Tolerizing Effects of Soluble CD137 on the CD137-CD137L Signaling Axis for Exploitation as a Therapeutic in Type 1 Diabetes

Manuel Eduardo Rojas Quintana, MD

Thesis presented as a requirement for the Degree of Doctor of Philosophy in Biomedical and Biological Sciences

DOCTORATE IN BIOMEDICAL AND BIOLOGICAL SCIENCES
UNIVERSIDAD DEL ROSARIO
BOGOTA
2026

Tolerizing Effects of Soluble CD137 on the CD137-CD137L Signaling Axis for Exploitation as a Therapeutic in Type 1 Diabetes

Student:
Manuel Eduardo Rojas Quintana, MD

Dissertation Advisor:
William M. Ridgway, MD
Chief, Division of Rheumatology, Allergy, and Clinical Immunology
University of California, Davis, School of Medicine

Co-director:
Carolina Ramírez-Santana, PhD
Director, Center for Autoimmune Diseases Research (CREA),
School of Medicine and Health Sciences, Universidad Del Rosario,
Bogota, Colombia

Thesis presented as a requirement for the Degree of Doctor of Philosophy in Biomedical and Biological Sciences
DOCTORATE IN BIOMEDICAL AND BIOLOGICAL SCIENCES
UNIVERSIDAD DEL ROSARIO
BOGOTA
2026

ACKNOWLEDGMENTS

First and foremost, I would like to express my deepest gratitude to my advisor, Dr. William Ridgway, for his invaluable mentorship, patience, and guidance over the past few years. Your dedication to scientific excellence has profoundly shaped my research approach, and I am incredibly grateful for the independence and support you provided me with.

I want to express my deep gratitude to my co-advisor, Dr. Carolina Ramírez-Santana. Despite the miles and time zones between us, her steady guidance, patience, and perspective were essential to the success of this work and to my development as a researcher.

My sincere thanks go to all past and present members of the Ridgway lab. Thank you for creating such a collaborative, stimulating, and vibrant working environment. I am particularly grateful to Luke, Weici, Patrick, and Nicolle for their scientific, academic, and technical guidance and to everyone who offered feedback during lab meetings and joint coffee breaks. Also, to my student Kate, who was incredibly supportive during all the late nights and stressful times.

Lastly, I want to express my profound gratitude to my family and friends. To my parents, thank you for your endless encouragement and sacrifices. To Madison, thank you for your unwavering love and patience, and for always keeping me grounded. I could not have completed this journey without you.

"Medicine is a science of uncertainty and an art of probability"

Sir. William Osler

TABLE OF CONTENTS

1. PUBLICATIONS LIST

This Doctoral dissertation corresponds to a compendium of scientific articles published in international journals and indexed in the Science Citation Reports. Below, all published articles are listed and attached to this document. Any supplementary information and/or tables will be included in compressed files organized according to the number assigned below:

Manuscript 1: Mariana Celis-Andrade, Victoria Morales-González, Manuel Rojas, Diana M. Monsalve, Yeny Acosta-Ampudia, Yhojan Rodríguez, Carolina Ramírez-Santana. Prevalence of Latent and Overt Polyautoimmunity in Type 1 Diabetes: A Systematic Review and Meta-analysis. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2024. DOI: https://doi.org/10.1016/j.dsx.2024.103087

Manuscript 2: Rojas Manuel, Yeny Acosta-Ampudia, Diana M. Monsalve, Carolina Ramírez-Santana, Luke S. Heuer, William Ridgway, Juan-Manuel Anaya, Zhe-Xiong Lian, M. Eric Gershwin. Autoimmunity, epitope analysis, and molecular mimicry. 2025. Current Opinion in Immunology. DOI: https://doi.org/10.1016/j.coi.2025.102681

Manuscript 3: Sixuan Liu, Yeny Acosta-Ampudia, Carolina Ramírez-Santana, Diana M. Monsalve, William Harper, Luke S. Heuer, Weici Zhang, William M. Ridgway, Rojas Manuel. Soluble CD137 in Health and Disease: A Systematic Review and Meta-Analysis. 2026. DOI: Under review - Autoimmunity reviews.

Manuscript 4: Rojas Manuel, Yeny Acosta-Ampudia, Luke S. Heuer, Weici Zang, Aftab A. Ansari, Diana M. Monsalve, Carolina Ramírez-Santana, Juan-Manuel Anaya, William M. Ridgway, M. Eric Gershwin. Antigen-Specific T Cells and Autoimmunity: A Comprehensive Review. Journal of Autoimmunity. 2023. DOI: https://doi.org/10.1016/j.jaut.2024.103303

Manuscript 5: Rojas Manuel, Luke Heuer, Weici Zhang, Yi-Guang Chen, William M. Ridgway. The Long and Winding Road: From Mouse Linkage Studies to a Novel Human Therapeutic Pathway in Type 1 Diabetes. Frontiers in Immunology. 2022. DOI: https://doi.org/10.3389/fimmu.2022.918837

Manuscript 6: Rojas Manuel, Luke S. Heuer, Weici Zhang, Nicolle Sweeney, Carolina Ramírez-Santana, Patrick S.C. Leung, Alvin Lam, Shraddha Kamat, Andrew R. Mendelsohn, Manley Huang, Bo Yu, Paulina Ackerman, Qisheng Wei, James W. Larrick, Yi-Guang Chen, William M. Ridgway. Alternate Splicing Converts Human CD137 from Costimulatory to Immunosuppressive Function. Journal of Autoimmunity. 2025. DOI: https://doi.org/10.1016/j.jaut.2025.103498

2. LIST OF FIGURES

3. ABSTRACT

Autoimmune diseases (ADs) represent a major and growing challenge for global health, with Type 1 Diabetes (T1D) serving as a prominent example. These diseases are characterized by a failure of immunological tolerance, resulting in the activation of pathogenic T cells that attack the body's own tissues. In this thesis, we describe the epidemiology of T1D, the incidence of polyautoimmunity (PolyA), and the role of soluble CD137 (sCD137) as a reliable biomarker for diagnosing and monitoring autoimmunity. In addition, we present the design and validation of a novel immunotherapy for autoimmunity that aims to avoid immunosuppression.

The first chapter describes the global epidemiology of T1D and how patients with this condition are at risk of developing additional ADs throughout their lives. Through a meta-analysis of 158 studies, we identified a striking pattern of PolyA. The data revealed that 8.50% of individuals with T1D have at least one other overt AD, while approximately 14.45% present with latent autoimmunity, which could predispose individuals to additional ADs in the future. This was confirmed by the meta-regression analysis, showing that the incidence of new-onset PolyA increases with each year lived with the disease. We then explored the main factors associated with this phenomenon and dived into the molecular mechanisms that may explain the onset of T1D, including PolyA. We propose that this vulnerability is often triggered by environmental factors, with molecular mimicry acting as a key driver. Here, we delve into how a case of mistaken identity, in which the immune system, while targeting a foreign microbe, mounts a response against a structurally similar self-protein, can ignite the initial spark of the autoimmune cascade observed at the population level.

Next, we focus on the central effector cell in autoimmunity: the antigen-specific T cell. In chapter 2, we follow the trajectory of these cells, from their escape from tolerance checkpoints to their activation and subsequent diversification through epitope spreading. We describe the traditional dogma of immunology, which holds that clonal expansion drives the selection of higher-affinity T-cell clones. However, novel evidence supports that low-affinity T cells perpetuate autoimmunity, whereas higher-affinity T cells are predominantly relevant in the early stages of disease, underscoring the critical need for novel biomarkers that allow the tracking of T cell activation. The CD137 is a strong marker of T cell activity, and recently, multiple studies included the serum measurement of sCD137 in several inflammatory conditions. To confirm the utility of this biomarker, we conducted a second meta-analysis of 47 studies. Our findings showed that sCD137, a splicing isoform of the costimulatory molecule CD137 produced by regulatory T cells (Tregs), is elevated in autoimmunity and may serve as a proxy marker for the immune system's attempt to restrain an enhanced immunological response. However, immunosuppressive therapies also lead to a marked drop in sCD137 levels, establishing

sCD137 as a sensitive indicator of the internal imbalance between regulatory and inflammatory mechanisms.

Finally, Chapter 3 aims to translate the genetic and clinical data into a tangible therapeutic strategy. We trace this story back to the T1D-protective *Idd9.3* genetic locus in the NOD mouse, pinpointing *Cd137* as the key gene. We found that the protective role of this locus is mediated by an increased production of the immunosuppressive sCD137 isoform. This pivotal insight from our mouse models was then extended to the human context. We successfully engineered and validated recombinant human Fc-sCD137 fusion proteins, confirming that human sCD137 is a potent immune checkpoint. Our mechanistic studies revealed that sCD137 downregulates the mTORC1 metabolic pathway, a central engine of T-cell growth and function. This work provides a robust foundation for a new therapeutic approach that strengthens one of the immune system's inherent anti-inflammatory routes rather than relying on global immunosuppression.

In essence, this work weaves a single, coherent thread from the large-scale clinical problem to a specific, molecular solution. It begins with the epidemiological scale of PolyA in T1D, then narrows its focus to the cellular and molecular drivers, validates a biomarker that traces T-cell activity, and culminates in a targeted, mechanism-driven immunotherapy. We expect that this work will lighten the path for future translational studies in humans and ultimately support the evaluation of the safety and efficacy of this therapy in T1D.

4. THEORETICAL FRAMEWORK

ADs are chronic and clinically heterogeneous conditions that affect up to 5% of the world's population (1,2), and their incidence is rising (3). Type 1 diabetes (T1D) is one of the most common ADs in children, characterized by the autoimmune destruction of insulin-producing β cells (4). The incidence and prevalence of this condition vary worldwide, possibly due to variations in environmental factors interacting with genetic risk loci, including HLA and non-*HLA* genes (4–6). During childhood, the frequency of T1D is uniformly distributed between sexes; however, the incidence in males is higher in young adults (15-25 years) (7). Globally, the prevalence of T1D is 9.5 cases per 10,000 people. Africa and Asia exhibit the lowest prevalence rates (3.5 and 6.9 per 10,000 people, respectively), whereas Europe (12.2 per 10,000 people) and the Americas (12-19 per 10,000 people) show the highest rates (5). Most cases in the Americas are found in the USA and Canada, particularly among individuals of Caucasian ancestry (8).

In addition, T1D incidence is increasing rapidly, especially in regions with already high disease prevalence, such as Europe and America. Recently, a study on Finland and Sardinia reported an increase in incidence from 40 cases to 60 cases per 100,000 children in recent years (9,10). A meta-regression analysis confirmed that this pattern is consistent worldwide, with an approximate 1.4-fold increase in incidence over the last 30 years (5). Given this upward trajectory, additional efforts to prevent and manage T1D are needed to reduce its impact on quality of life and the healthcare system.

A common feature of ADs is the failure to control peripheral autoreactive T cells, and most ADs are associated with dysfunctional Tregs (11). This T cell population constitutively and highly expresses CD25 (IL-2 receptor α chain) (12), and more specifically, the transcription factor Forkhead box P3 (FOXP3) (13–15). The clinical relevance of FOXP3 was first demonstrated in patients with immune dysregulation, polyendocrinopathy, enteropathy X-linked (IPEX) syndrome (16). More than 70 FOXP3 mutations have been described in these patients (17), who frequently present with PolyA, such as autoimmune thyroid disease, autoimmune cytopenias, or T1D (18–20). Polymorphisms in other genes implicated in Treg function, such as *IL2RA* and *CTLA4*, have also been associated with the development of endocrine and rheumatic ADs in humans (21). Collectively, this evidence highlights the crucial role of Tregs in maintaining immune homeostasis and preventing autoimmunity.

Current management of ADs is centered on immunosuppression. Multiple non-specific immunosuppressive therapies, such as methotrexate and leflunomide, are used to ameliorate autoreactivity and limit tissue damage. More recently, antibody-based treatments target specific molecules or cells involved in the immune response (e.g., anti-CD20 for B-cell depletion) (22). However, these approaches carry a major drawback: an increased susceptibility to infections. Recently, new therapeutics focusing on Tregs have

emerged. For instance, administration of IL-2 in patients with systemic lupus erythematosus (SLE) ameliorated disease activity by expanding Tregs without increasing infection risk, and low-dose IL-2 therapy is being investigated in T1D (23–26). Restoring Treg function may therefore provide a way to treat autoimmunity while reducing life-threatening adverse effects. Nonetheless, abnormal Treg function and conversion of Tregs into pathogenic Th17 cells have been reported as complications of Treg-based therapeutics (27–29). Thus, a deeper understanding of Treg biology is required.

Phase 1 clinical trials in early-onset T1D showed that infusion of autologous ex vivo-expanded $CD4^+CD25^+CD127^-$ Tregs was associated with reduced exogenous insulin requirements and preservation of β-cell function, with these effects persisting for up to 1 year after infusion and without severe adverse events (30,31). In a similar study, adult patients maintained stable C-peptide levels and insulin use for up to 2 years (32). However, this Treg-based strategy would require periodic reinfusions to maintain immunoregulation, and autologous Treg transplantation may be difficult in low-resource settings. In addition, these trials are still in early phases (1 and 2), and the observed effect sizes have been modest. Therefore, additional strategies are needed to boost peripheral Treg response and restore homeostasis.

The sCD137 produced by Tregs has emerged as a new candidate for restoring immune homeostasis by boosting peripheral immune regulation in both mice and humans (33). Interestingly, unlike many other therapeutic interventions, sCD137 delays the onset of end-stage T1D and even reverses the disease in mice (33). Furthermore, sCD137 inhibits human T-cell activation and has been identified as a serum biomarker of disease activity in T1D (33). Thus, it is tempting to speculate that sCD137 will be a step forward in controlling the disease in humans. However, the mechanisms by which sCD137 regulates immune response in humans remain poorly understood. In addition, new strategies to improve the stability and bioavailability of sCD137 are needed before it can be translated into clinical practice.

4.1 HLA and non-HLA susceptibility genes for T1D

The genetic risk of developing T1D is mainly determined by polymorphisms in the *HLA* region of the major histocompatibility complex (MHC) class II. Among these, the *HLA-DR* and *HLA-DQ* loci confer the greatest risk. Haplotypes of alleles in the *HLA-DRB1, HLA-DQA1,* and *HLA-DQB1* genes are associated with different levels of disease susceptibility. The haplotypes *DRB1*04:01* and *DRB1*04:05* carry the highest risk, whereas *DRB1*04:02* and *DRB1*04:04* are associated with a lower risk of T1D. Interestingly, the presence of *DRB1*04:03* transforms the haplotype into a protective one (34). These findings suggest that the specific combination of inherited haplotypes is critical for T1D development (i.e., summative effect) (8). Such combinations may differentiate the risk of the disease across ethnicities. For instance, the *DRB1*07-DQA1*03-*

*DQB1*02* haplotype confers a high T1D risk in people of African descent, whereas the *DRB1*07-DQA1*02:01-DQB1*02* is associated with low risk or even protection in individuals of European ancestry (35).

Since MHC II is highly associated with disease risk, antigen presentation to CD4+ T cells is altered, promoting autoreactivity or antigen cross-reactivity *via* molecular mimicry (36). Interestingly, haplotypes associated with T1D risk are also associated with seroconversion (i.e., positivity for islet-specific autoantibodies), but not with progression to overt disease (37). It suggests that despite autoreactivity and latent autoimmunity, additional environmental, immunological, and genetic factors outside the MCH II region are required to develop clinical T1D.

There also appears to be an independent relationship between certain MHC I alleles and T1D susceptibility. These class I molecules are expressed on nearly all nucleated cells and present antigens to $CD8^+$ T cells, which are critical for β-cell destruction (8). *HLA-A*24, B*18*, and *B*39* accelerate progression to overt T1D after seroconversion (38–40). MHC I and MHC II haplotypes can interact. For example, *HLA-A*24:02, B*39:01,* and *B*39:06* alleles were restricted to specific *HLA-DR/DQ* haplotypes in Finnish patients with T1D (41).

Multiple genes and polymorphisms associated with T1D have been identified outside the MCH region. Genome-wide association studies (GWAS) have identified several non-MHC polymorphisms associated with T1D risk. However, the causal genes remain uncertain due to strong linkage disequilibrium across several populations (8). Moreover, many of these genes are also associated with other ADs such as multiple sclerosis, rheumatoid arthritis (RA), coeliac disease, and SLE (42). This supports shared pathogenic mechanisms across ADs (i.e., autoimmune tautology) (43–46), and may explain the appearance of PolyA in children with T1D (47).

The development of systemic tolerance depends on the ectopic expression of numerous tissue-specific proteins in the thymus, which are presented during thymic selection and can mediate the deletion of autoreactive T cells. There are polymorphisms in genes involved in central tolerance in T1D. Single-nucleotide polymorphisms (SNPs), often linked to variable-number tandem repeats (VNTRs) in non-coding regions, are found in the insulin (INS) gene. VNTR variants associated with reduced INS expression in the thymus are linked to a higher risk of T1D (48).

Similarly, mutations in the autoimmune regulator (*AIRE*) gene have confirmed the role of central tolerance in T1D. *AIRE* controls the expression of autoantigens and the selection of autoreactive T cells in the thymus (18–20). AIRE dysfunction impairs the elimination of autoreactive T cells and may hinder the generation of Treg cells (18–20), as described in IPEX syndrome (16). Patients with such dysfunction exhibit a high frequency of PolyA, including T1D (18–20).

