

REVIEW

Open Access



Pancreatic draining lymph nodes (PLNs) serve as a pathogenic hub contributing to the development of type 1 diabetes

Fei Sun^{1,2†}, Chun-Liang Yang^{2†}, Fa-Xi Wang², Shan-Jie Rong², Jia-Hui Luo², Wan-Ying Lu², Tian-Tian Yue³, Cong-Yi Wang^{1,2*}  and Shi-Wei Liu^{1*}

Abstract

Type 1 diabetes (T1D) is a chronic, progressive autoinflammatory disorder resulting from the breakdown of self-tolerance and unrestrained β cell-reactive immune response. Activation of immune cells is initiated in islet and amplified in lymphoid tissues, especially those pancreatic draining lymph nodes (PLNs). The knowledge of PLNs as the hub of aberrant immune response is continuously being replenished and renewed. Here we provide a PLN-centered view of T1D pathogenesis and emphasize that PLNs integrate signal inputs from the pancreas, gut, viral infection or peripheral circulation, undergo immune remodeling within the local microenvironment and export effector cell components into pancreas to affect T1D progression. In accordance, we suggest that T1D intervention can be implemented by three major ways: cutting off the signal inputs into PLNs (reduce inflammatory β cell damage, enhance gut integrity and control pathogenic viral infections), modulating the immune activation status of PLNs and blocking the outputs of PLNs towards pancreatic islets. Given the dynamic and complex nature of T1D etiology, the corresponding intervention strategy is thus required to be comprehensive to ensure optimal therapeutic efficacy.

Keywords Type 1 diabetes (T1D), Pancreatic draining lymph nodes (PLNs), Signal inputs, PLN remodeling, Signal outputs

Introduction

As a prototypical autoimmune disease, type 1 diabetes (T1D) stems from the breakdown of self-tolerance and subsequent relentless immune attack which destroys pancreatic islet β cells, thereby leading to insulin deficiency [1–5]. The etiologies underlying T1D are yet to be fully addressed, but are associated with genetic predisposition, epigenetic reprogramming and environmental cues including diet, lifestyle change, microbiota alteration and infection of specific viral strains [6–9]. All these intrinsic abnormalities and extrinsic insults are deemed to initiate islet autoreactive immune responses. Damage associated molecular patterns (DAMPs) along with autoantigens released from dying β cells are the major drivers of autoimmune priming [10, 11]. On the other hand, disrupted integrity of the intestinal barrier allows translocation of

[†]Fei Sun and Chun-Liang Yang contributed equally to this work.

*Correspondence:

Cong-Yi Wang
wangcy@tjh.tjmu.edu.cn
Shi-Wei Liu
lswspring6@aliyun.com

¹ Shanxi Bethune Hospital, Shanxi Academy of Medical Science, Tongji Shanxi Hospital, Third Hospital of Shanxi Medical University, Taiyuan, China

² NHC Key Laboratory of Respiratory Diseases, Department of Respiratory and Critical Care Medicine, The Center for Biomedical Research, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

³ Division of Nutrition, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China



microbial components to the remote area, which then act as immunostimulatory adjuvants to exacerbate β cell destruction [12, 13]. In particular, viral infections could directly interfere with β cell function, but the deteriorative effect largely comes from infection-induced interferonopathy, a “spillover effect” of the anti-viral response [14, 15].

Single-cell techniques applied in peripheral blood, pancreatic draining lymph nodes (PLNs) and pancreas have greatly advanced our understanding of cell components involved in T1D development [16]. Strikingly, single-cell RNA sequencing (scRNA-seq) with 4-week, 8-week and 15-week old non-obese diabetic (NOD) mice found that the immune infiltration is already identifiable as early as 4-week of age, rapidly progresses at around 8-week old and peaks at 15-week old [17]. T1D is therefore acknowledged as a chronic progressive inflammatory disorder. In this case, the breakdown of immune tolerance is a gradually occurring process, but most critically, our body could exploit versatile approaches to counterbalance the overactive autoimmune responses to protect the residual β cell mass. ScRNA-seq of human pancreas revealed the unexpected immune regulatory function of ductal epithelial cells [18, 19], and upon IFN- γ stimulation, β cells actively upregulate PD-L1 expression to resist autoinflammatory assault [20]. Additional immunological self-limiting mechanisms are also found including regulatory T cell (Treg) adaptation, activation-induced cell death (AICD) and the exhaustion of effector T cells (Teff), which collectively put a brake on the derailed immune responses [21, 22].

Given that T1D is resulted from autoimmune destruction of islet β cells, the crosstalk between β cells and islet resident immune cells plays an initiative part and determines the tissue specificity of T1D, but the destructive autoimmune response is owing to the signaling amplified in organized lymphoid structures. Mounting studies have demonstrated the presence of tertiary lymphoid organs (TLOs) in the peri-islet milieu [23]. TLOs are formed in response to lymphotoxin signaling, and therefore, the removal of PLNs in NOD mice cannot entirely prevent T1D development [17, 24, 25]. In general, TLOs are normally visible at 14–20 weeks of age in NOD mice [24, 26]. As a result, it is very unlikely that TLOs could replace the role of PLNs in T1D pathogenesis, especially at the early stage of disease development. Indeed, excision of PLNs at 3 weeks almost completely protects NOD mice from insulinitis and diabetes, but the goal cannot be achieved once it is conducted at 10 weeks of age [17]. The knowledge of PLNs as a place of aberrant immune response is continuously being replenished and renewed, and what we presented here aims to piece together those valuable up-to-date findings, and to delineate the comprehensive

landscape of T1D pathogenesis from a PLN-centered perspective.

