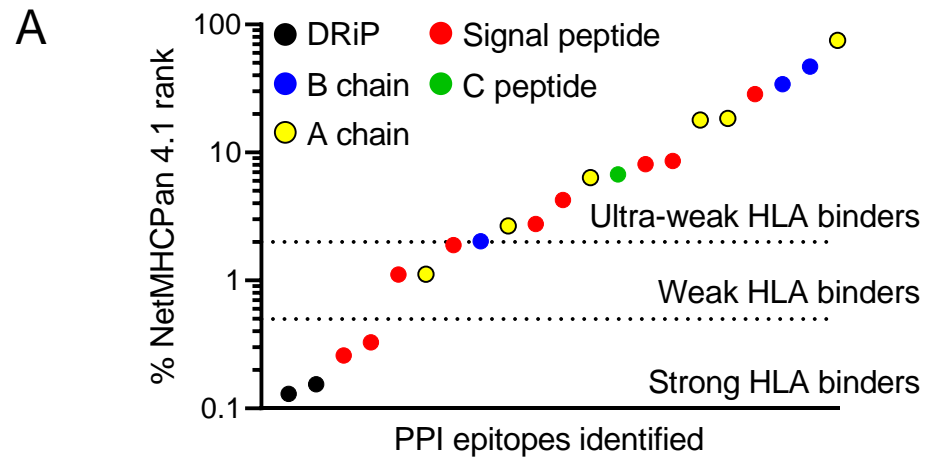


Figure 3

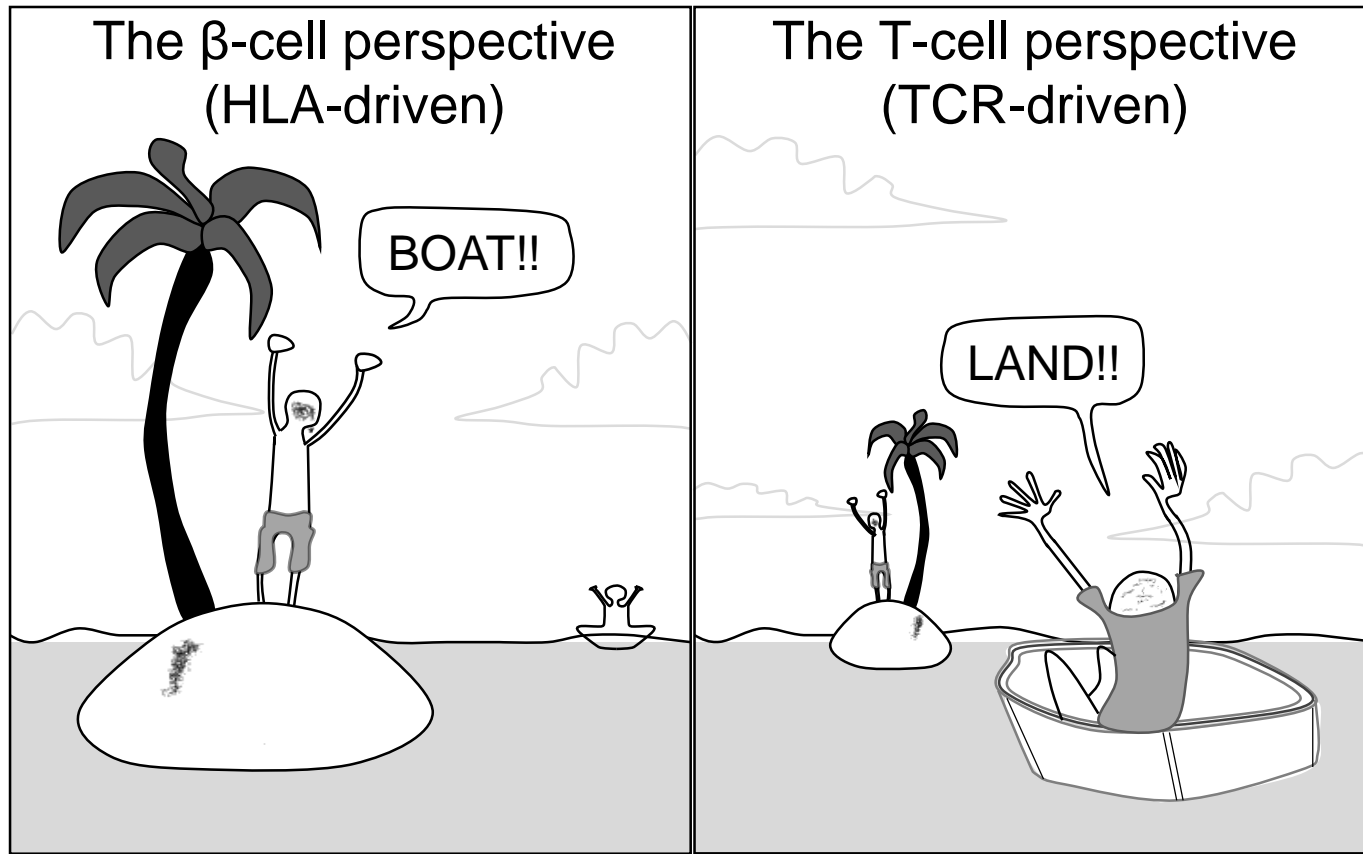


Figure 4