Additional genes implicated in T1D include protein tyrosine phosphatase non-receptor type 22 (*PTPN22*), which regulates antigen-driven activation of T and B cells and the survival of autoreactive cells in the thymus during central tolerance (49–51). Variants in the interleukin-2 receptor subunit alpha (*IL2RA*) gene impair IL-2 signaling and reduce Treg function in T1D (52). Furthermore, polymorphisms in the cytotoxic T-lymphocyte-associated protein 4 (*CTLA4*) gene, a key negative regulator of T-cell activation, are associated with increased T1D risk (53). Therefore, T1D is genetically complex and requires an integrated analysis of multiple genes with diverse immunological effects, along with their interactions with environmental factors.

4.2 Environmental factors and islet autoimmunity

Environmental factors play a crucial role in the onset of T1D in addition to the strong genetic influence on its development (54,55). Maternal age, vitamin D deficiency, infant diet, gut microbiota, and exposure to environmental chemicals contribute to its development (56–58). Infectious agents, particularly viruses, are considered leading triggers of T1D. Children genetically predisposed to T1D have been reported to exhibit a significantly higher risk of developing the disease following viral respiratory infections during the colder months, suggesting a seasonal pattern and supporting a pathogenic role for microbes in T1D (59–61).

Within this context, several enteroviruses have been linked to T1D. Epidemiological data indicate that enterovirus outbreaks are followed by an increase in T1D incidence (62). In a recent study assessing enterovirus RNA or viral protein levels in blood or stool, children with T1D were found to have evidence of infection ten times more frequently than controls, and patients with pre-diabetes showed a higher odds of infection than healthy individuals (63). Coxsackievirus has been associated with the onset of T1D in both animal models and individuals with recent-onset T1D (64–66). Other viruses linked to T1D include rotaviruses, rubella virus, parechoviruses, influenza virus, Ljungan virus, and mumps virus (67–71).

The discovery of similarity between the epitopes of pancreatic cells and viral components lends support for molecular mimicry in T1D (36). Viral antigens are thought to initially activate T lymphocytes that cross-react with pancreatic targets in T1D patients (61). This mechanism has been proven in transgenic mice expressing lymphocytic choriomeningitis virus (LCMV) glycoprotein in pancreatic β cells, in which LCMV infection induces $CD8^+$ T cell-mediated diabetes (72). Cross-reactivity between enteroviral protein 1 and the β-cell antigen tyrosine phosphatase IA-2 has been described, as well as epitope similarity between coxsackievirus and CMV proteins and the β-cell antigen glutamic acid decarboxylase 65 (GAD65) (73,74). In addition, the viruses may also act as innate immune triggers (particularly type I interferons/IFN-α/β, IFN-λ, and related pathways) that

can affect pancreatic β-cells or islets, potentially contributing to "innate autoimmunity" or accelerating T1D in susceptible individuals (75).

Infectious agents or disturbances in the gut microbiota may facilitate the release of pancreatic antigens, promoting phagocytosis by antigen-presenting cells (APCs) and, subsequently, the activation of autoreactive T cells in genetically susceptible individuals (8). However, this evidence is still a matter of debate. For instance, inoculation with coxsackievirus B3 in non-obese diabetic (NOD) mice conferred long-term protection against, rather than induction of, T1D (76). Collectively, these findings underscore the complexity of T1D pathogenesis and highlight the need for integrative analyses that consider multiple environmental and genetic factors simultaneously.

4.3 Insulitis and perpetuation of autoimmunity

The presentation of pancreatic antigens by APCs leads to the activation of autoreactive $CD4^+$ and $CD8^+$ T cells, which perpetuate insulitis (i.e., infiltration of immune cells into the pancreatic islets) and the destruction of β cells (77,78). In NOD mice, dendritic cells (DCs) and macrophages are the first cells to infiltrate pancreatic islets, where they produce inflammatory cytokines such as TNF-α (79). This early event is critical for subsequent T cell migration and accumulation. In addition, resident APCs are essential for sensing exogenous insults to the pancreas, which may constitute the first step in the inflammatory process (80).

A recent study in NOD mice using an anti-CSF-1 monoclonal antibody to deplete macrophages showed that treatment, whether initiated at the onset of autoimmunity or during established diabetes, led to a significant reduction in uncontrolled glycemia (81). In addition, they showed that the early entry of $CD4^+$ T cells and DCs into pancreatic islets was reduced, and that there was an impairment in the presentation of insulin epitopes by dispersed islet cells to T cells (81).

These findings are consistent with previous studies indicating that resident macrophages act as initial triggers of T cell infiltration and are critical for disease perpetuation (82). They also suggest that targeting innate immune responses would offer a novel strategy for managing T1D. For instance, treatment of NOD mice with an agonistic TLR4/MD-2 monoclonal antibody induced APC tolerance *in vitro* and in vivo (81), suggesting that educating the innate immune response, regardless of disease stage, may help control autoreactivity and disease progression (83).

Following the initial APC-driven inflammatory response, T cells migrate into the pancreas. $CD8^+$ T cells are the predominant infiltrating immune cells, although $CD4^+$ T cells and B cells are also present, especially in young children (84). Class I HLA hyperexpression is a hallmark of insulitis in all islet cells. This hyperexpression is consistent with effective

autoantigen presentation to cytotoxic $CD8^+$ T cells in the infiltrate and is connected to local interferon gamma production (85). Such infiltration is also found in the exocrine pancreas, which secretes digestive enzymes, ions, and water into the duodenum (86).

Both $CD4^+$ and $CD8^+$ T cells from T1D patients can recognize islet antigens associated with autoantibody production (e.g., GAD65 and IA-2) (87,88). Interestingly, the massive reaction against islet antigens is thought to be related to epitope spreading and bystander activation phenomena highly associated with environmental and infectious agents (89). The cytotoxic response against β-cells ultimately leads to the destruction of insulin-producing cells, resulting in overt diabetes.

In addition to $CD4^+$ and $CD8^+$ T-cell autoreactivity, Tregs fail to maintain peripheral tolerance (90). Patients with T1D show increased resting Tregs ($CD4^+CD25^+FOXP3^+CD45RA$) and decreased activated Tregs ($CD4^+CD25^+FOXP3^+CD45RO$) compared with healthy subjects. In addition, such low Treg levels were associated with an increased likelihood of lower residual C-peptide secretion and poorer HbA1C control (91). In other studies, Tregs from patients with T1D showed defective suppression of T cell proliferation *in vitro* (92). As further discussed for NOD mice, altered Tregs function is critical for perpetuating autoimmunity in T1D. Restoring or boosting Treg activity offers an exciting field of research for treating T1D.

The serum of T1D patients is frequently positive for autoantibodies that identify insulin (IAA), GADA, IA-2A, and zinc transporter 8 (ZnT8A). These autoantibodies are highly predictive of the disease, especially when combined to build predictive models (93). T1D can develop up to 20 years after seroconversion (94,95). These autoantibodies are also helpful for the stage-based classification of the disease. Stage 1 is defined as the presence of numerous autoantibodies with normal glucose levels; stage 2 is characterized by dysglycemia without clinical symptoms; stage 3 corresponds to symptomatic illness (96). This classification helps to correlate pathological findings with the clinical presentation of the disease.

4.4 The NOD strain and its implications for T1D research

The NOD mouse, which spontaneously develops autoimmune T1D, has long served as a model to delineate both genetic and immune mechanisms of T1D and its treatment. This model was established in 1980 by Makino *et al.* (97) and emerged from breeding a mouse strain that spontaneously developed cataracts (i.e., CTS strain) (98). Two groups of mice emerged: males with glucose intolerance but without glucosuria, later known as the non-obese non-diabetic (NON) strain, and females with polyuria, ketoacidosis, and glucosuria, subsequently known as the NOD strain (98). Histological examinations of NOD mice demonstrated lymphocyte infiltration of pancreatic islets (insulitis), as well as a decrease in β-cell number and islet size (97).

Typically, 80% of female NOD mice develop insulitis at three weeks and T1D at ~20 weeks (99). The *H2*^{g7} MHC haplotype essential for T1D development in NOD mice has the unique I-A allele (I-A^{g7}). I-A^{g7} encodes histidine and serine at positions 56 and 57 instead of the two usually conserved proline and aspartic acid residues found in other mouse strains (100). The diabetogenic variants of the human class II *HLA-DQβ* homolog also have non-aspartic acid substitutions at residue 57 (101). The genetic association of both MHC class I and class II with disease supports the pathogenic role of CD8$^+$ and CD4$^+$ T cells in the destruction of β-cells in humans and mice (102–105). Multiple autoantigens are targeted by autoreactive T cells (e.g., GAD, insulin, or HSP) (106). However, while the MHC II I-A^{g7} is a major susceptibility allele, it is not sufficient for the development of diabetes, as shown by complete T1D resistance in B10 mice expressing I-A^{g7} (107). In B6 congenic mice expressing I-A^{g7}, circulating T cells can recognize the same β-cell autoantigens as in NOD mice; however, no autoimmunity occurs. These B6.G7 congenic mice confirm the importance of non-MHC genes in controlling autoimmunity in the NOD genetic background (108,109).

In addition to CD4$^+$ and CD8$^+$ T cell autoreactivity, Tregs suppress the development of T1D in NOD mice. Depletion of CD4$^+$CD25$^+$ Treg cells at critical time points can accelerate T1D progression (110). Ablation of essential proliferative or co-stimulatory signals required for Treg cells, such as IL-2 or CD28, exacerbates T1D (111). NOD Treg quantity and functional capability are reduced, and increasing NOD Treg cell activity can prevent diabetes (112–114). These studies suggested that, in addition to the crucial role of T cell autoreactivity, immunological pathways related to Tregs could be genetically determined in the NOD model. Further studies showed shared susceptibility genes affecting Treg function between mice and humans for T1D (e.g., *IL2RA* and *CTLA-4*) (115–117). Thus, studying NOD Treg function and control may enable the development of novel therapeutics in humans. These considerations highlight the significance of identifying *non-HLA* genes implicated in immune regulatory function, Treg function and development, and T1D pathogenesis (117). Identifying genes in the B6/B10 genetic background that can control autoimmunity has thus been a major goal in this field.

4.5 Immunogenetic studies of NOD and Human T1D and translation to novel therapeutics

Before the advent of whole-genome sequencing, many non-*HLA* genomic regions associated with T1D were discovered by linkage analysis of the NOD genome (111,118,119). Identified genetic regions were confirmed to play a role in T1D pathogenesis by constructing congenic mice (120). Congenic mice were constructed by introgression of resistant insulin-dependent diabetes (*Idd*) loci/regions onto the NOD background. Backcrossing of NOD with B6 (C57BL/6J), B10 (C57BL/10Sn), and other T1D-resistant strains demonstrated that over 30 murine recessive *Idd* loci were

associated with protection from spontaneous diabetes (121) (Figure 1A-B). These studies allowed the classification of *Idd* intervals into two groups: those that confer both insulitis and diabetes resistance, and a second group that protects against T1D but has no effect on insulitis (118) (Figure 1A). For example, the *Idd3* locus on chromosome 3 was implicated in protection against insulitis and T1D, whereas the *Idd4* locus on chromosome 11 did not protect against insulitis but did prevent T1D (122). It suggested that genes within these regions exhibited differential effects on T1D development (i.e., T cell migration, cytotoxicity, or Treg function). The next step was to identify and confirm candidate genes within the introgressed genetic regions. This confirmation process ultimately has taken decades of work and the development of new technologies (e.g., whole-genome sequencing, CRISPR).

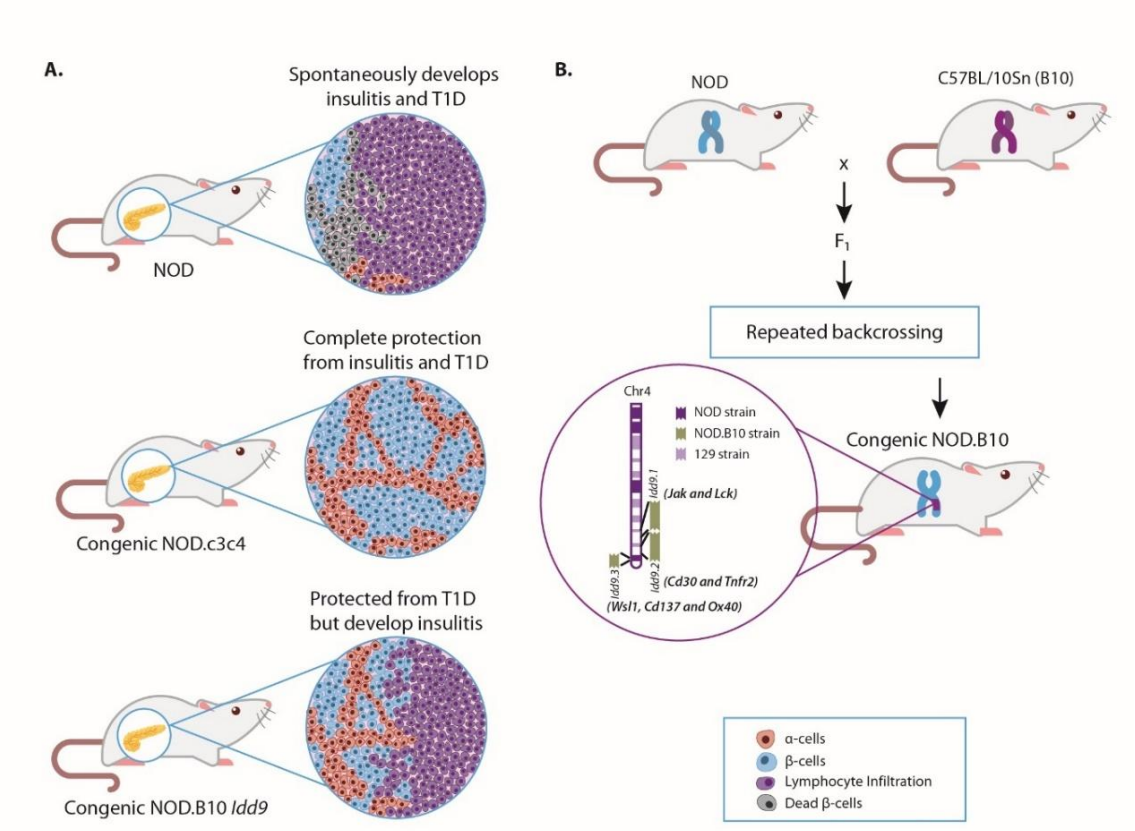

Figure 1. Functional and immunological roles of CD137. (A) Effects of congenic intervals on the clinical and histological phenotypes of NOD mice. (B) Breeding of NOD and B10 mice to produce congenic mice with *Idd9* regions. Chr: Chromosome; NOD: Non-obese diabetic; *Idd*: Insulin-dependent diabetes; T1D: Type 1 diabetes. Source: (123).

One of the first identified non-*HLA* candidate genes encodes interleukin 2 (IL-2). *Il2* is located in the Idd3 region and has profound effects on T cell and Treg function, making it a good candidate gene for T1D (124). NOD produces an altered IL-2 protein compared to the protective B10 allele, with a shortened tandem repeat sequence encoding a poly-glutamine stretch, plus an extra four amino acid insert, in the N-terminal coding region of IL-2 (111). These immunogenetic studies uncovered evidence of multiple genes with multiplicative effects on the immune response. For example, the *Idd3/Idd5* double congenic mice, comprising the *Il2* and *Ctla4* candidate genes, were completely protected from T1D, whereas, when studied alone, only ~20% and ~50% protection were observed, respectively (117,125,126).

Genetic studies in mice were compared with human T1D genetic studies, and strong similarities were observed. The genetic architecture of mouse and human T1D is remarkably similar, with variants affecting multiple immune genes and pathways in common between both species, including *IL-2*, *IL-2RA*, *CTLA-4*, *IL-10*, the *HLA* region, *PTPN22*, and *IL-7R* For example, SNPs in the human homologous *Il2* region were also associated with T1D susceptibility, identifying the IL-2 pathway as potentially shared in the pathogenesis of disease in both species (127,128). The NOD Il2 gene variant reduced IL-2 production, and elegant engineering of Il2 gene haploinsufficiency reproduced the NOD effect, yielding functionally deficient Tregs (129). Low-dose IL-2 therapy increased Tregs in mouse models, leading to human trials; however, while this boosted human Treg numbers, it did not affect T1D outcomes in initial trials (130). A variety of approaches have been tried to optimize IL-2-mediated immune modulation. IL-2 targeted *in vitro* expansion of Tregs is one approach that was effective in NOD mice, tying the IL-2 immunogenetic effects to the enhancement of Treg deficiencies in T1D (131). Large numbers of Tregs are needed for human trials, and *in vitro* expansion may overcome some of the deficiencies of earlier Treg trials (132). Clinical trials in human T1D are ongoing with low-dose IL-2 therapy (133) and Treg therapy (134), which have built upon these earlier results. CTLA-4 is another critical immune molecule with variants identified in mice and humans. The mouse locus (*Idd5*) overlaid the orthologous human *IDD12* locus (125).