PLNs integrate priming signals from diverse sources of input

Pancreas is the primary source of input signals for efficient triggering of immunological events within PLNs. Recurrent exposure to islet-specific antigens is deemed to contribute to the early initiation of T1D [27]. Pancreatic islet β cell-derived granules containing catabolized insulin peptide fragments (e.g., insulin B:12-20) are released into circulation or near the neighborhood, taken up, and presented by antigen-presenting cells (APCs), which ultimately enhances CD4⁺ T cell diabetogenicity in various lymphoid tissues, especially PLNs, as evidenced by the presence of insulin specific germinal centers (GCs) [28]. Dendritic cells (DCs) serve as a bridge linking β cell damage to the activation of adaptive immune system [29]. Defects in NOD DCs has been ascribed to the *Idd10/17/18* region, which hinders the generation of tolerogenic DCs and arrests DCs in a maturing phase, thereby producing more IL-12 but less IL-10 [30]. Prior to overt lymphocytic insulinitis, CD8a⁺ DCs accumulate at the edge of islet. The frequency of CD8a⁺ DCs reduces in the pre-diabetic pancreas rather than in the PLNs, and the expression of tolerogenic markers such as CCR5, CLEC9A, and IL-10, is down-regulated. These data indicate that alteration of DC state and loss of peri-islet tolerance might precede the breakdown of tolerance in PLNs [31]. There are two major subsets of islet resident DCs: CD103⁺ DCs derived from pre-DCs, and CD11b⁺ DCs originated from circulating monocytes. CD103⁺ DCs are adept at cross-presenting islet autoantigens by migrating towards PLNs, while CD11b⁺ DCs are more phagocytic and preferentially stay in the islet [32]. Physiological β cell death, occurring around 2 weeks of age in all mouse strains, goes awry in NOD mice, which provides primordial diabetogenic antigen to CD11b⁺ DCs and provokes T cell activation in PLNs [33]. In contrast, BATF3-dependent CD103⁺ DCs make up a minor population of islet APCs in newborn NOD mice; however, by 4 weeks of age, the proportion of CD103⁺ DCs surges in concomitant with the accession of T cells into islets. Ablation of BATF3 results in a lack of CD103⁺ DCs in both pancreas and PLNs, thereby preventing autoreactive T cell activation and T1D development [34]. An amplification loop is also identified between T cells and DCs, as islet infiltrating T cells are able to further upregulate the expression of CD40, CD80 and CCR7 on DC surface, which augments their potency to prime more autoreactive T cells in PLNs [35]. In addition to DCs, B cells partially contribute to T1D pathogenesis by immunoglobulin (Ig)-mediated antigen capture and the priming effect on

diabetogenic T cell response [36]. Therefore, although the pathogenic role of B cell-secreted autoantibodies (Ab) is an issue under debate, autoreactive B cells may act as APCs necessary for the initial activation of β cell reactive CD4 T cells [37].

Notably, intra-islet APCs capture antigenic peptides, get matured and obtain the migratory capacity towards PLNs via the afferent lymphatic vessels [38]. Lymph-angiogenesis represents a pathological feature commonly observed in chronic inflammatory disorders, particularly in the case of insulinitis in T1D setting. Vascular endothelial growth factors receptor 3 (VEGFR3) is critically involved in the above process, and VEGFR3 blockade reduces multiple low dose streptozotocin (MLDS)-induced immune responses in PLNs [39]. By injecting indocyanine green (ICG) into parenchyma in the anterior or posterior surface of the pancreas head, seven main pancreatic lymphatic drainage pathways were identified [40]. A similar technique may be applied to NOD mice to reveal the lymphatic draining pathways, given lymphatic system is tightly associated with the initiation or resolution of pancreatitis [41]. Unfortunately, relevant studies on whether targeting lymph-angiogenesis is a feasible approach for T1D treatment are lacking thus far.

Specific strains of viral infections also contribute to the motivation of PLNs and the priming of autoinflammatory reactions. Orally infected rhesus monkey rotavirus (RRV) makes its presence in PLNs by extra-intestinal spread, which activates regional APCs and elicits a Th1 biased adaptive immune response. Rotavirus infection in at-risk children positively correlates with T1D progression and accelerates T1D onset in a mouse model [42]. Mechanistically, rotavirus infection of NOD mice enhances the expression of MHC-I molecule on PLN B cells and promotes the proliferation of autoreactive T cells possibly through bystander activation [43]. In rats, Kilham rat virus (KRV) infection reproducibly induces acute T1D in genetically predisposed BB/Wor strain. By in situ hybridization, the tissue tropism of KRV infection was unraveled. Interestingly, KRV mRNA and DNA were readily detected in peripancreatic lymphoid tissues while were hardly seen in the pancreas following 5 days of infection [44]. Consistently, the T1D-inducing effect of KRV infection is attributed to B cell and plasmacytoid DC (pDC) activation in PLNs. Microarray analysis revealed that the upregulated genes elicited by KRV infection were predominantly IFN- γ -induced chemokines and genes associated with IL-1 pathways, interferon production, and downstream signaling molecules [45]. On the contrary, certain viral inputs may alleviate the progression of T1D. For instance, intraperitoneal or intranasal infection of murine gammaherpesvirus-68 (MHV-68) delays T1D

onset by reducing dendritic cell antigen presentation and rendering PLN autoreactive T cells at a naïve state [46]. Therefore, the immune regulatory role of viral infections may vary under the context of T1D.

Gut-derived signals are another important source of input that affects the immune status of PLNs. From the perspective of development, a preferential trafficking route exists from the gut to PLNs, and PLN resident T cells can also be activated by antigens drained from the peritoneum and the gastrointestinal tract [47]. An appealing hypothesis proposed that dietary intake of wheat gluten triggers T1D pathogenesis by releasing dipeptidyl peptidase IV (DPP4)-cleaved X-pro peptides. Gluten-derived peptides would be ingested by intestinal DCs, which are then recruited into PLNs by chemokines CCL19/CCL21 to activate β cell reactive lymphocytes [48]. A similar antigen mimicry approach is adopted by pathogenic gut microbiome. The *hprt4-18* peptide derived from the human gut commensal *Parabacteroides distasonis* activates T cell clones of T1D patients that are specifically directed at an epitope in the B-chain of insulin (insB:9-23), and as a result, the seroconversion rates are consistently higher in children whose microbiome harbors sequences capable of producing the *hprt4-18* peptide [49]. Moreover, the dysbiosis of gut microbiota, disruption of the intestinal barrier integrity and microbial translocation are construed as the key pathological events in T1D pathogenesis as well [50, 51]. For example, streptozotocin (STZ) treatment would cause a “leaky gut” permitting the translocation of microbial products into PLNs, where they are probed by the nucleotide-binding oligomerization domain containing 2 (NOD2), inducing pathogenic Th1 and Th17 response [52]. Additionally, functional and metabolic alterations of gut microbiome, featured by the decreased butyrate production and bile acid metabolism along with increased lipopolysaccharide biosynthesis, are observed in T1D children [53]. Particularly, the combination of 18 bacterial species and fecal metabolites provides prognostic value for T1D [53], which lays the foundation for microbiota-based T1D therapies including fecal microbiota transfer (FMT) [54] and the supplementation of beneficial bacterial species [55]. Taken together, although gut-associated lymph nodes are sources of intermediate diabetogenic lymphocytes, they are likely engaged in the early phase of T1D initiation [56].

PLNs constantly exchange cell components with the peripheral blood and circulating diabetogenic T cells tend to choose PLNs as the priority to habitat. Circulating B cells access into PLNs mainly by their surface expression of mucosal addressin cell adhesion molecule 1 (MAdCAM-1) and $\alpha 4\beta 7$ integrin, and partly by the presence of L-selectin or LFA-1. Upon their arrival in PLNs,

they sense, capture and present the drained autoantigens to T cells [57]. Using a T1D adoptive transfer model in NOD mice, by analysis of the transferred T cells in the pancreas and lymphoid organs including thymus, spleen, and lymph nodes from pancreatic, mesenteric, axillary, inguinal and combo-aortic areas, it was interestingly discovered that the transferred T cells are readily and predominantly infiltrated into PLNs, where they undergo the process of activation and acquisition of diabetogenicity [58]. This phenomenon is corroborated by the adoptive transfer of antigen-specific BDC2.5T cells. Before insulinitis is detectable, the transferred T cells are found to only proliferate in PLNs, indicating that β cell-derived antigens are similarly and predominantly transported into PLNs, although small amounts of antigens could be spread into remote areas [59]. Altogether, PLNs receive signals from the pancreas, gut, viral infection, and circulation (Fig. 1). These diverse external inputs are integrated in PLNs and finally transformed into abnormal islet autoreactive immune responses, which would be discussed in the following sections.

PLN remodeling is featured by the perturbation of immune microenvironment

In recent-onset T1D patients, histological examination revealed decreased primary B cell follicle frequency, fewer follicular dendritic cell (FDC, CD21, and CD35 positive) networks, and accumulation of extracellular matrix glycosaminoglycan hyaluronan (HA) and HA binding proteins in PLNs [60, 61]. By classifying 5-week old NOD mice into insulin autoantibody (IAA⁺) group and IAA⁻ group along with comparative analysis, it was found that the differentially expressed genes (DEGs) are

enriched in tissue reconfiguration and Th1 immunity, suggesting an early immunological rewiring in PLNs [62]. PLN remodeling is accompanied by a shift from immune tolerance to the state of immune activation. Breakdown of self-tolerance is a prerequisite for the autoreactive response, and anomalies in tolerizing mechanisms take the principal responsibility.

Generally, PLN remodeling is characterized by the alteration of stromal cells. Fibroblastic reticular cells (FRCs) form the scaffold to support the PLN architecture and physiologically present self-antigens to induce T cell tolerance. FRC networks in NOD PLNs display larger reticular pores than non-obese diabetes-resistant (NOR) controls, and thus engage with more T cells, which possibly serves as a compensatory anti-inflammatory mechanism [63]. Lymph node stromal cells (LNSCs) are also physiologically involved in T cell tolerance induction in human T1D patients, and similarly, NOD mice PLN-derived LNSCs display enhanced tolerogenic phenotype along with increased antigen-presenting potential to offset DC-induced T cell activation [64]. Deformed epidermal autoregulatory factor 1 (DEAF1) is a transcription regulator that promotes the expression of peripheral tissue antigens (PTA) in LNSCs. As forward of T1D progression, the alternatively spliced dominant-negative isoform DEAF1-Var1 is upregulated in PLNs (through splicing factor Srsf10 and Ptbp2), which reduces PTA expression and possibly promotes the loss of peripheral tolerance [65]. Reduction of DEAF1 function downregulates the expression of eukaryotic translation initiation factor 4 gamma 3 (Eif4g3), which modulates the translation of various genes involved in PTA presentation (such as aminopeptidase N), as revealed by the polysome

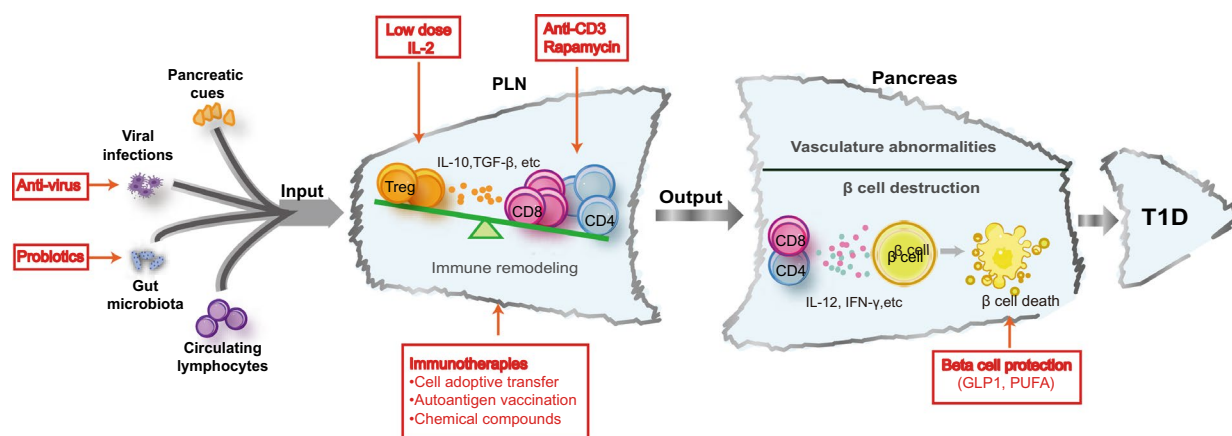


Fig. 1 Pancreatic draining lymph nodes (PLNs) integrate signal inputs from various sources and undergo substantial immune remodeling to elicit anti-islet response. Pancreas derived autoantigens (soluble or presented by APCs), viral infections, gut microbiota components and circulating lymphocytes get access into PLNs, leading to and/or boosting the imbalance of Treg/Teff. The primed autoreactive T cells then serve as the major PLN outputs that infiltrate the pancreas and are responsible for T1D initiation. APC antigen-presenting cell, Treg regulatory T cell, Teff effector T cell

profiling [66]. The expression of tissue-specific antigens (TSAs) mediated by the autoimmune regulator (AIRE) in the thymus is essential for central tolerance induction, while DEAF1 may serve as a master regulator manipulating the expression of PTAs and peripheral tolerance induction [67, 68]. Therefore, PTA-mediated peripheral tolerance induction plays an instructive role in T1D initiation [69].

It is worthy of note that PLN resident and immigratory APCs are decisive for the ultimate tolerance breakdown and priming of autoimmune reactions. Compared to DCs isolated from PLNs of control mice or axillary/inguinal (A/I) LN of NOD mice, DCs from NOD PLNs form larger clusters with T cells (increase with age) which comprise a major source of proliferating T cells. The cluster formation is specific, as NOD PLN DCs fail to cluster with A/I T cells and in turn A/I DCs fail to cluster with PLN T cells [70]. The DNAX-activating protein of 12 kDa (DAP12) is an adaptor molecule expressed on lymphoid and myeloid cells. DAP12 in DCs facilitates the activation of PLN Treg cells and serves as a tolerance mechanism to β cell-derived antigens. *DAP12* deficiency in BDC2.5/B6g7 TCR transgenic mice manifests higher activation of PLN T cells and more rapid T1D onset, implying the critical role of DC in dictating the direction of tolerance or immunity of PLNs [71]. B cells play an elusive part in T1D pathogenesis regarding the production of autoantibodies [72]. Eight-hundred sixty-three human IgG antibodies were cloned from 4092 single B cells from PLNs and peripheral blood. Surprisingly, only 2 clones showed reactivity to insulinoma-associated antigen 2 (IA-2), while the rest of them were negative for commonly known autoantigens including IA-2, GAD65 and zinc transporter 8 (ZnT8), indicating an infrequent presence of autoantigen-specific IgG⁺ B lymphocytes in PLNs from IAA-positive individuals [73]. Marginal zone B (MZB) cells are detected in almost 80% of NOD mice by 16-week old and the population expands along with T1D progression. These MZB cells are hyperresponsive to TLR, CD40 and S1P, and express MHC-II, CD80 and CD86, by which they serve as potent APCs to prime diabetogenic T cells within PLNs [74]. Therefore, B cells would probably assist DCs in the transition of PLN state from tolerance to immunity.