T1D susceptibility was also mapped to a non-coding region of human CTLA-4, resulting in lower levels of the CTLA-4 soluble splice variant; the mouse gene also demonstrated alterations in CTLA4 splicing (135). Human trials targeting CTLA-4 with a soluble form that blocks T cell activation appear promising (136). These therapies may be effective even though the human disease shows remarkably different patterns of insulitis than those in the mouse, with much less exuberant immune infiltrates (137). The difference in β-cell immune infiltration may explain why prevention of diabetes in NOD is readily achieved, whereas in humans, prevention trials have, until recently, failed. One successful approach to preventing human T1D has been achieved using anti-CD3 antibodies, which preferentially target CD8 effector cells (138). Notably, anti-CD3 antibodies were identified in NOD mice to reverse established disease (not just prevent it), demonstrating the

usefulness of therapeutic trials of acute T1D in NOD mice. Overall, these examples illustrate the rich insights and potential therapies resulting from T1D immunogenetic studies. The latest large-scale study identified 78 genetic regions linked to T1D (including 36 novel loci) and confirmed their strong association with immune function and their potential for clinical therapeutics (139). Thus, much more work is needed to apply immunogenetic studies to novel therapeutic pathways.

4.6 The role of Idd9 and its main candidate gene, Cd137, in NOD T1D

After identifying the *Idd9* region in linkage studies, the Wicker group constructed congenic mice with the B10 *Idd9* region introgressed onto the NOD background. The B10 *Idd9* region prevented the onset of spontaneous diabetes in NOD mice (less than 5% of female mice developed T1D) (140). However, most mice still developed insulitis, driven by T cells expressing CD30 and producing high levels of IL-4 (140) (Figure 1A). This confirmed that genes associated with lymphocyte infiltration were outside the *Idd9* interval but suggested that some genes within this region halted autoimmunity.

This hypothesis was validated in double-congenic mice comprising B6 (*Idd3, Idd17, Idd10,* and *Idd18*) from the chromosome 3 region and B10 (*Idd9*) from the B10 region (also known as the NOD.c3c4 strain). NOD.c3c4 mice were completely protected from diabetes, and only 10% of mice developed insulitis (140) (Figure 1A). This confirmed that spontaneous diabetes is a complex trait in which epistasis among multiple genes (HLA and non-HLA) is critical for its development, but it also suggested that the Idd9 interval contains genes associated with T cell activation and modulation.

The *Idd9* region, a 48 cM interval, was fine-mapped into three intervals (i.e., *Idd9.1, 9.2,* and *9.3*), with seven candidate genes (i.e., Jak1, Lck, Cd30, Tnfr2, Cd137, Wsl1, and Ox40). *Wsl1, Cd137,* and *Ox40* were initially proposed as candidate genes within the *Idd9.3* locus (140). However, B6 *Wsl1* did not exhibit sequence variations compared to NOD, and *Ox40* was subsequently found to lie outside the *Idd9.3* region and was excluded as a candidate gene (140). Thus, *Cd137* remained the key candidate for T1D protection within the *Idd9.3* locus. Jumping ahead 15 years, it was recently confirmed, using combined congenic mapping and nuclease-based gene targeting, that *Cd137* is the susceptibility gene within the *Idd9.3* locus critical for modulation of T1D (140,141).

Cd137 is located at 1.217-Mb of the *Idd9* locus (i.e., *Idd9.3*) (142), and *Idd9.3* conferred ~40% protection for T1D (140). Analysis of coding variants demonstrated two synonymous SNPs in NOD vs. B10 *Cd137*: a valine to alanine substitution at position 24 and a leucine to proline substitution at position 211 (near the transmembrane domain). There is also an alanine insertion in NOD between amino acids 174 and 175 (140) (Figure 2). These structural modifications suggested that membrane-bound CD137(mCD137) could be hypofunctional in NOD mice (136,140).Mouse *Cd137 (4-1bb or Tnfrsf9)* codes

for two CD137 isoforms: mCD137 and sCD137 (Figure 3) (143). Membrane CD137 is mostly expressed on $CD4^+$ and $CD8^+$ T cells, whereas sCD137 is produced by Tregs (83). The ligand for both isoforms, CD137L, is coded by *Tnfsf9* on chromosome 17 and is expressed on APCs and activated T cells (83).

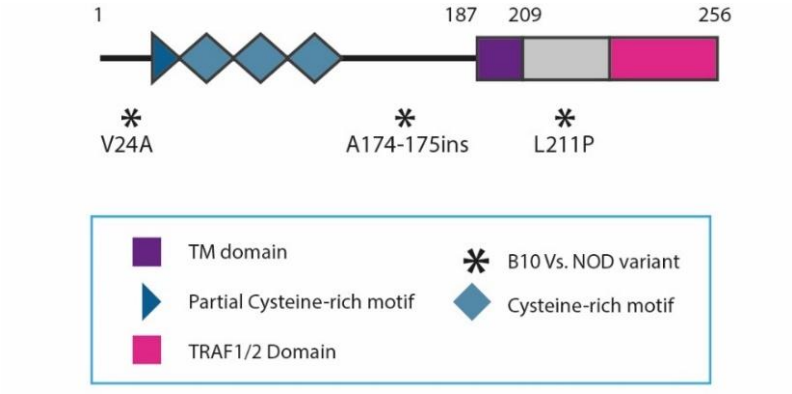

Figure 2. Non-synonymous SNPs of B10 *Cd137* vs. NOD. Valine to alanine substitution at position 24, leucine to proline substitution at position 211 (near the transmembrane domain). And alanine insertion in NOD between amino acids 174 and 175. SNPs: Single nucleotide polymorphisms; STP: Ser/Thr/Pro-rich; TM: Transmembrane domain; TRAF: Tumor necrosis factor receptor-associated factor. Source: Author.

Since the NOD *Cd137* SNPs suggested that mCD137 was hypofunctional compared to the NOD.B10 strain (140), Cannons *et al.*(142) evaluated T cell activation and proliferation to test this hypothesis. They confirmed that NOD and NOD.B10 mice showed similar mCD137 expression after anti-CD3 stimulation in Th1 and Th2 culture conditions. However, when T cells from NOD mice were costimulated with CD137L, they proliferated less and produced lower levels of IL-2 than T cells from mice carrying the B10 allele of *Cd137*. This strongly suggested that the NOD SNPs lead to a hypo-functional mCD137 protein, which could play a role in T1D pathogenesis. Over the last 15 years, our lab has begun to delineate the complex immune biology of CD137 and CD137L in T1D.

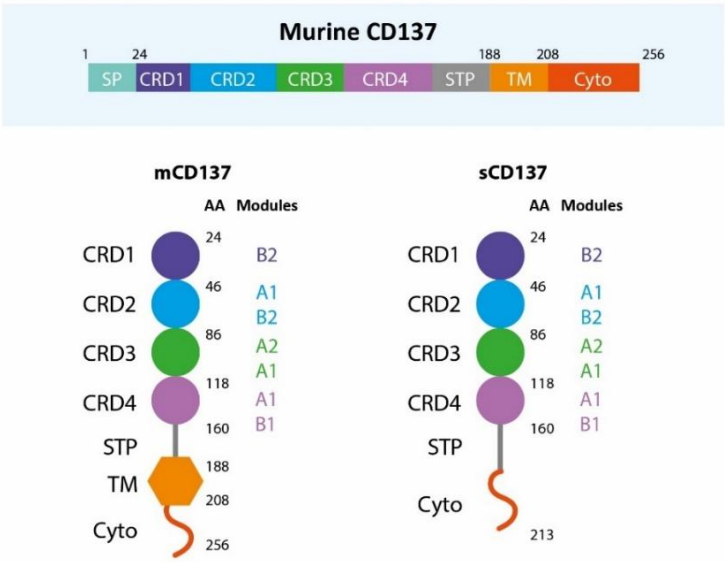

Figure 3. Murine mCD137 and sCD137 spliced variant structure. The modules represent the basic structure and configuration of CD137. As an integral member of the TNF receptor superfamily, the CDR and submodules configuration define this receptor's unique function. The CDR2 (modules A1 and B2) and CDR3 (module A2) are responsible for binding CD137L. These regions are homologous to mCD137 in humans. Structurally, the spliced variant lacks the transmembrane domain. SP: Signal peptide; CRD: Cysteine-rich domain; STP: Serine/threonine/proline-rich segment; TM: Transmembrane domain; Cyto: Cytoplasmic domain. Source: Author.

4.7 CD137 and CD137L: A double-edged sword in autoimmunity

CD137 is a glycoprotein belonging to the TNF receptor superfamily, and the membrane form is expressed on activated $CD4^+$ and $CD8^+$ T lymphocytes (144), Tregs (145–151), natural killer (NK) cells (152), and monocytes (153). mCD137 is also constitutively expressed on a subset of Tregs (150). CD137L (4-1BBL), its ligand, belongs to the TNF superfamily and is expressed on activated APCs, including macrophages, B cells, and DCs (154–156). Activated T cells also upregulate and express CD137L (157). mCD137 lacks intrinsic enzymatic activity in its intracellular domain and functions by binding to the TRAF1 and TRAF2 adaptor proteins, which enhance K63 polyubiquitination in the CD137 signalosome (158,159). CD137L aggregation, upon interaction with mCD137, induces mCD137 receptor clustering and TRAF-mediated activation of the ERK, JNK, p38, NF-kB, and MAPK intracellular signaling pathways, resulting in cell activation, proliferation, and T cell survival (160–167). Notably, signaling in the CD137:CD137L pathway is bidirectional: both the receptor and ligand signal into their respective cells (168,169). This

bidirectional signaling adds a layer of complexity to the analysis of the pathway's biological function.

4.7.1 T Cells mCD137/CD137L Signaling

The effects of mCD137 in T cell biology are diverse but with specific implications for inflammation and immune regulation. TCR-induced proliferation and cytokine production were enhanced after T cells were stimulated with agonistic anti-mCD137 antibodies (also known as 3H3), independent of B7-CD28/CTLA-4 interactions (170,171). mCD137 signaling results in NF-κB activation that promotes the expression of antiapoptotic genes, including Bcl-xL and Bfl-1 (170,171), and mitochondrial function and biogenesis, thereby improving T cell survival (172,173).

mCD137 has a prominent role in $CD8^+$ T-cell costimulation, influencing cytotoxicity in an IL-2-independent manner. Furthermore, $CD8^+$ T cells produce a greater amount of IFN-γ after mCD137 activation (174). *In vivo* experiments showed that knockout mCD137/CD137L mice exhibited a reduced memory $CD8^+$ T cell response to viruses (175–177) and decreased T cell survival (178). These findings pointed to a costimulatory role for mCD137 in long-lasting memory T-cell activation and the enhancement of cytotoxicity, and laid the basis for mCD137-based therapies for cancer (179–182). In contrast to these effects, when knockout mice were stimulated with anti-CD3, T cells showed hyperresponsiveness, indicating an additional immunosuppressive role for sCD137 (164).

4.7.2 APCs mCD137/CD137L Signaling

The expression of CD137L on APCs is increased at sites of inflammation *in vivo* (183,184). Intrinsic activation of APCs by CD137L upregulated B7-1 and B7-2, and increased IL-6 and IL-12 secretion (183). CD137L is upregulated on activated T cells, and CD137L signaling is critical for $CD8^+$ T cell survival via STAT3- and FAS-mediated pathways (185). $CD4^+$ T cell activation can also be modulated by CD137L- expressing APCs (*via* APC CD137L signaling through T cell mCD137) that stimulate IL-2 and IL-4 T cell production (170,171).

CD137L on APCs, therefore, affects the cytotoxic immune response and is critical for the survival of $CD8^+$ and $CD4^+$ T cells. Inhibition of mCD137 or CD137L might reduce inflammation *via* $CD8^+$ T cells but may, at the same time, affect $CD4^+CD25^+$ CD137-expressing Tregs. Indeed, *Cd137* is upregulated by *Foxp3* (186). CD137 is expressed by Tregs infiltrating the islets in T1D, suggesting an immunoregulatory role for $CD137^+$ Tregs (187). Thus, modulating CD137 or CD137L signaling in T effector cells could decrease

immunosuppression via Tregs, underscoring the intricacies of this pathway and the potential for double-edged effects.

4.7.3 Tregs mCD137/CD137L Signaling

Type 1 regulatory T (Tr1) cells are another type of regulatory T cell characterized by IL-10 production and the lack of constitutive *Foxp3* expression (188). Despite evidence of *CD137L* mRNA expression after stimulation (189), it is unknown whether these cells also exert their suppressive function via sCD137 or whether they play a role in NOD mice during T1D pathogenesis. Since *Cd137* is upregulated by *Foxp3* (186), Tr1 cells may not produce large quantities of sCD137. Further studies of this cell subset and their involvement in the mCD137/CD137L axis are warranted.

Agonistic anti-mCD137 antibodies induced the proliferation of $CD4^+CD25^+$ Tregs while maintaining their suppressive activity (149). Interestingly, the effects of agonistic anti-mCD137 antibodies are diverse and dependent on the target and the disease. Activating $CD8^+$ T cells by anti-mCD137 antibodies in cancer models leads to tumor cell elimination. In sharp contrast, in models of autoimmunity, e.g., murine models of SLE (189,190), experimental autoimmune encephalomyelitis (191), collagen-induced arthritis (178,192), Sjögren's syndrome-like sialadenitis (193), and inflammatory bowel disease (194), anti-mCD137 antibody treatment leads to immunoregulation and disease amelioration (150). For example, anti-mCD137 administration in the SLE murine model reversed disease, reduced autoantibody production (i.e., dsDNA antibodies), and decreased immune complex deposition (190). Induction of T cell anergy by anti-mCD137 antibodies might play a role in some of these models (190,195).

4.8 Anti-CD137 Antibodies prevented T1D via Treg expansion but accelerated T1D in the absence of Tregs

Since *Cd137* was a candidate gene in T1D, we initiated an investigation into its role in T1D using agonistic anti-mCD137 antibodies. We showed that anti-mCD137 antibodies in NOD mice prevented the development of T1D but did not ameliorate insulitis, consistent with residual insulitis in NOD congenic mice protected from T1D by the B10 *Idd9.3* region (150). We found that anti-mCD137 expanded $CD4^+CD25^+$ Tregs, and their transfer to NOD-*scid* mice completely prevented T1D (150). However, T1D progressed more rapidly when NOD-*scid* mice were treated with anti-mCD137 after pathogenic $CD4^+$ and $CD8^+$ T cell transfer in the absence of Tregs. Therefore, in the absence of Tregs, mCD137 stimulation could potentiate pancreatic destruction through $CD8^+$ T-cell cytotoxicity. This is similar to the effect of anti-mCD137 administered in the context of autoimmune thyroiditis, which worsens the disease (196).

Due to this dual effect, activation of effector T cells in acute autoimmunity may preclude the use of agonistic mCD137 antibodies in clinical autoinflammatory states, including T1D, because activated T cells upregulate mCD137 in these settings. In contrast, mCD137 antibodies in non-inflammatory states (e.g., pre-diabetes) might prevent autoimmunity, as they target Tregs that constitutively express mCD137 without activating T cell effector cells. This dual effect led us to explore alternative approaches to therapeutically target the mCD137/CD137L pathway in T1D.

We turned our attention to sCD137, which is formed by alternative splicing (157,197) and exists as a dimer (198). Murine sCD137 differs from humans. In mice, only the exon coding for the transmembrane domain of CD137 is spliced out (Figure 2-3), whereas in the latter, two splice variants are observed (199,200) (Figure 4).

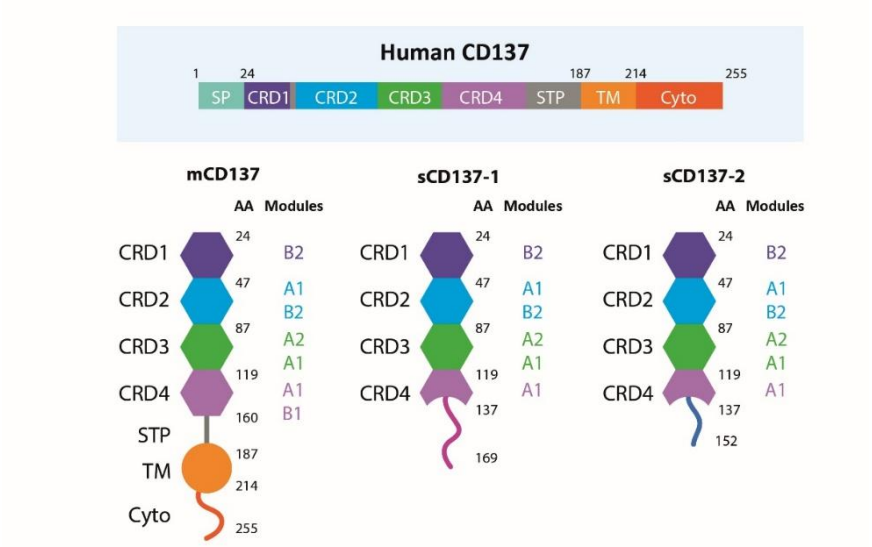

Figure 4. Structures of human mCD137 and sCD137 spliced isoforms. The modules represent the basic structure and configuration of CD137. As an integral member of the TNF receptor superfamily, the CDR and submodules configuration define this receptor's unique function. The CDR2 (modules A1 and B2) and CDR3 (module A2) are responsible for binding CD137L. These regions are homologous to mCD137 in mice. Structurally, the spliced variants lack CRD4 module B1. However, it would not interfere with binding to CD137L. SP: Signal peptide; CRD: Cysteine-rich domain; STP: Serine/threonine/proline-rich segment; TM: Transmembrane domain; Cyto: Cytoplasmic domain. Source: Author.

The larger spliced variant (i.e., 162 amino acids), the sCD137-1, lacks the region from nucleotide 414 to 545, which encodes the serine/threonine/proline-rich segment of

mCD137. This deletion results in a frame-shift mutation and a translational stop codon 96 nucleotides downstream. Thus, this spliced variant lacks the transmembrane domain but retains a terminal sequence that differs from the native mCD137 cytoplasmic domain (Figure 4) (199). In the smaller spliced variant (152 amino acids), the deletion starts at the same nucleotide as in sCD137-1. However, it extends to nucleotide 681, comprising the exon for the transmembrane domain and the 5` upstream exon (Figure 4) (199). The terminal section differs from mCD137 and sCD137-1 (Figure 4). Whether these differences between spliced variants are biologically significant remains unknown. The sCD137 is preferentially secreted by $CD4^+$ T cells, whereas $CD8^+$ T cells express higher amounts of mCD137 (201). We found that the major source of sCD137 is $CD4^+CD25^+CD137^+$ Tregs (151).

Spliced variants are critical for modulating the immune response (202). The induction of alternative splicing is poorly understood but may occur as a response to environmental signals. In autoimmunity, splicing also occurs in the modulation of immune responses (202). Changes in the immunological environment (i.e., T cell autoreactivity and a pro-inflammatory milieu) induce Treg cells to produce sCD137. We have demonstrated that activating Treg cells increases sCD137 production by Tregs in mice and humans (33).

We previously demonstrated that the mouse sCD137 is produced as a homodimer, which is capable of suppressing total $CD4^+$ and $CD8^+$ T cells, preventing and reversing T1D in NOD mice (33,198). Although the ultrastructure of human sCD137 remains unknown, there is some evidence pointing to differences between species. Unlike its human counterpart, mouse CD137L forms a covalent dimer (203). As mouse CD137L only binds to two receptor molecules (i.e., CD137) due to its dimeric structure, this leads to a 2:2 stoichiometry in the CD137/CD137L complex (two CD137L molecules bind to two CD137 molecules) rather than the 3:3 stoichiometry as observed in humans (200). Since mouse CD137L can only bind to two CD137 molecules, it may not be sufficient to trigger strong signaling on its own. Mouse Gal-9 helps to bring multiple CD137 molecules together, forming larger clusters that are necessary for efficient signaling *via* interaction with glycosylated spots in their CRD4 region. In this line, the mouse sCD137 could either prevent CD137L multimerization or induce a negative signal, as we previously demonstrated (33).

On the other hand, human CD137L forms non-covalent bonds, giving rise to homotrimers (200). Interestingly, it was found that the human CD137 receptor interaction is dominated by a single monomer within the trimer with little interaction with the adjacent ligand protomer (200). In addition, such interaction requires Gal-9, which facilitates CD137 aggregation, signaling, and functional activity in T cells, dendritic cells, and natural killer cells (204). This has significant implications for our understanding of human sCD137 biology since the sCD137 isoforms (i.e., sCD137-1 and sCD137-2) lack the glycosylation sites in the CRD4 region, which are relevant for CD137/Gal-9 interaction in mice (203).

Further studies uncovering the functional implication of the CRD4 modifications in the human sCD137 isoforms, their ultrastructure (e.g., homodimer or homotrimer), the modulation of Gal-9, and the sCD137/CD137L interaction are necessary.

Inflammatory environmental changes may explain the origin of spliced variants of CD137 from Tregs as a homeostatic response to ameliorate inflammation. A similar process is seen with soluble CTLA4 (sCTLA4), a spliced variant of membrane-bound CTLA4 mainly produced by Foxp3$^+$ Tregs (205). sCTLA4 particularly suppresses early T-cell activation by preventing the interaction of CD80/CD86 with the costimulatory receptor CD28 (206). In addition, it inhibits IFN-α, IL-2, IL-7, and IL-13 production while activating TGF-β and IL-10 release (207). Silencing *sCTLA-4* mRNA by RNA interference accelerated the onset of T1D in NOD mice and impaired Treg ability to downregulate DC costimulation (205). Both spliced variants, sCTLA4 and sCD137, may be critical for effective Treg function in the pathogenesis of T1D.

What is the role of sCD137? Our hypothesis was that sCD137, like sCTLA4, functions as a negative feedback mechanism to downregulate immune response mediated by mCD137 and CD137L (143,208). sCD137 reduces the production of IL-10 and IL-12 from activated splenocytes (201). In addition, T cell proliferation and IL-2 release were inhibited when sCD137 was administered to these cells (209). These initial reports clarified the effects of CD137 in different conditions (i.e., cancer and autoimmunity) and suggested that sCD137 was the missing link in understanding the dual effects of the mCD137/CD137L axis.

To confirm the role of CD137 in the *Idd9.3* locus, we evaluated the function (immunosuppressive effects) and quantity of CD137$^+$ Treg cells in NOD.*Idd9.3* congenic mice (151). When compared to NOD mice, the NOD.*Idd9.3* strain had significantly higher percentages of CD4$^+$CD25$^+$CD137$^+$Foxp3$^+$ Tregs in the thymus and spleen, and the numbers increased with age. This supported the hypothesis that the hypofunctional NOD *Cd137* allele led to decreased Treg survival, consistent with the known effects of mCD137 on cell survival. CD137$^+$ Tregs showed superior immunosuppression compared to CD4$^+$CD25$^+$CD137$^-$ Tregs, directly showing an effect of CD137 on Treg function. Thus, increased numbers of CD137$^+$ Tregs, mediated by the protective allele, led to increased overall suppressive capacity. Importantly, CD137$^+$ Tregs showed suppressive capability in an independent contact assay. This supported our continued focus on the possible immunosuppressive role of sCD137 in T1D.

4.9 sCD137 is produced by Tregs and inhibits T cell autoreactivity in a paracrine fashion

We first confirmed that sCD137 was mainly produced by CD4$^+$CD25$^+$CD137$^+$ Tregs and in higher amounts in NOD.*Idd9.3* congenic mice (151). Next, we demonstrated that

sCD137 primarily exists as a ~55 kDa homodimer under non-reducing and a ~35 kDa monomer under reducing conditions (198). Next, we showed that administering recombinant sCD137 to NOD mice prevented diabetes and reduced insulitis by preserving insulin$^+$ islets (198). Since CD4$^+$CD25$^+$CD137$^+$ Tregs inhibited T cells in a contact-independent manner (151), we evaluated the role of sCD137 in T cell inhibition. We demonstrated that sCD137 inhibited activated T cells by binding to CD137L (198). In addition, sCD137 can directly stop the proliferation of effector CD4$^+$CD25$^-$CD137$^-$ T cells in the absence of APCs, and without inducing cell death (198) (Figure 5).

In addition to its crucial role in immunosuppression, reports suggest that mCD137, like other costimulatory molecules, plays a nonredundant role in maintaining the pathogenic activity of β-cell-autoreactive T cells in NOD mice. We found that, compared with wild-type mice, T1D development is reduced in NOD Cd137-/- mice. *Cd137*$^{-/-}$ and their T cells are less capable of inducing T1D in NOD.*Rag1*$^{-/-}$ recipients (210). This, at first, seemed contradictory to our data on the immunoregulatory properties of CD137$^+$ Tregs and sCD137. As sCD137 produced by Tregs is suppressive, evaluating the distinctive role of mCD137 in CD4$^+$ and CD8$^+$ T cells was crucial. Isolated T cells from NOD and NOD.*Cd137*$^{-/-}$ mice were transferred into NOD.*Rag1*$^{-/-}$ recipients. The T cell adoptive transfer studies revealed that mCD137 expression in CD8$^+$ T cells was required for the development of T1D in NOD mice, whereas mCD137 expression in CD4$^+$ T cells was diabetes-protective (210). Specifically, mCD137 expression on CD4$^+$ Tregs is important for their T1D suppression function. We further demonstrated that mCD137 cell-intrinsically stimulates the accumulation and proliferation of autoreactive CD8$^+$ T lymphocytes within the islets, suggesting a role for mCD137 in the diabetogenic activity of CD8$^+$ T cells. However, sCD137 suppressed the proliferation of CD8$^+$ T cells. These experiments supported the concept that the T1D protection conferred by the *Idd9.3* locus is mediated by sCD137 produced by Tregs.

As the sCD137/CD137L interaction modulates effector CD8$^+$ T cells, clarifying the role of CD137L in T1D immunomodulation is essential. CD137L-deficient NOD mice exhibited less insulitis and a delayed onset of T1D (211). Interestingly, CD137L expression on myeloid APCs appeared to be necessary for the survival of β-cell–autoreactive CD8$^+$ T cells and T1D progression, but CD137L has no effect on the formation or homeostasis of Foxp3$^+$ Tregs (211). It remains to be determined if mCD137 in Tregs modulates their function and whether Tregs capable of producing sCD137 but not mCD137 are sufficient to suppress T1D.

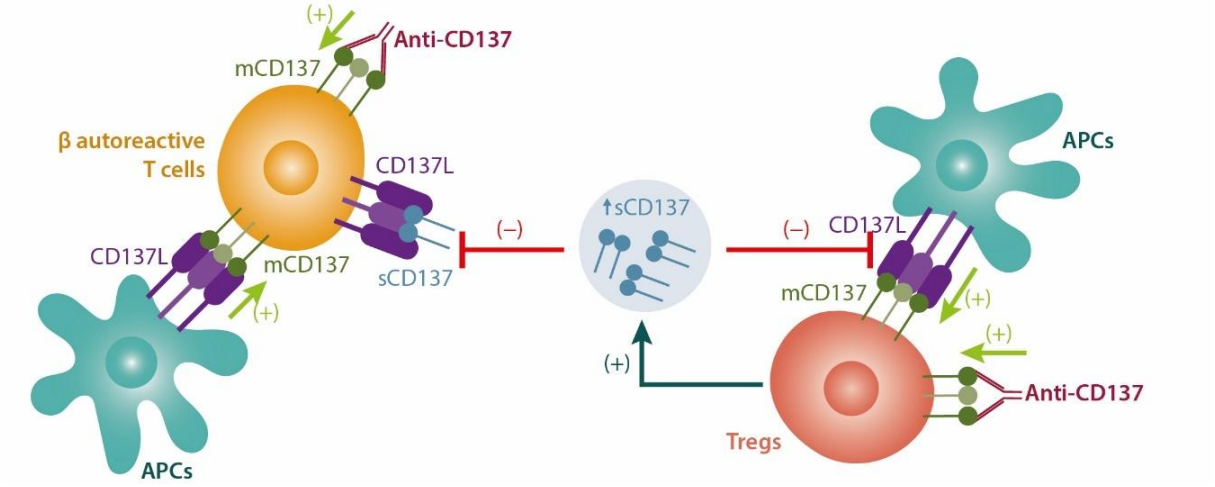

Figure 5. Tregs produce sCD137 as a dimer. sCD137 induces altered CD137L signaling in APCs and autoreactive T cells (compared to mCD137), reducing inflammation and damage in the pancreas. In contrast to sCD137, anti-mCD137 antibodies activate Tregs (a strong immune regulatory effect), but they may also increase autoreactive T cell survival and proliferation, thereby perpetuating inflammation and autoimmunity. APCs: Antigen-presenting cells; CD137L: CD137 Ligand; mCD137: Membrane-bound CD137; sCD137: Soluble CD137; Tregs: T regulatory cells. Source: (123).

4.10 sCD137 directly suppresses T cells in an APC and Treg-independent manner, inducing anergy

It is relatively easy to prevent T1D in NOD mice, and a much more stringent target is the reversal of actual acute T1D. Thus, we treated NOD mice with new-onset T1D with recombinant sCD137 (33). This experiment confirmed that sCD137 could not only prevent T1D but also halt acute T1D and avert the development of end-stage diabetes. In effectively treated mice, β-cell immunohistochemistry revealed considerable preservation of insulin$^+$ β cells and a rise in insulin$^+$ islets (33). In this setting, T cells showed downregulation of mTORC1, developed an anergic phenotype (reversed by IL-2), and were suppressed by sCD137 in antigen-experienced and activated memory T cells. CD8$^+$ effector memory cells also showed a reduction in the production of inflammatory cytokines in the presence of sCD137 (i.e., IFN-γ) (33).

In pediatric patients with human T1D, we found lower levels of sCD137 than in age-matched non-diabetic controls during acute flares (hospital admissions for hyperglycemia). We also confirmed that human Tregs were the primary source of sCD137 (33). Furthermore, human peripheral CD4$^+$ T cells activated by sCD137 were inhibited. These results were analogous to those in NOD congenic strains, supporting the notion that these murine models are useful and relevant to affecting the autoimmune phenomena

driving human T1D. This evidence showed that sCD137 is associated with autoimmunity in humans with T1D and that low sCD137 levels may serve as a biomarker for T1D. Further studies are required to confirm the role of sCD137 in reversing established destructive insulitis and the pathways underlying this phenomenon.

Surprisingly, sCD137 is reported to be increased in patients with RA (199,212,213) and multiple sclerosis (214), and the levels were directly correlated with the severity of the disease (212). Increased levels of sCD137 could be a homeostatic attempt by Tregs to modulate inflammation in these conditions. However, it also raises the possibility that, in the presence of a substantial inflammatory substrate, the stoichiometric ratio of sCD137 to CD137L could be reduced, thereby reducing sCD137's efficacy or possibly indicating that higher sCD137 doses would be required. New strategies to improve the half-life and potency of sCD137 could be critical to enhancing their therapeutic effects in human autoimmunity.

In summary, Linkage studies and the construction of congenic mice enabled the identification of candidate genes implicated in the pathogenesis of T1D. The cumulative evidence suggests that *Cd137* and its coding isoforms are crucial for T1D development, and the CD137-CD137L pathway is a promising therapeutic target. Treg-generated sCD137 modulates the mCD137/CD137L axis, reduces insulitis, and halts T1D in the NOD mouse. The ability to effectively halt acute T1D with exogenous sCD137 is an exciting development with attractive therapeutic potential. Prevention studies in humans are difficult to implement, and those attempted so far have failed (215). Therefore, treating acute disease is a more appealing strategy, but the current approved therapeutic landscape is limited. The use of antibodies targeting the CD137-CD137L axis is appealing; however, while anti-mCD137 antibodies are protective in some models of autoimmune disease by activating Tregs, they can also enhance $CD8^+$ T cell killing activity in the absence of Tregs. sCD137, on the other hand, only suppresses $CD4^+$ and $CD8^+$ T cell activation and may therefore be safer than an anti-mCD137-based approach. In human studies, low levels of sCD137 during T1D flares and the inhibition of activated $CD8^+$ T cells in vitro after sCD137 stimulation support its further translational use. Soluble CD137 suppresses autoreactive $CD8^+$ T cells by inducing anergy. However, little is known about sCD137's role in innate immunity. The mechanistic role of sCD137 on CD137L-expressing myeloid APCs should be explored to determine if there will be lasting effects on innate immune function. In addition, it is unknown whether mCD137 on Tregs drives their differentiation to a more robust inhibitory phenotype. This could have therapeutic implications, particularly for the pharmacokinetics and pharmacodynamics of human sCD137.

5. AIMS

Aim

1. To evaluate the tolerizing effects of sCD137 on the mCD137-CD137L signaling in humans for the treatment of T1D.

Specific aims

2. To characterize the epidemiological landscape of T1D and describe the possible mechanisms implicated in its development.

3. To investigate the cellular and molecular characteristics of pathogenic antigen-specific T cells and validate sCD137 as a dynamic biomarker of immune dysregulation in ADs.

4. To translate the immunogenetic insights of CD137 from animal models to human immunology and functionally validate recombinant human Fc-sCD137 as a novel, mechanism-based immunotherapeutic agent.

6. CHAPTERS

CHAPTER I: To characterize the epidemiological landscape of T1D and describe the possible mechanisms implicated in its development.

This chapter integrates two major studies: a comprehensive meta-analysis on the prevalence of polyautoimmunity in T1D and a state-of-the-art review on the mechanisms of molecular mimicry in this condition.

Manuscript 1: Prevalence of Latent and Overt Polyautoimmunity in Type 1 Diabetes: A Systematic Review and Meta-Analysis

This study analyzed data from 158 articles involving 270,890 individuals with T1D to define the landscape of co-occurring autoimmune diseases. We found that the pooled prevalence of overt PolyA (clinical coexistence of two or more ADs fulfilling classification criteria) in T1D patients is 8.50% (95% CI: 6.77 to 10.62), with North America exhibiting the highest prevalence of overt polyautoimmunity at 14.50%. The most common co-occurring AD was autoimmune Thyroid Disease (AITD) with a prevalence of 7.44%.

On the other hand, latent PolyA (the presence of autoantibodies unrelated to an index AD, without clinical criteria fulfillment) was significantly higher at 14.45% (95% CI: 11.17 to 18.49). Asia exhibited the highest rates of latent polyautoimmunity (23.29%), followed by Oceania (21.53%). The most frequently detected autoantibodies in these asymptomatic patients were Rheumatoid Factor (RF), followed by Ro52, Thyroid Peroxidase (TPO), and Thyroglobulin (TG) antibodies.

There was a significant positive association between the duration of T1D and the frequency of both latent (β: 0.0456, p=0.0140) and overt (β: 0.0373, p=0.0152) PolyA. This suggests that the risk of developing additional autoimmune conditions is dynamic and increases progressively as patients age. Individuals with T1D should regularly undergo assessments to identify potential concurrent autoimmune diseases, especially as they age.