The breakdown of self-tolerance is followed by unrestrained autoreactive T cell response, which contributes to the long-lasting and unresolved T1D progression [75, 76]. PLN memory CD4⁺ T cells and pancreatic memory CD4⁺ T cells share restricted TCR β usage, and the majority of public clonotypes express TRBV13-2 (V β 8.2) gene segment. Further analysis of CDR3 β sequences revealed rare clones of well-identified diabetes-related clonotypes, including those recognizing IGRP, insulin B:9–23

and chromogranin, which reflects the potential occurrence of intra- or inter-molecular epitope spreading and the hypermutation nature of TCR [77]. A high degree of clonal expansion was observed in PLNs from long-term diabetic patients [78]. However, despite the promiscuous TCR clones within PLNs, the disease-causing clonotypes may be limited [79]. In NOD mice, T cells specifically recognizing HIP2.5 epitope (a fusion of insulin C-peptide and chromogranin A fragment) account for around 40% of islet-infiltrating T cells at both prediabetic and diabetic stages [80]. In humans, GAD65 reactive TCR is present in 38.9% of examined patients, which contributes >25% reactive TCR β (TRB) within the conventional T cells isolated from PLNs [81].

Crosstalk between APCs and T cells is indispensable for efficient T cell priming. NOD mice harbor a unique MHC-II genotype (I-Ag7), which presents β cell-derived naturally processed peptides mainly coming from proteins associated with neuronal or neuro-endocrine cell types (e.g. synaptotagmin, neuromodulin, and amyloid β) or proteins associated with secretory granules (e.g. secretogranin and chromogranin) to CD4⁺ T cells [82]. Replacement of I-Ag7 by I-E on DCs of NOD mice promotes the differentiation of autoreactive CD4⁺ T cells into antidiabetogenic autoregulatory T cells and protects against T1D progression [83]. Among different effector CD4⁺ T cell (Teff) subsets, Th1 is the most pathogenic one. Adoptive transfer of Th1 cells from BDC2.5 transgenic mice induces T1D in NOD/SCID mice. However, the transferred Th17 cells readily upregulate T-bet and secrete IFN- γ upon exposure to IL-12, and neutralization of IFN- γ instead of IL-17 prevents T1D induced by the transfer of purified Th17 cells [84]. On the other hand, priming of diabetogenic CD8⁺ T cells requires the cross-presentation activity of DCs. Cross-presentation of islet antigens is inactive during neonatal life and gradually available when the inflammatory response becomes obvious [85]. NOD BMDCs pulsed with freeze-thawed insulinoma cells activate diabetogenic CD8⁺ T cells in the presence of TLR9 agonist and anti-CD40. Specifically, TLR9 affects the function of pDCs in PLNs, which produce type 1 interferons to participate in CD8⁺ T cell activation [86]. Notably, adoptive transfer of autoreactive CD8⁺ T cells alone results in clonal deletion in draining lymph nodes [87, 88], while co-delivery of autoreactive CD4⁺ T cells is required to provide essential help for the optimal activation of CD8⁺ T cells [89].

Immune regulatory cells are also present in PLNs to serve as a homeostatic mechanism to put a brake on the overactive immune response. Teff cells play a double-faceted role in T1D development. Teff cells other than induce islet destruction, they also boost Treg cell expansion to enhance their suppressive function in PLNs

[90]. The number of PLN Tregs dramatically drops in NOD mice due to the impaired retention caused by the downregulation of SDF-1/CXCR4 axis [91]. Similarly, the frequency of T follicular regulatory (Tfr) cells, a specialized regulatory counterpart of T follicular helper (Tfh) cells, is reduced in PLNs of T1D patients, and re-supplementation of Tfr cells delays T1D development in mice [92]. The unbalanced immune status of human T1D is featured by functional defects in CD4⁺CD25⁺ Tregs in PLNs but not in peripheral blood [93, 94]. PLN Tregs inhibit in situ differentiation of islet-reactive CD8⁺ T cells, and the suppression is mediated by the TGF- β /TGF- β RII axis, as Treg cells could not control naive or activated islet-reactive CD8⁺ T cells bearing a dominant-negative TGF- β RII genotype following adoptive transfer [95]. Likewise, a study argued that Treg function is not compromised during T1D initiation, rather conventional T cells showed reduced susceptibility to Treg-mediated suppression [96]. Such resistance of T cells to Tregs is mediated by the elevated IL-21 levels in PLNs, which probably contributes to the enhanced DC migratory capacity [97]. In addition to Treg cells, other regulatory cells are also involved in the modulation of PLN immune activation state. For instance, mice deficient in mast cells are more prone to multiple low dose STZ-induced insulinitis, and adoptive transfer of mast cells confers resistance to T1D by promoting Treg cells and suppressing Th17 cells in PLNs [98]. NKT cells activated by alpha-galactosyl ceramide (alpha-GalCer) could induce the maturation of disease-protective DCs, which tolerizes pathogenic T cells in the PLNs. As a result, alpha-GalCer pretreatment reduces T1D incidence in mice [99, 100].

In brief, intrinsic defects along with external inputs synergistically contribute to tolerance breakdown and immune activation in PLNs. The co-existence of both effector and regulatory mechanisms suggests that T1D pathogenesis is an outcome of immune imbalance gambled by the promiscuous immunological events, which explain the chronic and relapsing nature of the disease (Fig. 2).