Manuscript 2: Autoimmunity, Epitope Analysis, and Molecular Mimicry

This review redefines the traditional understanding of molecular mimicry by incorporating modern structural biology and environmental triggers. We proposed a novel classification beyond simple sequence matching:

A. Direct Mimicry: Exact protein-level identity (e.g., viral hijacking).
B. Linear Homology: Shared sequential peptide epitopes.
C. Structural Mimicry: Convergence of 3D shapes (tertiary/quaternary) without sequence similarity.

D. Functional Mimicry: Non-peptide molecules (metabolites, glycans) mimicking immune signals.

Structural and functional mimicry could account for more than 40% of cross-reactive responses in autoimmunity. Most of these occur as mimotopes, defined as 3D molecular analogs that escape tolerance because of structural rather than linear resemblance to self-antigens. In addition, besides structural homology, we identified three molecular pathways involved in breaking tolerance:

1. Polyspecific TCR recognition: A single TCR interacts with both microbial and self-antigens.
2. Dual TCR expression: A single T cell carries two different TCRs.
3. Chimeric TCR assembly: Hybrid α/β chain combinations.

In addition, there is a novel field focused on the formation of new epitopes and the modification of existing ones that could trigger autoimmunity, including post-translational modifications (PTMs) and neo-epitopes. Factors such as smoking or specific infections (e.g., P. gingivalis) induce PTMs (e.g., citrullination) that create new targets for the immune system. In T1D, the covalent cross-linking of proinsulin peptides creates neo-epitopes that bypass thymic tolerance and activate autoreactive T cells.

Molecular mimicry often acts as the "initial spark." The subsequent tissue damage leads to the release of more self-antigens, causing the immune response to diversify (spread), which eventually leads to chronic clinical disease. Mimicry alone may be innocuous; clinical autoimmunity requires additional random events, such as changes in regulatory T cell function or cytokine production thresholds.

Novel computational models like AlphaFold2 & ColabFold have revolutionized the field by providing high-confidence 3D structural predictions of proteins and their isoforms, allowing the identification of "cryptic mimotopes" that lack linear sequence similarity but share tertiary topologies. In addition, combining structural predictions with Cryo-Electron Microscopy (Cryo-EM) data helps visualize the physical docking between TCRs and peptide-MHC (pMHC) complexes. Other software, such as NetMHCpan-4.1 and HPEPDOCK, are considered leading tools for predicting MHC antigen presentation by integrating mass spectrometry (MS)- eluted ligand data, which is crucial for identifying T1D-relevant neo-epitopes. This may enable the study of molecular mimicry beyond peptide structure and the development of novel immunotherapies.

CHAPTER II: To investigate the cellular and molecular characteristics of pathogenic antigen-specific T cells and validate sCD137 as a dynamic biomarker of immune dysregulation in ADs.

This chapter focuses on the cellular drivers of autoimmunity and the validation of sCD137 as a systemic signature of immune dysregulation.

Manuscript 3: Soluble CD137 in Health and Disease: A Systematic Review and Meta-Analysis

This meta-analysis of 47 articles (4,854 individuals) confirms that sCD137 levels are significantly higher in patients with disease than in healthy controls (Standardized mean difference [SMD]: 1.08; 95% CI: 0.71 to 1.44). The highest elevations were found in infectious diseases (SMD: 1.78, 95% CI, 0.08 to 3.47) and gastrointestinal/genitourinary syndromes (SMD: 1.30, 95% CI, 0.12 to 2.48), while autoimmune diseases (SMD: 0.73, 95% CI, 0.03 to 1.43) and cancer (SMD: 0.79, 95% CI, 0.23 to 1.34) showed lower but significant elevations. In autoimmune contexts, successful therapy significantly reduced sCD137 levels (SMD: -0.73, 95% CI, -1.24 to -0.23), whereas cancer immunotherapies (e.g., anti-PD-1) increased them (SMD: 1.14, 95% CI, 0.60 to 1.67), reflecting its role as a marker of immunoregulatory pressure. The biomarker's reliability was not influenced by age, geographical location, or the specific measurement technique (ELISA vs. high-throughput proteomics). This study confirms that sCD137 could be used in the diagnosis and follow-up of inflammatory conditions and cancer.

Manuscript 4: Antigen-Specific T Cells and Autoimmunity

The central dogma of immunity states that clonal expansion drives the selection of higher-affinity T-cell clones; however, novel evidence supports the Involvement of low-affinity T cells in the perpetuation of autoimmunity. Initial islet destruction is likely triggered by high-affinity T-cell clones that recognize primary autoantigens. Then, chronic, long-term insulitis is sustained by low-affinity T-cell clones. These clones are often missed by standard MHC II tetramer assays but are identified through novel 2D affinity tests (micropipette-based adhesion). Effective T1D treatment should target both high- and low-affinity repertoires to prevent continuous β-cell destruction.

The most common T-cell targets in T1D include Pre-proinsulin (PPI), Glutamate decarboxylase (GAD), Zinc-transporter 8 (ZnT8), and Islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP). In addition, there are neo-epitopes formed by the fusion of insulin fragments with other secretory granules. They avoid thymic negative selection and are recognized by highly pathogenic $CD4^+$ T cells in both NOD mice and human T1D patients. Targeting these specific clones (e.g., via CAR-Tregs or altered peptide ligands) offers a path to treat autoimmunity without the risks of systemic immunosuppression.

CHAPTER III: To translate the immunogenetic insights of CD137 from animal models to human immunology and functionally validate recombinant human Fc-sCD137 as a novel, mechanism-based immunotherapeutic agent.

This chapter traces the translational journey from the NOD mouse model to the development of human Fc-sCD137.

Manuscript 5: The Long and Winding Road: From Mouse Linkage Studies to a Novel Human Therapeutic Pathway in T1D

In the NOD mouse model of T1D, *Cd137* was mapped within the *Idd9.3* region, which we proved prevented T1D. The B6 variant of *Idd9.3* protected from T1D by enhancing the survival of $CD137^+$ Tregs, which were more immunosuppressive than $CD137^-$ Tregs. We subsequently demonstrated that *Cd137* was the *Idd9.3* susceptibility gene. Production of mouse sCD137 was increased in mice with the B6 allele of *Idd9.3* and in mice effectively treated with an agonist anti-CD137 antibody, suggesting a possible immunomodulatory role of mouse sCD137. Treatment of acute diabetic NOD mice with sCD137 ameliorates the disease. Mouse sCD137 delayed the onset of end-stage disease, maintained insulin-producing β-cells in the islets, and, in some cases, prevented progression to end-stage T1D. The immunological mechanism of sCD137 was anergy induction in $CD4^+$T cells (reversed by exogenous IL-2), resulting in reduced antigen-specific T cell proliferation and IL-2/IFN-γ secretion *via* modulation of the mTOR complex 1 (mTORC1) pathway. Additionally, sCD137 significantly reduced the production of inflammatory cytokines by $CD8^+$ effector memory T cells, which play a key role in β-cell damage. These results suggested that, unlike mCD137, sCD137 induced a counter-regulatory immunosuppressive response that opposed the initial costimulatory action of mCD137. We observed that human T1D patients had lower serum sCD137 levels than age-matched controls. These results suggested that sCD137 could be a promising avenue for treating T1D and other T cell-mediated ADs.

Manuscript 6: Alternate Splicing Converts Human CD137 from Costimulatory to Immunosuppressive Function

We found that human *TNFRSF* is alternatively spliced to produce two isoforms, sCD137-1/2, which lack the CRD4 region and form unique structures compared to mCD137. Human activated Tregs produce both *CD137* splice isoforms, which are rapidly upregulated after cell activation, and identify an activated Treg phenotype along with *FOXP3*, *CTLA4*, and *sCTLA4*. We engineered recombinant Fc-Hu-sCD137 variants, which were immunosuppressive, inhibiting IFN-γ secretion and proliferation in purified $CD4^+$ and $CD8^+$ T cells in an APC-independent manner. These effects are mediated by the downregulation of S6 and 4EBP1 of the mTOR complex 1 pathway. Human sCD137 variants, unlike the membrane-bound form, are immunosuppressive and may represent a novel treatment for inflammation and autoimmunity.

We found that human macrophages differentiated with M-CSF and Fc-sCD137 variants exhibited lower membrane expression of M1/M2 markers (i.e., CD86, CD163, and CD206), the CD137 ligand, and a diminished response to LPS, as measured by reduced TNF-α production while continuing to produce IL-10. Additionally, Fc-sCD137 variants reduced the extracellular acidification rate of differentiated monocytes, indicating reduced glycolytic capacity. These changes were modulated by downregulation of mTORC1, and STAT-3 pathways. Human sCD137 variants regulate adaptive and innate immune responses, highlighting their potential as therapeutic agents for controlling inflammatory and autoimmune diseases.

7. CONCLUSIONS

CHAPTER I:

- ✓ 14.45% of patients with T1D present latent PolyA, and 8.50% have overt polyautoimmunity, with AITD as the most frequent disease.
- ✓ The presence of rheumatoid factor, Ro52, and thyroid autoantibodies is the most common cause of latent PolyA in T1D.
- ✓ Routine screening for AITD patients with T1D is necessary for overall management, especially as the patients age.
- ✓ Structural mimicry, rather than simple sequence homology, is a key driver of T1D initiation, as well as the development of additional ADs (i.e., PolyA).
- ✓ Environmental triggers like viruses utilize molecular mimicry to break immune tolerance in susceptible individuals.
- ✓ Endogenous neo-epitopes, such as Hybrid Insulin Peptides, are primary targets for autoreactive T cells in T1D.
- ✓ Advanced AI-driven modeling (i.e., AlphaFold2) is essential to identify structural mimotopes. In addition, docking and affinity analyses will allow the detection of novel pathogenic peptides and the design of targeted therapies for ADs.

CHAPTER II:

- ✓ ADs pathogenesis follows a "Two-Hit" model involving both high and low-affinity T-cell clones.
- ✓ High-affinity T cells initiate the early destruction of tissues, while low-affinity T-cell clones are responsible for the chronic, long-term persistence of autoimmunity.
- ✓ Standard MHC II tetramer assays often fail to detect the full spectrum of low-affinity pathogenic T cells. However, the novel 2D affinity tests (i.e., micropipette-based adhesion) allow the detection of such T-cell clones.
- ✓ sCD137, produced by activated Tregs, is significantly elevated in inflammatory conditions, including ADs and cancer, which could serve as a diagnostic biomarker.
- ✓ On the other hand, sCD137 decreases with immunosuppressive treatment but increases in the presence of cancer immunotherapy. This suggests that sCD137 could also work as a response/failure therapeutic biomarker in both conditions.

CHAPTER III:

- ✓ The *Cd137* gene is the definitive susceptibility factor within the *Idd9.3* locus in NOD mice, and administration of sCD137 restored self-tolerance in this model, mediated by the downregulation of the mTORC1 pathway in antigen-specific effector T cells.
- ✓ Human Tregs produce sCD137-1/2 which differ from mCD137 due to altered CRD4-STP domain.
- ✓ The human *sCD137* isoforms transcripts could be used as markers for an activated Treg phenotype, similarly to *FOXP3*, *CTLA4*, and *sCTLA4.*
- ✓ The novel human Fc-sCD137 variants reduced the proliferation and production of cytokines *via* the modulation of mTORC1.
- ✓ Human recombinant Fc-sCD137 represents a promising, targeted therapeutic strategy for T1D and other T-cell mediated ADs.
- ✓ The effect of the variants is not T-cell dependent. This new treatment modifies monocyte activity and differentiation, further enhancing the overall effect of the therapy

8. PERSPECTIVES

- ✓ Cohort studies are necessary to confirm the progression from latent to overt PolyA in T1D. This will enable the generation of prediction models based on autoantibody positivity and may also require analysis of other covariates, such as disease duration, sex, and HLA genotypes.

- ✓ It is still unknown whether having any PolyA is related to adverse outcomes in T1D. The study of this phenotype is of major clinical relevance in patients with this condition.

- ✓ We found geographical variations in the prevalence of latent and overt PolyA in T1D. It warrants further study to identify genetic variants associated with the onset of additional ADs in T1D.

- ✓ Despite the strong results of sCD137 in differentiating healthy controls from diseased patients, further studies are required to evaluate the utility of this biomarker in the severity of the disease, and in the follow-up of flares in diseases like SLE, RA, or MS, as well as its correlation with cell subtypes like Tregs $FOXP3^{+}CD137^{+}$ and multivariate modeling with other soluble markers such as sCTLA4.

- ✓ Even though the techniques for sCD137 detection are well validated, and we found no difference between them (i.e., ELISA, Olink proteomic assay, Multiplex bead-based immunoassay), it is still unknown which isoform is detected. There is still a need to define which isoform is more clinically relevant.

- ✓ We demonstrated that soluble Fc-sCD137 variants are strong suppressors of immune cells; however, it is still unknown which of the isoforms is more relevant in the immune modulation by Tregs. Studies using CRISPR-edited Tregs (knockout of sCD137-1 or -2 *via* splice-specific guides) will confirm the biological relevance of each isoform and guide future Immunotherapeutics.

- ✓ We found that sCD137 isoforms exhibit modifications in the CRD4 domain. This region is of major relevance for mCD137/CD137L complex formation and activation in humans via the interaction with Galectin-9 (Gal-9). Further studies on the functional implications of CRD4 modifications in human sCD137 isoforms, their ultrastructure (e.g., homodimer or homotrimer), modulation of Gal-9, and the sCD137/CD137L interaction are necessary.

- ✓ It is critical to define the efficacy of the variants in animal models to further move into clinical trials in humans. Conditions such as graft-versus-host disease and pancreatic islet transplantation models will allow us to test the efficacy of this novel therapy.

9. DISSERTATION PRODUCTS

The products generated during the development of this doctoral dissertation are listed below:

PUBLICATION LIST:

Manuscript 1: Mariana Celis-Andrade, Victoria Morales-González, Manuel Rojas, Diana M. Monsalve, Yeny Acosta-Ampudia, Yhojan Rodríguez, Carolina Ramírez-Santana. Prevalence of Latent and Overt Polyautoimmunity in Type 1 Diabetes: A Systematic Review and Meta-analysis. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2024. DOI: https://doi.org/10.1016/j.dsx.2024.103087

Manuscript 2: Rojas Manuel, Yeny Acosta-Ampudia, Diana M. Monsalve, Carolina Ramírez-Santana, Luke S. Heuer, William Ridgway, Juan-Manuel Anaya, Zhe-Xiong Lian, M. Eric Gershwin. Epitope Analysis and Molecular Mimicry. 2025. Current Opinion in Immunology. DOI: https://doi.org/10.1016/j.coi.2025.102681

Manuscript 3: Sixuan Liu, Yeny Acosta-Ampudia, Carolina Ramírez-Santana, Diana M Monsalve, William Harper, Luke S. Heuer, Weici Zhang, William M. Ridgway, Rojas Manuel. Soluble CD137 in Health and Disease: A Systematic Review and Meta-Analysis. 2026. DOI: Submitted - Autoimmunity reviews.

Manuscript 4: Rojas Manuel, Yeny Acosta-Ampudia, Luke S. Heuer, Weici Zang, Aftab A. Ansari, Diana M. Monsalve, Carolina Ramírez-Santana, Juan-Manuel Anaya, William M. Ridgway, M. Eric Gershwin. Antigen-Specific T Cells and Autoimmunity: A Comprehensive Review. Journal of Autoimmunity. 2023. DOI: https://doi.org/10.1016/j.jaut.2024.103303

Manuscript 5: Rojas Manuel, Luke Heuer, Weici Zhang, Yi-Guang Chen, William M. Ridgway. The Long and Winding Road: From Mouse Linkage Studies to a Novel Human Therapeutic Pathway in Type 1 Diabetes. Frontiers in Immunology. 2022. DOI: https://doi.org/10.3389/fimmu.2022.918837

Manuscript 6: Rojas Manuel, Luke S. Heuer, Weici Zhang, Nicolle Sweeney, Carolina Ramírez-Santana, Patrick S.C. Leung, Alvin Lam, Shraddha Kamat, Andrew R. Mendelsohn, Manley Huang, Bo Yu, Paulina Ackerman, Qisheng Wei, James W. Larrick, Yi-Guang Chen, William M. Ridgway. Alternate Splicing Converts Human CD137 from Costimulatory to Immunosuppressive Function. Journal of Autoimmunity. 2025. DOI: https://doi.org/10.1016/j.jaut.2025.103498

ACADEMIC CONFERENCES:

- **UC Davis Department of Internal Medicine Research Symposium**

Type of conference: Congress.
Scope: International (Sacramento, USA).
Modality: Poster.
Date: October 2024.
Title: *'Human Alternately Spliced Soluble CD137 Isoforms Suppress Immune Responses via the mTORC1 Pathway'*

- **XVII Simposio Institucional Cayre IPS**
  Type of conference: Congress.
  Scope: National (Bogota, Colombia).
  Modality: Plenary session.
  Date: February 2025.
  Title: *'El empalme alternativo convierte la función de CD137 de coestimuladora a inmunosupresora en humanos'*

- **XV Congreso Colombiano de Alergia, Asma e Inmunología y el VII Encuentro de la Asociación Colombiana de Inmunología**
  Type of conference: Congress.
  Scope: National (Bogota, Colombia).
  Modality: Plenary session.
  Date: September 2025.
  Title: *'El empalme alternativo convierte la función de CD137 de coestimuladora a inmunosupresora en humanos'*

INTERNATIONAL INTERNSHIP:

- **Division of Rheumatology, Allergy, and Clinical Immunology**
  UC Davis School of Medicine, Davis, USA.
  Department of Internal Medicine
  Date: 2022 - 2025.

AWARDS:

- **Richard C. Woodard Diabetes Research Award, 2025 (Link)**
  UC Davis School of Medicine, Sacramento, USA.
  Department of Internal Medicine
  Date: May 2025.

SUPPLEMENTAL MATERIAL:

- To access supplementary material, please Click Here.

10. REFERENCES

1. Anaya JM, Shoenfeld Y, Rojas-Villarraga A, Levy RA, Cervera R, editors. Autoimmunity: From Bench to Bedside. Autoimmunity: From Bench to Bedside. Bogota (Colombia): Rosario University Pres; 2013.
2. Rojas-Villarraga A, Amaya-Amaya J, Rodriguez-Rodriguez A, Mantilla RD, Anaya JM. Introducing polyautoimmunity: secondary autoimmune diseases no longer exist. Autoimmune Dis. 2012;2012:254319.
3. Lerner A, Jeremias P, Matthias T. The World Incidence and Prevalence of Autoimmune Diseases is Increasing. Int J Celiac Dis. 2015;3:151–5.
4. Norris JM, Johnson RK, Stene LC. Type 1 diabetes—early life origins and changing epidemiology. Lancet Diabetes Endocrinol. 2020;8:226–38.
5. Mobasseri M, Shirmohammadi M, Amiri T, Vahed N, Hosseini Fard H, Ghojazadeh M. Prevalence and incidence of type 1 diabetes in the world: a systematic review and meta-analysis. Heal Promot Perspect. 2020/04/17. 2020;10(2):98–115.
6. Bao YK, Weide LG, Ganesan VC, Jakhar I, McGill JB, Sahil S, et al. High prevalence of comorbid autoimmune diseases in adults with type 1 diabetes from the HealthFacts database. J Diabetes. 2019;11:273–9.
7. Diaz-Valencia PA, Bougnères P, Valleron AJ. Global epidemiology of type 1 diabetes in young adults and adults: a systematic review. BMC Public Health. 2015 Mar;15:255.
8. Ilonen J, Lempainen J, Veijola R. The heterogeneous pathogenesis of type 1 diabetes mellitus. Nat Rev Endocrinol. 2019 Nov;15(11):635–50.
9. Harjutsalo V, Sjöberg L, Tuomilehto J. Time trends in the incidence of type 1 diabetes in Finnish children: a cohort study. Lancet (London, England). 2008 May;371(9626):1777–82.
10. Songini M, Mannu C, Targhetta C, Bruno G. Type 1 diabetes in Sardinia: facts and hypotheses in the context of worldwide epidemiological data. Acta Diabetol. 2017 Jan;54(1):9–17.
11. Sakaguchi S, Mikami N, Wing JB, Tanaka A, Ichiyama K, Ohkura N. Regulatory T Cells and Human Disease. Annu Rev Immunol. 2020;38:541–66.
12. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol. 1995;155:1151–64.
13. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol. 2003;4:330–6.
14. Khattri R, Cox T, Yasayko SA, Ramsdell F. An essential role for Scurfin in CD4+CD25+ T regulatory cells. Nat Immunol. 2003;4:337–42.
15. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science (80-). 2003;299:1057–61.

16. Jamee M, Zaki-Dizaji M, Lo B, Abolhassani H, Aghamahdi F, Mosavian M, et al. Clinical, Immunological, and Genetic Features in Patients with Immune Dysregulation, Polyendocrinopathy, Enteropathy, X-linked (IPEX) and IPEX-like Syndrome. J allergy Clin Immunol Pract. 2020;8:2747-2760.e7.
17. Bacchetta R, Barzaghi F, Roncarolo MG. From IPEX syndrome to FOXP3 mutation: a lesson on immune dysregulation. Ann N Y Acad Sci. 2018;1417:5–22.
18. Schmidt RE, Grimbacher B, Witte T. Autoimmunity and primary immunodeficiency: two sides of the same coin? Nat Rev Rheumatol. 2017 Dec;14(1):7–18.
19. Guo CJ, Leung PSC, Zhang W, Ma X, Gershwin ME. The immunobiology and clinical features of type 1 autoimmune polyglandular syndrome (APS-1). Autoimmun Rev. 2018 Jan;17(1):78–85.
20. Amaya-Uribe L, Rojas M, Azizi G, Anaya JM, Gershwin ME. Primary immunodeficiency and autoimmunity: A comprehensive review. J Autoimmun. 2019;99:52–72.
21. Todd JA, Walker NM, Cooper JD, Smyth DJ, Downes K, Plagnol V, et al. Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. Nat Genet. 2007;39:857–64.
22. Barnas JL, Looney RJ, Anolik JH. B cell targeted therapies in autoimmune disease. Curr Opin Immunol. 2019;61:92–9.
23. He J, Zhang R, Shao M, Zhao X, Miao M, Chen J, et al. Efficacy and safety of low-dose IL-2 in the treatment of systemic lupus erythematosus: a randomised, double-blind, placebo-controlled trial. Ann Rheum Dis. 2020;79:141–9.
24. von Spee-Mayer C, Siegert E, Abdirama D, Rose A, Klaus A, Alexander T, et al. Low-dose interleukin-2 selectively corrects regulatory T cell defects in patients with systemic lupus erythematosus. Ann Rheum Dis. 2016;75:1407–15.
25. Hartemann A, Bensimon G, Payan CA, Jacqueminet S, Bourron O, Nicolas N, et al. Low-dose interleukin 2 in patients with type 1 diabetes: a phase 1/2 randomised, double-blind, placebo-controlled trial. lancet Diabetes Endocrinol. 2013;1:295–305.
26. Pham MN, von Herrath MG, Vela JL. Antigen-Specific Regulatory T Cells and Low Dose of IL-2 in Treatment of Type 1 Diabetes. Front Immunol. 2016;6.
27. Wan YY. Regulatory T cells: immune suppression and beyond. Cell Mol Immunol. 2010;7:204–10.
28. Zhou L, Lopes JE, Chong MMW, Ivanov II, Min R, Victora GD, et al. TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing RORgammat function. Nature. 2008;453:236–40.
29. Yang XO, Nurieva R, Martinez GJ, Kang HS, Chung Y, Pappu BP, et al. Molecular antagonism and plasticity of regulatory and inflammatory T cell programs. Immunity. 2008;29:44–56.
30. Marek-Trzonkowska N, Mysliwiec M, Dobyszuk A, Grabowska M, Techmanska I, Juscinska J, et al. Administration of CD4+CD25highCD127- regulatory T cells

preserves β-cell function in type 1 diabetes in children. Diabetes Care. 2012;35:1817–20.
31. Marek-Trzonkowska N, Myśliwiec M, Dobyszuk A, Grabowska M, Derkowska I, Juścińska J, et al. Therapy of type 1 diabetes with CD4(+)CD25(high)CD127-regulatory T cells prolongs survival of pancreatic islets - results of one year follow-up. Clin Immunol. 2014;153:23–30.
32. Bluestone JA, Buckner JH, Fitch M, Gitelman SE, Gupta S, Hellerstein MK, et al. Type 1 diabetes immunotherapy using polyclonal regulatory T cells. Sci Transl Med. 2015;7:315ra189.
33. Itoh A, Ortiz L, Kachapati K, Wu Y, Adams D, Bednar K, et al. Soluble CD137 Ameliorates Acute Type 1 Diabetes by Inducing T Cell Anergy. Front Immunol. 2019;10:2566.
34. Erlich H, Valdes AM, Noble J, Carlson JA, Varney M, Concannon P, et al. HLA DR-DQ haplotypes and genotypes and type 1 diabetes risk: analysis of the type 1 diabetes genetics consortium families. Diabetes. 2008 Apr;57(4):1084–92.
35. Todd JA, Mijovic C, Fletcher J, Jenkins D, Bradwell AR, Barnett AH. Identification of susceptibility loci for insulin-dependent diabetes mellitus by trans-racial gene mapping. Nature. 1989 Apr;338(6216):587–9.
36. Rojas M, Restrepo-Jiménez P, Monsalve DM, Pacheco Y, Acosta-Ampudia Y, Ramírez-Santana C, et al. Molecular mimicry and autoimmunity. J Autoimmun. 2018 Dec;95:100–23.
37. Ilonen J, Kiviniemi M, Lempainen J, Simell O, Toppari J, Veijola R, et al. Genetic susceptibility to type 1 diabetes in childhood - estimation of HLA class II associated disease risk and class II effect in various phases of islet autoimmunity. Pediatr Diabetes. 2016 Jul;17 Suppl 2:8–16.
38. Noble JA, Valdes AM, Varney MD, Carlson JA, Moonsamy P, Fear AL, et al. HLA class I and genetic susceptibility to type 1 diabetes: results from the Type 1 Diabetes Genetics Consortium. Diabetes. 2010 Nov;59(11):2972–9.
39. Tait BD, Colman PG, Morahan G, Marchinovska L, Dore E, Gellert S, et al. HLA genes associated with autoimmunity and progression to disease in type 1 diabetes. Tissue Antigens. 2003 Feb;61(2):146–53.
40. Mbunwe E, Van der Auwera BJ, Weets I, Van Crombrugge P, Crenier L, Coeckelberghs M, et al. In antibody-positive first-degree relatives of patients with type 1 diabetes, HLA-A*24 and HLA-B*18, but not HLA-B*39, are predictors of impending diabetes with distinct HLA-DQ interactions. Diabetologia. 2013 Sep;56(9):1964–70.
41. Mikk ML, Heikkinen T, El-Amir MI, Kiviniemi M, Laine AP, Härkönen T, et al. The association of the HLA-A*24:02, B*39:01 and B*39:06 alleles with type 1 diabetes is restricted to specific HLA-DR/DQ haplotypes in Finns. HLA. 2017 Apr;89(4):215–24.
42. Pociot F, Lernmark Å. Genetic risk factors for type 1 diabetes. Lancet (London,

England). 2016 Jun;387(10035):2331–9.
43. Diaz-Gallo LM, Oke V, Lundström E, Elvin K, Ling Wu Y, Eketjäll S, et al. Four Systemic Lupus Erythematosus Subgroups, Defined by Autoantibodies Status, Differ Regarding HLA-DRB1 Genotype Associations and Immunological and Clinical Manifestations. ACR open Rheumatol. 2022 Jan;4(1):27–39.
44. Pacheco Y, Barahona-Correa J, Monsalve DM, Acosta-Ampudia Y, Rojas M, Rodríguez Y, et al. Cytokine and autoantibody clusters interaction in systemic lupus erythematosus. J Transl Med. 2017 Nov;15(1):239.
45. Botello A, Herrán M, Salcedo V, Rodríguez Y, Anaya JM, Rojas M. Prevalence of latent and overt polyautoimmunity in autoimmune thyroid disease: A systematic review and meta-analysis. Clin Endocrinol (Oxf). 2020 Oct;93(4):375–89.
46. Anaya JM. The autoimmune tautology. A summary of evidence. Jt bone spine. 2017 May;84(3):251–3.
47. Malagón C, Gomez MDP, Mosquera C, Vargas C, Gonzalez T, Arango C, et al. Juvenile polyautoimmunity in a rheumatology setting. Autoimmun Rev. 2019 Apr;18(4):369–81.
48. Vafiadis P, Bennett ST, Todd JA, Nadeau J, Grabs R, Goodyer CG, et al. Insulin expression in human thymus is modulated by INS VNTR alleles at the IDDM2 locus. Nat Genet. 1997 Mar;15(3):289–92.
49. Bottini N, Vang T, Cucca F, Mustelin T. Role of PTPN22 in type 1 diabetes and other autoimmune diseases. Semin Immunol. 2006 Aug;18(4):207–13.
50. Lempainen J, Laine AP, Hammais A, Toppari J, Simell O, Veijola R, et al. Non-HLA gene effects on the disease process of type 1 diabetes: From HLA susceptibility to overt disease. J Autoimmun. 2015 Jul;61:45–53.
51. Törn C, Hadley D, Lee HS, Hagopian W, Lernmark Å, Simell O, et al. Role of Type 1 Diabetes-Associated SNPs on Risk of Autoantibody Positivity in the TEDDY Study. Diabetes. 2015 May;64(5):1818–29.
52. Garg G, Tyler JR, Yang JHM, Cutler AJ, Downes K, Pekalski M, et al. Type 1 diabetes-associated IL2RA variation lowers IL-2 signaling and contributes to diminished CD4+CD25+ regulatory T cell function. J Immunol. 2012 May;188(9):4644–53.
53. de Jong VM, Zaldumbide A, van der Slik AR, Laban S, Koeleman BPC, Roep BO. Variation in the CTLA4 3'UTR has phenotypic consequences for autoreactive T cells and associates with genetic risk for type 1 diabetes. Genes Immun. 2016;17(1):75–8.
54. Wang Z, Xie Z, Lu Q, Chang C, Zhou Z. Beyond Genetics: What Causes Type 1 Diabetes. Clin Rev Allergy Immunol. 2017 Apr;52(2):273–86.
55. Borchers AT, Uibo R, Gershwin ME. The geoepidemiology of type 1 diabetes. Autoimmun Rev. 2010 Mar;9(5):A355-65.
56. Ilonen J, Vaarala O, Akerblom HK, Knip M. Environmental factors and primary prevention in type 1 diabetes. Pediatr Endocrinol Diabetes Metab.

2009;15(4):227–32.
57. Gülden E, Wong FS, Wen L. The gut microbiota and Type 1 Diabetes. Clin Immunol. 2015 Aug;159(2):143–53.
58. Rose K, Penna-Martinez M, Klahold E, Kärger D, Shoghi F, Kahles H, et al. Influence of the vitamin D plasma level and vitamin D-related genetic polymorphisms on the immune status of patients with type 1 diabetes: a pilot study. Clin Exp Immunol. 2013 Feb;171(2):171–85.
59. Karvonen M, Jäntti V, Muntoni S, Stabilini M, Stabilini L, Muntoni S, et al. Comparison of the seasonal pattern in the clinical onset of IDDM in Finland and Sardinia. Diabetes Care. 1998 Jul;21(7):1101–9.
60. Rasmussen T, Witsø E, Tapia G, Stene LC, Rønningen KS. Self-reported lower respiratory tract infections and development of islet autoimmunity in children with the type 1 diabetes high-risk HLA genotype: the MIDIA study. Diabetes Metab Res Rev. 2011 Nov;27(8):834–7.
61. Coppieters KT, Wiberg A, von Herrath MG. Viral infections and molecular mimicry in type 1 diabetes. APMIS. 2012 Dec;120(12):941–9.
62. Wagenknecht LE, Roseman JM, Herman WH. Increased incidence of insulin-dependent diabetes mellitus following an epidemic of Coxsackievirus B5. Am J Epidemiol. 1991 May;133(10):1024–31.
63. Yeung WCG, Rawlinson WD, Craig ME. Enterovirus infection and type 1 diabetes mellitus: systematic review and meta-analysis of observational molecular studies. BMJ. 2011 Feb;342:d35.
64. Dotta F, Censini S, van Halteren AGS, Marselli L, Masini M, Dionisi S, et al. Coxsackie B4 virus infection of beta cells and natural killer cell insulitis in recent-onset type 1 diabetic patients. Proc Natl Acad Sci U S A. 2007 Mar;104(12):5115–20.
65. Al-Hello H, Paananen A, Eskelinen M, Ylipaasto P, Hovi T, Salmela K, et al. An enterovirus strain isolated from diabetic child belongs to a genetic subcluster of echovirus 11, but is also neutralised with monotypic antisera to coxsackievirus A9. J Gen Virol. 2008 Aug;89(Pt 8):1949–59.
66. Yoon JW, Austin M, Onodera T, Notkins AL. Isolation of a virus from the pancreas of a child with diabetic ketoacidosis. N Engl J Med. 1979 May;300(21):1173–9.
67. Coppieters KT, Boettler T, von Herrath M. Virus infections in type 1 diabetes. Cold Spring Harb Perspect Med. 2012 Jan;2(1):a007682.
68. Nilsson AL, Vaziri-Sani F, Broberg P, Elfaitouri A, Pipkorn R, Blomberg J, et al. Serological evaluation of possible exposure to Ljungan virus and related parechovirus in autoimmune (type 1) diabetes in children. J Med Virol. 2015 Jul;87(7):1130–40.
69. Honeyman MC, Laine D, Zhan Y, Londrigan S, Kirkwood C, Harrison LC. Rotavirus infection induces transient pancreatic involution and hyperglycemia in weanling mice. PLoS One. 2014;9(9):e106560.