Autoreactive lymphocytes are exported from PLNs and infiltrate into the islet

After remodeling, PLNs become a formidable “military base” to store arsenal of weapons for β cell killing. Translocation of lymphocytes from PLNs to pancreatic islets (consisting of PLN egress, lymphocyte trafficking and islet infiltration) is crucial for T1D initiation. The BDC-Idd9 mice harbor BDC2.5 TCR transgenic T cells containing the *Idd9* genomic region originated from diabetes-resistant B10 mice. Unlike BDC T cells that predominantly accumulate in PLNs and pancreas, BDC-Idd9 T cells gather in splenic periarteriolar

lymphatic sheaths, but both of them are comparable in terms of development, functional activation and proliferation [101]. Similarly, the NOD-Idd22 mice carry the diabetes-resistant ALR strain-derived *Idd22* genomic region (Chromosome8: D8Mit293-D8Mit137). This ALR-derived *Idd22* locus does not affect immune cell diabetogenicity, β cell resistance to cytotoxicity or proliferation of transferred CTLs in PLNs. However, β cell autoreactive T cells accumulate less in pancreatic islets due to the lower adhesion molecule expression on vascular endothelial cells and the consequent weaker adherence of T cells [102]. Vasculature abnormalities are indeed essentially implicated in T1D pathogenesis. Through contrast-enhanced ultrasound measurement, researchers found that islet microvasculature reorganization and blood flow dynamics precede T1D onset in various pre-clinical models, and islets have a denser microvasculature during diabetes progression [103]. Comparative microarray analysis revealed that genes involved in angiogenesis are specifically activated in NOD islets of 2–4 weeks of age [104]. In particular, VEGFR2 is upregulated in inflamed islets and, as a result, inhibition of VEGFR2 ameliorates T1D progression, which supports that VEGFR2 is likely responsible for the enhanced vascularity and lymphocyte infiltration [105].

Adhesion molecules and chemokine–chemokine receptors, which are present on activated PLN-derived lymphocytes, are indispensable for the development of lymphocytic insulinitis [106]. Mucosal addressin cell adhesion molecule-1 (MAdCAM-1) is expressed on islet vessels of NOD mice early during lymphocyte accumulation in islets. Integrin $\alpha 4\beta 7^{\text{hi}}$ T cells in NOD mice are mainly come from PLNs or spleen, rather than mucosal lymphoid tissue, which infiltrate into islet through binding to MAdCAM-1 [107]. Alternatively, high endothelial venules (HEVs) in inflamed islets co-express CCL21 and CCL19, which recruit CCR7⁺ T cells from bloodstream into islets. Blockade of CCR7 abolishes 70% of T cell infiltration while not affecting B cells [108]. Intravital two-photon imaging demonstrated that peri-vascular CD11c⁺ cells govern T cell extravasation by secreting plentiful and redundant chemokines. For this reason, depletion of peri-vascular CD11c⁺ cells, instead of blocking limited chemokine–chemokine receptor signaling pathways, is more efficient in preventing the entrance of lymphocytes into islets [109]. Intriguingly, activated T cells could upregulate the expression of insulin receptors (IRs). IR positivity not only helps sense insulin for enhanced metabolic activity but also serves as an atypical chemokine receptor that directs the migration of T cells towards islets following the concentration gradient of insulin [110].

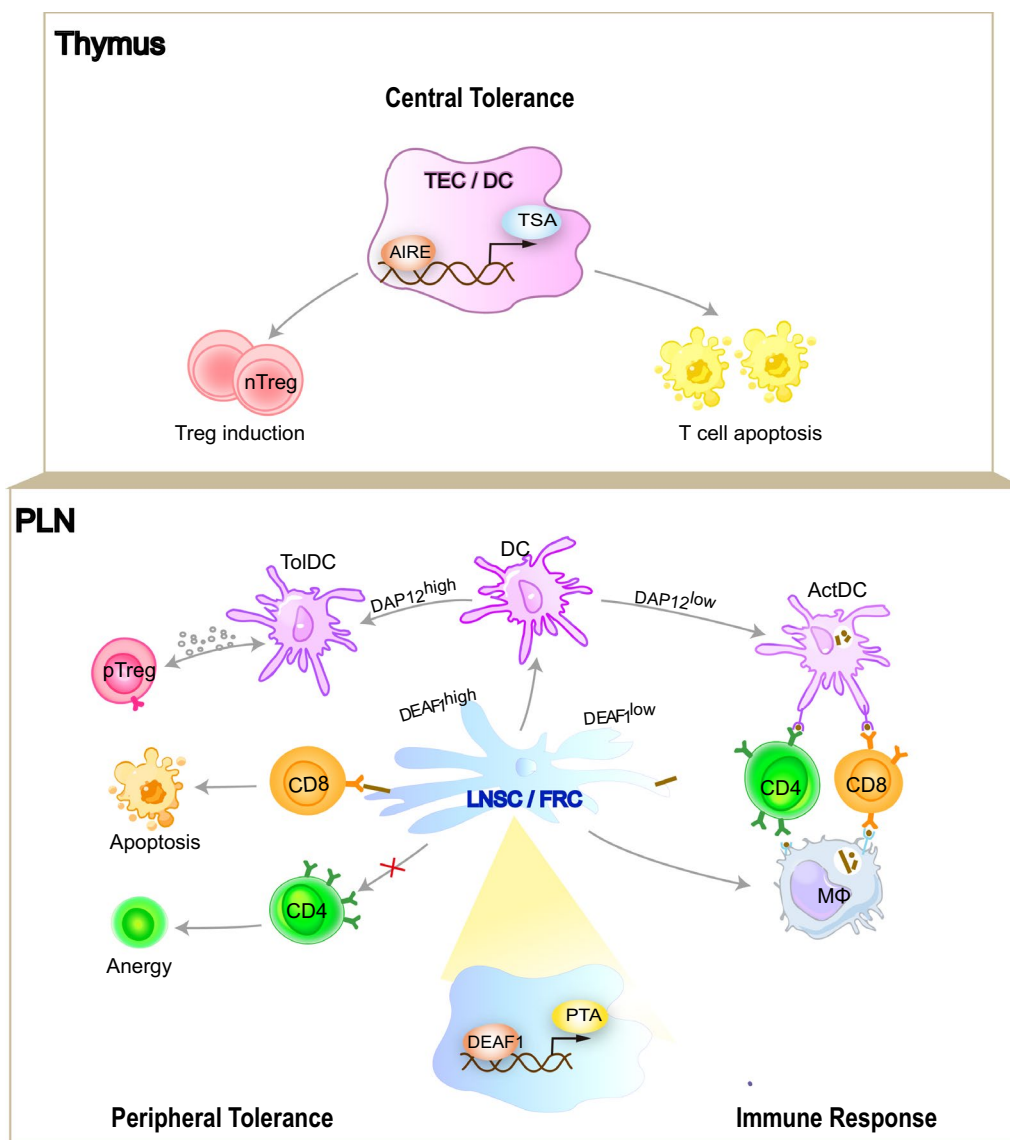


Fig. 2 Breakdown of self-tolerance mechanism underpins T1D etiology. Briefly, self-tolerance is achieved at two different but related levels. Within the thymus, AIRE promotes the ectopic expression of tissue-specific antigens (TSAs) in thymic epithelial cells (TECs) and together with the presence of thymus resident DC, central tolerance is established through depleting autoreactive T cells (negative selection) and the induction of antigen-specific Treg cells. In parallel, within PLN, DEAF1 drives the ectopic expression of peripheral tissue antigens (PTAs) in lymph node stromal cells (LNSCs)/fibroblastic reticular cells (FRCs) and together with DAP12^{hi} DCs, peripheral tolerance is established to further solidify immune homeostasis. Abnormalities in organismal tolerizing mechanism is thus fundamental to the pathogenesis of autoimmune disorders including T1D. *Tol-DC* tolerogenic dendritic cell, *Act-DC* activated dendritic cell, *DAP12* DNAX-activating protein of 12 kDa, *DEAF1* deformed epidermal autoregulatory factor 1

The stepwise, continuous spectrum of immune cell infiltration is best exemplified by CD8⁺ T cells, which experience distinct states of naïve, effector, memory, stem-like memory, or exhaustion. After leaving PLNs and arriving at islets, CD8⁺ T cells gradually gain higher expression of the cytotoxic effector markers, granzyme B, IFN-γ, and CD107a [111]. Activated CD8⁺ T cells face up with the fate of either becoming exhausted or dead

after killing [21, 112]. TCF1^{hi} stem-like memory CD8⁺ T cells are a minor but unique cell population that possesses the characteristics of both memory cells and stem cells [113]. They reside in PLN and provide a persistent output of autoreactive CD8⁺ T cells that enter the islet and replenish the depleted mission-completed ones [113, 114]. The presence of TCF1^{hi} stem-like CD8⁺ T cells is also confirmed in conditions like tumors, and cDC1 is

required for their maintenance [115]. Therefore, it is not surprising to observe decreased PLN cellularity and T cell number in NOD mice after disease onset [116], and the turnover of autoreactive lymphocytes may contribute to the remission-relapsing phases of T1D progression (Fig. 3).

As part of the compensatory protective mechanism, Treg cells also migrate from PLNs to the pancreas. In response to IFN- γ produced by Teff cells, antigen-specific ICOS⁺ Treg cells preferentially express CXCR3 in PLNs and are chemoattracted by CXCL9, CXCL10, and CXCL11 derived from intra-pancreatic APC populations and β cells, serving as a homeostatic mechanism to slow down T1D progression [117]. Upon arrival at pancreas, it is possible that Treg cells further undergo phenotypic and functional adaptations in the new microenvironment. By crossing Foxp3 scurfy mice with BDC2.5 mice, it is found that the absence of Treg does not affect T1D initiation but accelerates T1D progression. Additionally,

the transcriptome profiling between PLN Treg and intra-islet Treg is different, suggesting that Treg cells primarily impinge on autoimmune diabetes by restraining destructive T cells inside the islets [118]. PLN-derived Treg cells are extremely potent and a mere 2,000 cells are capable of preventing diabetes development [119]. However, a study showed that miR-125a-5p is specifically hyper-expressed in Treg cells isolated from PLNs of donors with T1D. Upregulated miR-125a-5p is associated with reduced CCR2 level, which hinders the attraction of CCR2⁺ Treg cells by islet-derived CCL2 [120]. For the therapeutic purpose, butyrate administration induces colonic Treg cells and upregulates their surface expression of $\alpha 4\beta 7$, CCR9, and GPR15, thereby directing their migration to PLNs and then pancreas [121]. The direct transfer of Treg cells suppresses the function of macrophages and inhibits effector T cell function in islets in a TGF- β -dependent manner, which lays the rational foundation of Treg-based T1D therapies [122].

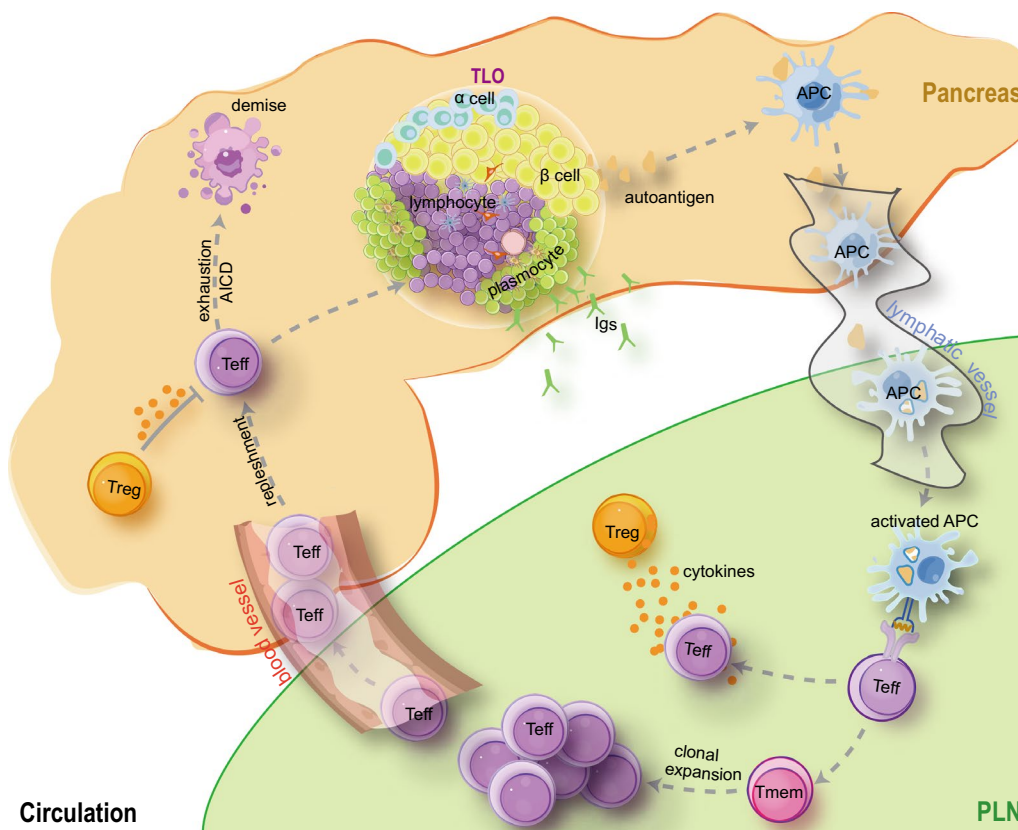


Fig. 3 Pancreatic draining lymph nodes (PLNs) orchestrate and perpetuate the vicious cycle of islet-specific autoimmune reaction. Effector T cells (Teff) are efficient in β cell killing and are organized into specialized tertiary lymphoid organs (TLOs) with chronic T1D progression. Autoantibodies (Igs) produced by plasmocytes act as the early immune biomarker of T1D initiation and the autoantigens generated from dead islet β cells are presented by antigen presenting cells (APCs) to prime autoreactive T cells in PLNs. A minor population of autoreactive T cells are present in the form of stem like memory T cells (Tmem) to continuously fuel anti-islet immunity, considering that Teff have a short lifespan and would not persist once arriving at the islet niche

The intervention of T1D development by strategies targeting PLNs

From a PLN-centered view, T1D intervention strategy can be implemented by three major ways: cutting off the signal inputs into PLNs (reduce inflammatory β cell damage, enhance gut integrity and get rid of pathogenic viral infections), modulating the immune activation status of PLNs, and blocking the outputs of PLNs towards pancreatic islets.