70. Valdés C, Unanue N, Hernández M, García R, Castro M, Vásquez L, et al. Is there a link between influenza and type I diabetes? Increased incidence of TID during the pandemic H1N1 influenza of 2009 in Chile. Pediatr Endocrinol Rev. 2013 Dec;11(2):161–6.
71. Kondrashova A, Nurminen N, Patrikainen M, Huhtala H, Lehtonen J, Toppari J, et al. Influenza A virus antibodies show no association with pancreatic islet autoantibodies in children genetically predisposed to type 1 diabetes. Diabetologia. 2015 Nov;58(11):2592–5.
72. Ohashi PS, Oehen S, Buerki K, Pircher H, Ohashi CT, Odermatt B, et al. Ablation of “tolerance” and induction of diabetes by virus infection in viral antigen transgenic mice. Cell. 1991 Apr;65(2):305–17.
73. Hiemstra HS, Schloot NC, van Veelen PA, Willemen SJ, Franken KL, van Rood JJ, et al. Cytomegalovirus in autoimmunity: T cell crossreactivity to viral antigen and autoantigen glutamic acid decarboxylase. Proc Natl Acad Sci U S A. 2001 Mar;98(7):3988–91.
74. Härkönen T, Lankinen H, Davydova B, Hovi T, Roivainen M. Enterovirus infection can induce immune responses that cross-react with beta-cell autoantigen tyrosine phosphatase IA-2/IAR. J Med Virol. 2002 Mar;66(3):340–50.
75. Akhbari P, Richardson SJ, Morgan NG. Type 1 Diabetes: Interferons and the Aftermath of Pancreatic Beta-Cell Enteroviral Infection. Microorganisms [Internet]. 2020 Sep 15;8(9). Available from: http://www.ncbi.nlm.nih.gov/pubmed/32942706
76. Tracy S, Drescher KM, Chapman NM, Kim KS, Carson SD, Pirruccello S, et al. Toward testing the hypothesis that group B coxsackieviruses (CVB) trigger insulin-dependent diabetes: inoculating nonobese diabetic mice with CVB markedly lowers diabetes incidence. J Virol. 2002 Dec;76(23):12097–111.
77. Wållberg M, Cooke A. Immune mechanisms in type 1 diabetes. Trends Immunol. 2013;34:583–91.
78. Turley S, Poirot L, Hattori M, Benoist C, Mathis D. Physiological beta cell death triggers priming of self-reactive T cells by dendritic cells in a type-1 diabetes model. J Exp Med. 2003;198:1527–37.
79. Dahlén E, Dawe K, Ohlsson L, Hedlund G. Dendritic cells and macrophages are the first and major producers of TNF-alpha in pancreatic islets in the nonobese diabetic mouse. J Immunol. 1998;160:3585–93.
80. Roep BO, Thomaidou S, van Tienhoven R, Zaldumbide A. Type 1 diabetes mellitus as a disease of the β-cell (do not blame the immune system?). Nat Rev Endocrinol. 2021 Mar;17(3):150–61.
81. Carrero JA, McCarthy DP, Ferris ST, Wan X, Hu H, Zinselmeyer BH, et al. Resident macrophages of pancreatic islets have a seminal role in the initiation of autoimmune diabetes of NOD mice. Proc Natl Acad Sci U S A [Internet]. 2017 Nov 28;114(48):E10418–27. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29133420

82. Zirpel H, Roep BO. Islet-Resident Dendritic Cells and Macrophages in Type 1 Diabetes: In Search of Bigfoot's Print. Front Endocrinol (Lausanne). 2021;12:666795.
83. Itoh A, Ridgway WM. Targeting innate immunity to downmodulate adaptive immunity and reverse type 1 diabetes. ImmunoTargets Ther. 2017;6:31–8.
84. Leete P, Willcox A, Krogvold L, Dahl-Jørgensen K, Foulis AK, Richardson SJ, et al. Differential Insulitic Profiles Determine the Extent of β-Cell Destruction and the Age at Onset of Type 1 Diabetes. Diabetes. 2016 May;65(5):1362–9.
85. Richardson SJ, Rodriguez-Calvo T, Gerling IC, Mathews CE, Kaddis JS, Russell MA, et al. Islet cell hyperexpression of HLA class I antigens: a defining feature in type 1 diabetes. Diabetologia. 2016 Nov;59(11):2448–58.
86. Rodriguez-Calvo T, Ekwall O, Amirian N, Zapardiel-Gonzalo J, von Herrath MG. Increased immune cell infiltration of the exocrine pancreas: a possible contribution to the pathogenesis of type 1 diabetes. Diabetes. 2014 Nov;63(11):3880–90.
87. Roep BO, Peakman M. Antigen targets of type 1 diabetes autoimmunity. Cold Spring Harb Perspect Med. 2012 Apr;2(4):a007781.
88. Coppieters KT, Dotta F, Amirian N, Campbell PD, Kay TWH, Atkinson MA, et al. Demonstration of islet-autoreactive CD8 T cells in insulitic lesions from recent onset and long-term type 1 diabetes patients. J Exp Med. 2012 Jan;209(1):51–60.
89. Pacheco Y, Acosta-Ampudia Y, Monsalve DM, Chang C, Gershwin ME, Anaya JM. Bystander activation and autoimmunity. J Autoimmun. 2019 Sep;103:102301.
90. ElEssawy B, Li XC. Type 1 diabetes and T regulatory cells. Pharmacol Res. 2015;98:22–30.
91. Okubo Y, Torrey H, Butterworth J, Zheng H, Faustman DL. Treg activation defect in type 1 diabetes: correction with TNFR2 agonism. Clin Transl Immunol. 2016 Jan;5(1):e56.
92. Lindley S, Dayan CM, Bishop A, Roep BO, Peakman M, Tree TIM. Defective suppressor function in CD4(+)CD25(+) T-cells from patients with type 1 diabetes. Diabetes. 2005 Jan;54(1):92–9.
93. Ziegler AG, Rewers M, Simell O, Simell T, Lempainen J, Steck A, et al. Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. JAMA. 2013 Jun;309(23):2473–9.
94. Knip M, Selvenius J, Siljander H, Veijola R. Reclassification of asymptomatic beta cell autoimmunity: a critical perspective. Diabetologia. 2017 Jan;60(1):39–42.
95. Vehik K, Lynch KF, Schatz DA, Akolkar B, Hagopian W, Rewers M, et al. Reversion of β-Cell Autoimmunity Changes Risk of Type 1 Diabetes: TEDDY Study. Diabetes Care. 2016 Sep;39(9):1535–42.
96. Insel RA, Dunne JL, Atkinson MA, Chiang JL, Dabelea D, Gottlieb PA, et al. Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the Endocrine Society, and the American Diabetes Association. Diabetes Care. 2015 Oct;38(10):1964–74.

97. Makino S, Kunimoto K, Muraoka Y, Mizushima Y, Katagiri K, Tochino Y. Breeding of a non-obese, diabetic strain of mice. Jikken Dobutsu. 1980;29:1–13.
98. Mullen Y. Development of the Nonobese Diabetic Mouse and Contribution of Animal Models for Understanding Type 1 Diabetes. Pancreas. 2017;46:455–66.
99. Ridgway WM. Dissecting genetic control of autoimmunity in NOD congenic mice. Immunol Res. 2006;36:189–95.
100. Acha-Orbea H, McDevitt HO. The first external domain of the nonobese diabetic mouse class II I-A beta chain is unique. Proc Natl Acad Sci U S A. 1987;84(8):2435–9.
101. Todd JA, Acha-Orbea H, Bell JI, Chao N, Fronek Z, Jacob CO, et al. A molecular basis for MHC class II--associated autoimmunity. Science (80-). 1988;240(4855):1003–9.
102. Hamilton-Williams EE, Serreze D V, Charlton B, Johnson EA, Marron MP, Mullbacher A, et al. Transgenic rescue implicates beta2-microglobulin as a diabetes susceptibility gene in nonobese diabetic (NOD) mice. Proc Natl Acad Sci U S A. 2001;98:11533–8.
103. Nejentsev S, Howson JMM, Walker NM, Szeszko J, Field SF, Stevens HE, et al. Localization of type 1 diabetes susceptibility to the MHC class I genes HLA-B and HLA-A. Nature. 2007;450:887–92.
104. Singer SM, Tisch R, Yang XD, McDevitt HO. An Abd transgene prevents diabetes in nonobese diabetic mice by inducing regulatory T cells. Proc Natl Acad Sci U S A. 1993;90:9566–70.
105. Slattery RM, Kjer-Nielsen L, Allison J, Charlton B, Mandel TE, Miller JF. Prevention of diabetes in non-obese diabetic I-Ak transgenic mice. Nature. 1990;345:724–6.
106. Han S, Donelan W, Wang H, Reeves W, Yang LJ. Novel autoantigens in type 1 diabetes. Am J Transl Res. 2013;5:379–92.
107. Wicker LS, Miller BJ, Coker LZ, McNally SE, Scott S, Mullen Y, et al. Genetic control of diabetes and insulitis in the nonobese diabetic (NOD) mouse. J Exp Med. 1987;165:1639–54.
108. Koarada S, Wu Y, Olshansky G, Ridgway WM. Increased nonobese diabetic Th1:Th2 (IFN-gamma:IL-4) ratio is CD4+ T cell intrinsic and independent of APC genetic background. J Immunol. 2002;169(11):6580–7.
109. Koarada S, Wu Y, Ridgway WM. Increased entry into the IFN-gamma effector pathway by CD4+ T cells selected by I-Ag7 on a nonobese diabetic versus C57BL/6 genetic background. J Immunol. 2001;167(3):1693–702.
110. Ellis JS, Wan X, Braley-Mullen H. Transient depletion of CD4+ CD25+ regulatory T cells results in multiple autoimmune diseases in wild-type and B-cell-deficient NOD mice. Immunology. 2013;139:179–86.
111. Pérol L, Lindner JM, Caudana P, Nunez NG, Baeyens A, Valle A, et al. Loss of immune tolerance to IL-2 in type 1 diabetes. Nat Commun. 2016;7:13027.

112. Salomon B, Lenschow DJ, Rhee L, Ashourian N, Singh B, Sharpe A, et al. B7/CD28 costimulation is essential for the homeostasis of the CD4+CD25+ immunoregulatory T cells that control autoimmune diabetes. Immunity. 2000;12:431–40.
113. Wu AJ, Hua H, Munson SH, McDevitt HO. Tumor necrosis factor-alpha regulation of CD4+CD25+ T cell levels in NOD mice. Proc Natl Acad Sci U S A. 2002;99:12287–92.
114. D'Alise AM, Auyeung V, Feuerer M, Nishio J, Fontenot J, Benoist C, et al. The defect in T-cell regulation in NOD mice is an effect on the T-cell effectors. Proc Natl Acad Sci U S A. 2008;105:19857–62.
115. Rainbow DB, Esposito L, Howlett SK, Hunter KM, Todd JA, Peterson LB, et al. Commonality in the genetic control of Type 1 diabetes in humans and NOD mice: variants of genes in the IL-2 pathway are associated with autoimmune diabetes in both species. Biochem Soc Trans. 2008;36:312–5.
116. Wicker LS, Clark J, Fraser HI, Garner VES, Gonzalez-Munoz A, Healy B, et al. Type 1 diabetes genes and pathways shared by humans and NOD mice. J Autoimmun. 2005;25 Suppl:29–33.
117. Ridgway WM, Peterson LB, Todd JA, Rainbow DB, Healy B, Burren OS, et al. Gene-gene interactions in the NOD mouse model of type 1 diabetes. Adv Immunol. 2008;100:151–75.
118. Ghosh S, Palmer SM, Rodrigues NR, Cordell HJ, Hearne CM, Cornall RJ, et al. Polygenic control of autoimmune diabetes in nonobese diabetic mice. Nat Genet. 1993;4:404–9.
119. McAleer MA, Reifsnyder P, Palmer SM, Prochazka M, Love JM, Copeman JB, et al. Crosses of NOD mice with the related NON strain. A polygenic model for IDDM. Diabetes. 1995;44:1186–95.
120. Rogner UC, Avner P. Congenic mice: cutting tools for complex immune disorders. Nat Rev Immunol. 2003;3:243–52.
121. Driver JP, Serreze D V, Chen YG. Mouse models for the study of autoimmune type 1 diabetes: a NOD to similarities and differences to human disease. Semin Immunopathol. 2011;33:67–87.
122. Todd JA, Aitman TJ, Cornall RJ, Ghosh S, Hall JR, Hearne CM, et al. Genetic analysis of autoimmune type 1 diabetes mellitus in mice. Nature. 1991;351:542–7.
123. Rojas M, Heuer LS, Zhang W, Chen YG, Ridgway WM. The long and winding road: From mouse linkage studies to a novel human therapeutic pathway in type 1 diabetes. Front Immunol. 2022;13:918837.
124. Lyons PA, Armitage N, Argentina F, Denny P, Hill NJ, Lord CJ, et al. Congenic mapping of the type 1 diabetes locus, Idd3, to a 780-kb region of mouse chromosome 3: identification of a candidate segment of ancestral DNA by haplotype mapping. Genome Res. 2000;10:446–53.
125. Hill NJ, Lyons PA, Armitage N, Todd JA, Wicker LS, Peterson LB. NOD Idd5 locus

controls insulitis and diabetes and overlaps the orthologous CTLA4/IDDM12 and NRAMP1 loci in humans. Diabetes. 2000;49:1744–7.
126. Robles DT, Eisenbarth GS, Dailey NJM, Peterson LB, Wicker LS. Insulin autoantibodies are associated with islet inflammation but not always related to diabetes progression in NOD congenic mice. Diabetes. 2003;52:882–6.
127. Driver JP, Chen YG, Mathews CE. Comparative genetics: synergizing human and NOD mouse studies for identifying genetic causation of type 1 diabetes. Rev Diabet Stud. 2012;9(4):169–87.
128. Nyaga DM, Vickers MH, Jefferies C, Perry JK, O'Sullivan JM. The genetic architecture of type 1 diabetes mellitus. Mol Cell Endocrinol. 2018;477:70–80.
129. Yamanouchi J, Rainbow D, Serra P, Howlett S, Hunter K, Garner VE, et al. Interleukin-2 gene variation impairs regulatory T cell function and causes autoimmunity. Nat Genet. 2007;39(3):329–37.
130. Long SA, Buckner JH, Greenbaum CJ. IL-2 therapy in type 1 diabetes: "Trials" and tribulations. Clin Immunol. 2013;149(3):324–31.
131. Izquierdo C, Ortiz AZ, Presa M, Malo S, Montoya A, Garabatos N, et al. Treatment of T1D via optimized expansion of antigen-specific Tregs induced by IL-2/anti-IL-2 monoclonal antibody complexes and peptide/MHC tetramers. Sci Rep. 2018;8(1):8106.
132. Jarvis LB, Rainbow DB, Coppard V, Howlett SK, Georgieva Z, Davies JL, et al. Therapeutically expanded human regulatory T-cells are super-suppressive due to HIF1A induced expression of CD73. Commun Biol. 2021;4(1):1186.
133. Marcovecchio ML, Wicker LS, Dunger DB, Dutton SJ, Kopijasz S, Scudder C, et al. Interleukin-2 Therapy of Autoimmunity in Diabetes (ITAD): a phase 2, multicentre, double-blind, randomized, placebo-controlled trial. Wellcome Open Res. 2020;5:49.
134. Bettini M, Bettini ML. Function, Failure, and the Future Potential of Tregs in Type 1 Diabetes. Diabetes. 2021;70(6):1211–9.
135. Ueda H, Howson JM, Esposito L, Heward J, Snook H, Chamberlain G, et al. Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. Nature. 2003;423(6939):506–11.
136. Rachid O, Osman A, Abdi R, Haik Y. CTLA4-Ig (abatacept): a promising investigational drug for use in type 1 diabetes. Expert Opin Investig Drugs. 2020;29(3):221–36.
137. In't Veld P. Insulitis in human type 1 diabetes: a comparison between patients and animal models. Semin Immunopathol. 2014;36(5):569–79.
138. Herold KC, Bundy BN, Long SA, Bluestone JA, DiMeglio LA, Dufort MJ, et al. An Anti-CD3 Antibody, Teplizumab, in Relatives at Risk for Type 1 Diabetes. N Engl J Med. 2019;381(7):603–13.
139. Robertson CC, Inshaw JRJ, Onengut-Gumuscu S, Chen WM, Santa Cruz DF, Yang H, et al. Fine-mapping, trans-ancestral and genomic analyses identify causal