Cell-based therapies: transfusion of tolerance-inducing cells is a feasible approach to restoring immune balance in PLNs. Apart from Treg cell transfer mentioned above, infused double-negative (DN) T cells preferentially home to PLNs, where they could suppress the function of CD4⁺ T cells and reverse new-onset T1D once applied in combination with anti-thymocyte serum (ATS) [123]. Similarly, intraperitoneal administration of IDO (indoleamine 2,3-dioxygenase) overexpressed fibroblasts manifested potency to attenuate islet inflammation by inducing Treg cells and decreasing autoreactive CD8⁺ T cells following migrating to local lymph nodes [124]. Moreover, DCs delivered by intravenous and/or intraperitoneal injection are predominantly drained to PLNs [125, 126]. Adoptively transferred IL-4 overexpressing BMDCs accumulate in PLNs, normalize the abnormal gene expression profile, and delay T1D progression [127]. 1,25-Dihydroxyvitamin D3 (1,25(OH)2D3) treatment induces tolerogenic dendritic cells (ToIDCs) in both diabetes-prone NOD mice and diabetes-resistant C57BL/6 mice. Once the induced ToIDCs are co-transferred with activated CD4⁺ T cells into NOD/SCID recipients, they dampen the proliferation of autoreactive T cells in PLNs [128].

Chemical-based therapies: small chemical compounds can be applied to T1D treatment and their action modes vary. One class of drugs works by disrupting the process of islet lymphocytic infiltration. Tellurium compounds, including AS101 and SAS, inhibit the activity of $\alpha 4\beta 7$ integrin, thereby preventing autoreactive lymphocytes from migrating to the pancreas [129]. Tested in LEW.1AR1-IDDM spontaneous rat T1D model, S1P1 agonist FTY720 (fingolimod) promotes the retention of activated T cells in PLNs and hinders their islet infiltration [130]. By blocking the egress of lymphocytes and maintaining the integrity of peri-islet TLSs, FTY720 prevents diabetes development even at a time of significant insulinitis in the spontaneous T1D model of NOD mice [24]. Alternative S1P1 receptor (S1P1R) modulator, ponesimod, inhibits the spreading of T cell responses and demonstrates a potential therapeutic effect when combined with an anti-CD3 antibody [131]. The other set of chemicals works by inducing tolerance in PLNs. Cytopylyne from the plant *Bidens Pilosa* causes T cell apoptosis and elevates the Th2/Th1 ratio in PLNs [132].

Additionally, treatment with AHR ligand, 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), expands Treg population and reduces pancreatic islet insulinitis [133]. Administration of complete Freund's adjuvant (CFA) alone increases Treg percentage in PLNs and reverses new-onset T1D in 38% of NOD mice. The therapeutic effect is further boosted to 86% once it combines with the glucagon-like peptide-1 (GLP-1) analog exendin-4, which potently stimulates β cell replication [134]. Sulfatide reactive type II NKT cells (sulfatide/CD1d-tetramer⁺) are an anti-inflammatory subset differing from type I NKT cells. Administration of sulfatide C24:0 enlarges the type II NKT cell population, educates DCs to secrete more IL-10 and suppresses the activation of diabetogenic T cells [135]. Capsaicin, through binding to vanilloid receptor 1 (VR1), promotes anti-inflammatory macrophages in PLNs, which express IL-10 and PD-L1, and suppresses the activation of autoreactive T cells [136].

Vaccination-based therapies: vaccination has the advantage of inducing antigen-specific immune tolerance. Oral administration of recombinant insulin induces Treg cells in PLNs and shifts the Th1 response to Th2 by promoting the expression of IL-4 [137]. In addition, oral vaccination with live attenuated *Salmonella* that simultaneously delivers autoantigens and TGF- β induces tolerogenic DC throughout secondary lymphoid tissues and suppresses autoreactive T cell proliferation [138]. Moreover, delivery of microparticle formulation of RA (retinoic acid) plus TGF- β 1 with the presence of islet autoantigen on the surface could induce tolerogenic DCs in PLNs, thereby preventing the progression of mid-stage autoimmunity to overt T1D [139]. Zymosan, the immunoregulatory adjuvant, bolsters the generation of tolerogenic DC subset via binding to TLR2 and Dectin1. Injection of NOD mice with β cell autoantigen and zymosan protects against T1D by facilitating the production of antigen-specific PLN Treg cells [140]. Moreover, intra-lymphatic administration of GAD-alum together with oral intake of vitamin D results in partial T1D remission in human patients, an effect ascribed to the elevated IL-10 secretion and reduced CD8⁺ T cell activation [141]. Autoantigen vaccination combined with nanotechnology and other immunoregulatory agents, therefore, represents a promising direction in the field of T1D treatment.

Conclusions and perspectives

Finally, we conclude that PLNs serve as a pivotal hub linking various pathogenic inputs to islet β cell autoimmune damage. T1D intervention can be achieved by reducing pathogenic inputs/outputs and restoring the immune tolerant microenvironment of PLNs. Immunotherapies based on cell adoptive transfer, autoantigen vaccination, or chemical compounds should be combined

with other therapeutic approaches, including probiotics that enhance gut integrity, β cell-protective agents (GLP-1) and those regulating vascular or lymphatic function. Regarding the dynamic and complex nature of T1D pathogenesis, the corresponding intervention strategy is better to be comprehensive.

To further extend the above-mentioned concept, T1D should be regarded as a systemic disease when organ/tissue communications are considered. Firstly, patients with T1D suffer from subclinical exocrine insufficiency and acinar atrophy although they are not as apparent as endocrine impairment [142]. A high degree of fibrosis is detected in the exocrine part while the precise mechanism is elusive, but suggested to be associated with global pancreatic inflammation, autoimmunity targeting the exocrine pancreas, vascular and neural anomalies, and the putative involvement of pancreatic stellate cells [143, 144]. Pancreatic exocrine function decreases in a majority of young at-risk children and precedes the onset of islet autoimmunity, as indicated by the measurement of exocrine biomarker, fecal elastase-1 (FE-1) [145]. Secondly, except for PLNs, spontaneous anti-insulin germinal centers (GC) are formed throughout lymphoid tissues [146]. Before the clinical onset of T1D, autoreactive T cells accumulate in the bone marrow and can respond to islet-derived antigen stimulation. Adoptively transferred bone marrow autoreactive T cells home back to PLNs and pancreas, which implies the complex systemic recycling of islet autoreactive T cells [147]. Thirdly, T1D is also subjected to neuronal regulation. Vagal nerves project to PLNs and pancreas and impact immune response. Pancreatic nerve electrical stimulation (PNES) retains T/B cells in PLNs and down-regulates the pro-inflammatory reaction to halt T1D progression in diabetic mice [148, 149]. Lastly, lymph node sharing accomplished by co-drainage of pancreas, liver and the upper small intestine (duodenum) has perplexed the regulation of pancreatic autoimmunity at the organismal level [150], and on the other way round, the involvement of PLNs in type 2 diabetes (T2D) associated hepatic/intestinal pathology should not be negated. Collectively, these lines of evidence bring about novel insights and remind a conceptual update on our current understanding of T1D pathogenesis.