variants, cells, genes and drug targets for type 1 diabetes. Nat Genet. 2021;53(7):962–71.
140. Lyons PA, Hancock WW, Denny P, Lord CJ, Hill NJ, Armitage N, et al. The NOD Idd9 genetic interval influences the pathogenicity of insulitis and contains molecular variants of Cd30, Tnfr2, and Cd137. Immunity. 2000;13:107–15.
141. Forsberg MH, Foda B, Serreze D V, Chen YG. Combined congenic mapping and nuclease-based gene targeting for studying allele-specific effects of Tnfrsf9 within the Idd9.3 autoimmune diabetes locus. Sci Rep. 2019;9:4316.
142. Cannons JL, Chamberlain G, Howson J, Smink LJ, Todd JA, Peterson LB, et al. Genetic and functional association of the immune signaling molecule 4-1BB (CD137/TNFRSF9) with type 1 diabetes. J Autoimmun. 2005;25:13–20.
143. Luu K, Shao Z, Schwarz H. The relevance of soluble CD137 in the regulation of immune responses and for immunotherapeutic intervention. J Leukoc Biol. 2020;107:731–8.
144. Pollok KE, Kim YJ, Zhou Z, Hurtado J, Kim KK, Pickard RT, et al. Inducible T cell antigen 4-1BB. Analysis of expression and function. J Immunol. 1993;150:771–81.
145. Kwon BS, Weissman SM. cDNA sequences of two inducible T-cell genes. Proc Natl Acad Sci U S A. 1989;86:1963–7.
146. Gavin MA, Clarke SR, Negrou E, Gallegos A, Rudensky A. Homeostasis and anergy of CD4(+)CD25(+) suppressor T cells in vivo. Nat Immunol. 2002;3:33–41.
147. McHugh RS, Whitters MJ, Piccirillo CA, Young DA, Shevach EM, Collins M, et al. CD4(+)CD25(+) immunoregulatory T cells: gene expression analysis reveals a functional role for the glucocorticoid-induced TNF receptor. Immunity. 2002;16:311–23.
148. Choi BK, Bae JS, Choi EM, Kang WJ, Sakaguchi S, Vinay DS, et al. 4-1BB-dependent inhibition of immunosuppression by activated CD4+CD25+ T cells. J Leukoc Biol. 2004;75:785–91.
149. Zheng G, Wang B, Chen A. The 4-1BB costimulation augments the proliferation of CD4+CD25+ regulatory T cells. J Immunol. 2004;173:2428–34.
150. Irie J, Wu Y, Kachapati K, Mittler RS, Ridgway WM. Modulating protective and pathogenic CD4+ subsets via CD137 in type 1 diabetes. Diabetes. 2007;56:186–96.
151. Kachapati K, Adams DE, Wu Y, Steward CA, Rainbow DB, Wicker LS, et al. The B10 Idd9.3 locus mediates accumulation of functionally superior CD137(+) regulatory T cells in the nonobese diabetic type 1 diabetes model. J Immunol. 2012;189:5001–15.
152. Melero I, Johnston J V, Shufford WW, Mittler RS, Chen L. NK1.1 cells express 4-1BB (CDw137) costimulatory molecule and are required for tumor immunity elicited by anti-4-1BB monoclonal antibodies. Cell Immunol. 1998;190:167–72.
153. Stoll A, Bruns H, Fuchs M, Völkl S, Nimmerjahn F, Kunz M, et al. CD137 (4-1BB) stimulation leads to metabolic and functional reprogramming of human

monocytes/macrophages enhancing their tumoricidal activity. Leukemia [Internet]. 2021 Dec;35(12):3482–96. Available from: http://www.ncbi.nlm.nih.gov/pubmed/34021248
154. Pollok KE, Kim YJ, Hurtado J, Zhou Z, Kim KK, Kwon BS. 4-1BB T-cell antigen binds to mature B cells and macrophages, and costimulates anti-mu-primed splenic B cells. Eur J Immunol. 1994;24:367–74.
155. Chitnis T, Khoury SJ. Role of costimulatory pathways in the pathogenesis of multiple sclerosis and experimental autoimmune encephalomyelitis. J Allergy Clin Immunol. 2003;112:837–49; quiz 850.
156. Lee SW, Park Y, So T, Kwon BS, Cheroutre H, Mittler RS, et al. Identification of regulatory functions for 4-1BB and 4-1BBL in myelopoiesis and the development of dendritic cells. Nat Immunol. 2008;9:917–26.
157. Goodwin RG, Din WS, Davis-Smith T, Anderson DM, Gimpel SD, Sato TA, et al. Molecular cloning of a ligand for the inducible T cell gene 4-1BB: a member of an emerging family of cytokines with homology to tumor necrosis factor. Eur J Immunol. 1993;23:2631–41.
158. Zapata JM, Perez-Chacon G, Carr-Baena P, Martinez-Forero I, Azpilikueta A, Otano I, et al. CD137 (4-1BB) Signalosome: Complexity Is a Matter of TRAFs. Front Immunol. 2018;9:2618.
159. Etxeberria I, Glez-Vaz J, Teijeira Á, Melero I. New emerging targets in cancer immunotherapy: CD137/4-1BB costimulatory axis. ESMO open. 2020;4:e000733.
160. Cannons JL, Lau P, Ghumman B, DeBenedette MA, Yagita H, Okumura K, et al. 4-1BB ligand induces cell division, sustains survival, and enhances effector function of CD4 and CD8 T cells with similar efficacy. J Immunol. 2001;167:1313–24.
161. Arch RH, Thompson CB. 4-1BB and Ox40 are members of a tumor necrosis factor (TNF)-nerve growth factor receptor subfamily that bind TNF receptor-associated factors and activate nuclear factor kappaB. Mol Cell Biol. 1998;18:558–65.
162. Jang IK, Lee ZH, Kim YJ, Kim SH, Kwon BS. Human 4-1BB (CD137) signals are mediated by TRAF2 and activate nuclear factor-kappa B. Biochem Biophys Res Commun. 1998;242:613–20.
163. Cannons JL, Hoeflich KP, Woodgett JR, Watts TH. Role of the stress kinase pathway in signaling via the T cell costimulatory receptor 4-1BB. J Immunol. 1999;163:2990–8.
164. Cannons JL, Choi Y, Watts TH. Role of TNF receptor-associated factor 2 and p38 mitogen-activated protein kinase activation during 4-1BB-dependent immune response. J Immunol. 2000;165:6193–204.
165. Wang C, Wen T, Routy JP, Bernard NF, Sekaly RP, Watts TH. 4-1BBL induces TNF receptor-associated factor 1-dependent Bim modulation in human T cells and is a critical component in the costimulation-dependent rescue of functionally impaired HIV-specific CD8 T cells. J Immunol. 2007;179:8252–63.

166. Sabbagh L, Andreeva D, Laramée GD, Oussa NAE, Lew D, Bisson N, et al. Leukocyte-specific protein 1 links TNF receptor-associated factor 1 to survival signaling downstream of 4-1BB in T cells. J Leukoc Biol. 2013;93:713–21.
167. Sabbagh L, Pulle G, Liu Y, Tsitsikov EN, Watts TH. ERK-dependent Bim modulation downstream of the 4-1BB-TRAF1 signaling axis is a critical mediator of CD8 T cell survival in vivo. J Immunol. 2008;180:8093–101.
168. Kwon B. CD137-CD137 Ligand Interactions in Inflammation. Immune Netw. 2009;9:84–9.
169. Kwon B. Regulation of Inflammation by Bidirectional Signaling through CD137 and Its Ligand. Immune Netw. 2012;12:176–80.
170. Hurtado JC, Kim YJ, Kwon BS. Signals through 4-1BB are costimulatory to previously activated splenic T cells and inhibit activation-induced cell death. J Immunol. 1997;158:2600–9.
171. DeBenedette MA, Shahinian A, Mak TW, Watts TH. Costimulation of CD28- T lymphocytes by 4-1BB ligand. J Immunol. 1997;158:551–9.
172. Menk A V, Scharping NE, Rivadeneira DB, Calderon MJ, Watson MJ, Dunstane D, et al. 4-1BB costimulation induces T cell mitochondrial function and biogenesis enabling cancer immunotherapeutic responses. J Exp Med. 2018;215:1091–100.
173. Teijeira A, Labiano S, Garasa S, Etxeberria I, Santamaría E, Rouzaut A, et al. Mitochondrial Morphological and Functional Reprogramming Following CD137 (4-1BB) Costimulation. Cancer Immunol Res. 2018;6:798–811.
174. Shuford WW, Klussman K, Tritchler DD, Loo DT, Chalupny J, Siadak AW, et al. 4-1BB costimulatory signals preferentially induce CD8+ T cell proliferation and lead to the amplification in vivo of cytotoxic T cell responses. J Exp Med. 1997;186:47–55.
175. DeBenedette MA, Wen T, Bachmann MF, Ohashi PS, Barber BH, Stocking KL, et al. Analysis of 4-1BB ligand (4-1BBL)-deficient mice and of mice lacking both 4-1BBL and CD28 reveals a role for 4-1BBL in skin allograft rejection and in the cytotoxic T cell response to influenza virus. J Immunol. 1999;163:4833–41.
176. Tan JT, Whitmire JK, Ahmed R, Pearson TC, Larsen CP. 4-1BB ligand, a member of the TNF family, is important for the generation of antiviral CD8 T cell responses. J Immunol. 1999;163:4859–68.
177. Kwon BS, Hurtado JC, Lee ZH, Kwack KB, Seo SK, Choi BK, et al. Immune responses in 4-1BB (CD137)-deficient mice. J Immunol. 2002;168:5483–90.
178. Foell JL, Diez-Mendiondo BI, Diez OH, Holzer U, Ruck P, Bapat AS, et al. Engagement of the CD137 (4-1BB) costimulatory molecule inhibits and reverses the autoimmune process in collagen-induced arthritis and establishes lasting disease resistance. Immunology. 2004;113:89–98.
179. Melero I, Shuford WW, Newby SA, Aruffo A, Ledbetter JA, Hellström KE, et al. Monoclonal antibodies against the 4-1BB T-cell activation molecule eradicate established tumors. Nat Med. 1997;3:682–5.

180. Taraban VY, Rowley TF, O'Brien L, Chan HTC, Haswell LE, Green MHA, et al. Expression and costimulatory effects of the TNF receptor superfamily members CD134 (OX40) and CD137 (4-1BB), and their role in the generation of anti-tumor immune responses. Eur J Immunol. 2002;32:3617–27.
181. May KFJ, Chen L, Zheng P, Liu Y. Anti-4-1BB monoclonal antibody enhances rejection of large tumor burden by promoting survival but not clonal expansion of tumor-specific CD8+ T cells. Cancer Res. 2002;62:3459–65.
182. Martinez-Perez AG, Perez-Trujillo JJ, Garza-Morales R, Loera-Arias MJ, Saucedo-Cardenas O, Garcia-Garcia A, et al. 4-1BBL as a Mediator of Cross-Talk between Innate, Adaptive, and Regulatory Immunity against Cancer. Int J Mol Sci. 2021;22.
183. Futagawa T, Akiba H, Kodama T, Takeda K, Hosoda Y, Yagita H, et al. Expression and function of 4-1BB and 4-1BB ligand on murine dendritic cells. Int Immunol. 2002;14:275–86.
184. Laderach D, Wesa A, Galy A. 4-1BB-ligand is regulated on human dendritic cells and induces the production of IL-12. Cell Immunol. 2003;226:37–44.
185. Zhang B, Zhang Y, Niu L, Vella AT, Mittler RS. Dendritic cells and Stat3 are essential for CD137-induced CD8 T cell activation-induced cell death. J Immunol. 2010;184:4770–8.
186. Marson A, Kretschmer K, Frampton GM, Jacobsen ES, Polansky JK, MacIsaac KD, et al. Foxp3 occupancy and regulation of key target genes during T-cell stimulation. Nature. 2007;445:931–5.
187. Chen Z, Herman AE, Matos M, Mathis D, Benoist C. Where CD4+CD25+ T reg cells impinge on autoimmune diabetes. J Exp Med. 2005;202:1387–97.
188. Song Y, Wang N, Chen L, Fang L. Tr1 Cells as a Key Regulator for Maintaining Immune Homeostasis in Transplantation. Front Immunol. 2021;12:671579.
189. Sun Y, Chen HM, Subudhi SK, Chen J, Koka R, Chen L, et al. Costimulatory molecule-targeted antibody therapy of a spontaneous autoimmune disease. 2002;8:1405–13.
190. Foell J, McCausland M, Burch J, Corriazzi N, Yan XJ, Suwyn C, et al. CD137-mediated T cell co-stimulation terminates existing autoimmune disease in SLE-prone NZB/NZW F1 mice. Ann N Y Acad Sci. 2003;987:230–5.
191. Sun Y, Lin X, Chen HM, Wu Q, Subudhi SK, Chen L, et al. Administration of agonistic anti-4-1BB monoclonal antibody leads to the amelioration of experimental autoimmune encephalomyelitis. 2002;168:1457–65.
192. Seo SK, Choi JH, Kim YH, Kang WJ, Park HY, Suh JH, et al. 4-1BB-mediated immunotherapy of rheumatoid arthritis. Nat Med. 2004;10:1088–94.
193. Zhou J, You BR, Yu Q. Agonist-induced 4-1BB activation prevents the development of Sjögren's syndrome-like sialadenitis in non-obese diabetic mice. Biochim Biophys acta Mol basis Dis. 2020;1866:165605.
194. Lee J, Lee EN, Kim EY, Park HJ, Chang CY, Jung DY, et al. Administration of

agonistic anti-4-1BB monoclonal antibody leads to the amelioration of inflammatory bowel disease. Immunol Lett. 2005;101:210–6.
195. Mittler RS, Bailey TS, Klussman K, Trailsmith MD, Hoffmann MK. Anti-4-1BB monoclonal antibodies abrogate T cell-dependent humoral immune responses in vivo through the induction of helper T cell anergy. J Exp Med. 1999;190:1535–40.
196. Morris GP, Chen L, Kong Y chi M. CD137 signaling interferes with activation and function of CD4+CD25+ regulatory T cells in induced tolerance to experimental autoimmune thyroiditis. Cell Immunol. 2003;226:20–9.
197. Setareh M, Schwarz H, Lotz M. A mRNA variant encoding a soluble form of 4-1BB, a member of the murine NGF/TNF receptor family. Gene. 1995;164:311–5.
198. Kachapati K, Bednar KJ, Adams DE, Wu Y, Mittler RS, Jordan MB, et al. Recombinant soluble CD137 prevents type one diabetes in nonobese diabetic mice. J Autoimmun. 2013;47:94–103.
199. Michel J, Langstein J, Hofstädter F, Schwarz H. A soluble form of CD137 (ILA/4-1BB), a member of the TNF receptor family, is released by activated lymphocytes and is detectable in sera of patients with rheumatoid arthritis. Eur J Immunol. 1998;28:290–5.
200. Chin SM, Kimberlin CR, Roe-Zurz Z, Zhang P, Xu A, Liao-Chan S, et al. Structure of the 4-1BB/4-1BBL complex and distinct binding and functional properties of utomilumab and urelumab. Nat Commun. 2018 Nov;9(1):4679.
201. Shao Z, Sun F, Koh DR, Schwarz H. Characterisation of soluble murine CD137 and its association with systemic lupus. Mol Immunol. 2008;45:3990–9.
202. Yabas M, Elliott H, Hoyne GF. The Role of Alternative Splicing in the Control of Immune Homeostasis and Cellular Differentiation. Int J Mol Sci. 2015;17(1).
203. Bitra A, Doukov T, Wang J, Picarda G, Benedict CA, Croft M, et al. Crystal structure of murine 4-1BB and its interaction with 4-1BBL support a role for galectin-9 in 4-1BB signaling. J Biol Chem. 2018 Jan;293(4):1317–29.
204. Madireddi S, Eun SY, Lee SW, Nemčovičová I, Mehta AK, Zajonc DM, et al. Galectin-9 controls the therapeutic activity of 4-1BB-targeting antibodies. J Exp Med. 2014 Jun;211(7):1433–48.
205. Gerold KD, Zheng P, Rainbow DB, Zernecke A, Wicker LS, Kissler S. The soluble CTLA-4 splice variant protects from type 1 diabetes and potentiates regulatory T-cell function. Diabetes. 2011;60(7):1955–63.
206. Saverino D, Simone R, Bagnasco M, Pesce G. The soluble CTLA-4 receptor and its role in autoimmune diseases: an update. Auto Immun Highlights. 2010;1(2):73–81.
207. Simone R, Pesce G, Antola P, Rumbullaku M, Bagnasco M, Bizzaro N, et al. The soluble form of CTLA-4 from serum of patients with autoimmune diseases regulates T-cell responses. Biomed Res Int. 2014;2014:215763.
208. Michel J, Schwarz H. Expression of soluble CD137 correlates with activation-induced cell death of lymphocytes. Cytokine. 2000;12:742–6.

209. Hurtado JC, Kim SH, Pollok KE, Lee ZH, Kwon BS. Potential role of 4-1BB in T cell activation. Comparison with the costimulatory molecule CD28. J Immunol. 1995;155:3360–7.
210. Forsberg MH, Ciecko AE, Bednar KJ, Itoh A, Kachapati K, Ridgway WM, et al. CD137 Plays Both Pathogenic and Protective Roles in Type 1 Diabetes Development in NOD Mice. J Immunol. 2017;198:3857–68.
211. Foda BM, Ciecko AE, Serreze D V, Ridgway WM, Geurts AM, Chen YG. The CD137 Ligand Is Important for Type 1 Diabetes Development but Dispensable for the Homeostasis of Disease-Suppressive CD137(+) FOXP3(+) Regulatory CD4 T Cells. J Immunol. 2020;204:2887–99.
212. Jung HW, Choi SW, Choi JIL, Kwon BS. Serum concentrations of soluble 4-1BB and 4-1BB ligand correlated with the disease severity in rheumatoid arthritis. Exp Mol Med. 2004;36:13–22.
213. Rioja I, Hughes FJ, Sharp CH, Warnock LC, Montgomery DS, Akil M, et al. Potential novel biomarkers of disease activity in rheumatoid arthritis patients: CXCL13, CCL23, transforming growth factor alpha, tumor necrosis factor receptor superfamily member 9, and macrophage colony-stimulating factor. Arthritis Rheum. 2008;58:2257–67.
214. Liu GZ, Gomes AC, Putheti P, Karrenbauer V, Kostulas K, Press R, et al. Increased soluble 4-1BB ligand (4-1BBL) levels in peripheral blood of patients with multiple sclerosis. Scand J Immunol. 2006;64:412–9.
215. Beik P, Ciesielska M, Kucza M, Kurczewska A, Kuźmińska J, Maćkowiak B, et al. Prevention of Type 1 Diabetes: Past Experiences and Future Opportunities. J Clin Med. 2020 Aug;9(9).