Abbreviations

| | |
|-----------|-------------------------------------|
| T1D | Type 1 diabetes |
| LN | Lymph node |
| PLN | Pancreatic draining lymph node |
| DAMP | Damage associated molecular pattern |
| NOD | Non-obese diabetic |
| scRNA-seq | Single-cell RNA sequencing |
| Treg | Regulatory T cell |
| AICD | Activation-induced cell death |
| Teff | Exhaustion of effector T cells |

| | |
|-----------|--|
| TLO | Tertiary lymphoid organ |
| APC | Antigen-presenting cell |
| GC | Germinal center |
| DC | Dendritic cell |
| VEGFR3 | Vascular endothelial growth factors receptor 3 |
| MLDS | Multiple low dose streptozotocin |
| RRV | Rhesus monkey rotavirus |
| KRV | Kilham rat virus |
| pDC | Plasmacytoid DC |
| MHV-68 | Murine gammaherpesvirus-68 |
| DPP4 | Dipeptidyl peptidase IV |
| NOD2 | Nucleotide-binding oligomerization domain containing 2 |
| MAdCAM-1 | Mucosal addressin cell adhesion molecule 1 |
| FDC | Follicular dendritic cell |
| HA | Hyaluronan |
| IAA | Insulin autoantibody |
| DEG | Differentially expressed gene |
| FRC | Fibroblastic reticular cell |
| NOR | Non-obese diabetes-resistant |
| LNSC | Lymph node stromal cell |
| DEAF1 | Deformed epidermal autoregulatory factor 1 |
| PTA | Peripheral tissue antigens |
| Eif4g3 | Eukaryotic translation initiation factor 4 gamma 3 |
| AIRE | Autoimmune regulator |
| DAP12 | DNAX-activating protein of 12 k Da |
| IA-2 | Insulinoma-associated antigen 2 |
| ZnT8 | Zinc transporter 8 |
| MZB | Marginal zone B |
| STZ | Streptozotocin |
| MAdCAM-1 | Mucosal addressin cell adhesion molecule-1 |
| HEVs | High endothelial venules |
| IR | Insulin receptor |
| DN | Double-negative |
| IDO | Indoleamine 2,3-dioxygenase |
| 1,25OH2D3 | 1,25-Dihydroxyvitamin D3 |
| ToIDC | Tolerogenic dendritic cell |
| GLP-1 | Glucagon-like peptide-1 |
| VR1 | Vanilloid receptor 1 |
| RA | Retinoic acid |
| PNES | Pancreatic nerve electrical stimulation |

Acknowledgements

We thank Dr. Shu Zhang (Tongji Hospital, Tongji Medical College) for the kind suggestions on our manuscript.

Author contributions

FS and CL-Y wrote the manuscript; FX-W, TT-Y, JH-L, SJ-R, SZ and WY-L gave us valuable suggestions and made critical revisions. SW-L and CY-W conceptualized and supervised the preparation of this manuscript. All authors contributed to the manuscript and approved the submitted version.

Funding

Our study was supported by the National Key R&D Program of China (2022YFA0806101) from the Ministry of Science and Technology of China, the National Natural Science Foundation of China (82130023, 81920108009, 82100892, 82070808, 81873656, 82100823, 82100931, 91749207, 81770823, 82200923, 82270885 and 81800068), Department of Science and Technology of Hubei Province Program project (2020DCD014), the Postdoctoral Science Foundation of China (54000-0106540081 and 54000-0106540080), Hubei Health Committee Program (WJ2021ZH0002), the Integrated Innovative Team for Major Human Diseases Program of Tongji Medical College, Huazhong University of Science and Technology, and the Innovative Funding for Translational Research from Tongji Hospital.

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 8 March 2023 Accepted: 21 August 2023

Published online: 28 August 2023

References

- Bottazzo GF, et al. In situ characterization of autoimmune phenomena and expression of HLA molecules in the pancreas in diabetic insulinitis. *N Engl J Med*. 1985;313(6):353–60. <https://doi.org/10.1056/NEJM198508083130604>.
- Gepts W. Islet changes suggesting a possible immune aetiology of human diabetes mellitus. *Acta Endocrinol Suppl (Copenh)*. 1976;205:95–106.
- Roep BO. The role of T-cells in the pathogenesis of type 1 diabetes: from cause to cure. *Diabetologia*. 2003;46(3):305–21. <https://doi.org/10.1007/s00125-003-1089-5>.
- Yue T, et al. The AHR signaling attenuates autoimmune responses during the development of type 1 diabetes. *Front Immunol*. 2020;11:1510. <https://doi.org/10.3389/fimmu.2020.01510>.
- Li Y, et al. Revisiting the antigen-presenting function of beta cells in T1D pathogenesis. *Front Immunol*. 2021;12: 690783. <https://doi.org/10.3389/fimmu.2021.690783>.
- Dooley J, et al. Genetic predisposition for beta cell fragility underlies type 1 and type 2 diabetes. *Nat Genet*. 2016;48(5):519–27. <https://doi.org/10.1038/ng.3531>.
- Yue T, et al. MBD2 acts as a repressor to maintain the homeostasis of the Th1 program in type 1 diabetes by regulating the STAT1-IFN-gamma axis. *Cell Death Differ*. 2022;29(1):218–29. <https://doi.org/10.1038/s41418-021-00852-6>.
- Rewers M, Ludvigsson J. Environmental risk factors for type 1 diabetes. *Lancet*. 2016;387(10035):2340–8. [https://doi.org/10.1016/S0140-6736\(16\)30507-4](https://doi.org/10.1016/S0140-6736(16)30507-4).
- Bluestone JA, Herold K, Eisenbarth G. Genetics, pathogenesis and clinical interventions in type 1 diabetes. *Nature*. 2010;464(7293):1293–300. <https://doi.org/10.1038/nature08933>.
- Pugliese A. Autoreactive T cells in type 1 diabetes. *J Clin Invest*. 2017;127(8):2881–91. <https://doi.org/10.1172/JCI94549>.
- Kim TK, Lee MS. Innate immune receptors in type 1 diabetes: the relationship to cell death-associated inflammation. *Biochem Soc Trans*. 2020;48(3):1213–25. <https://doi.org/10.1042/BST20200131>.
- Sorini C, et al. Loss of gut barrier integrity triggers activation of islet-reactive T cells and autoimmune diabetes. *Proc Natl Acad Sci USA*. 2019;116(30):15140–9. <https://doi.org/10.1073/pnas.1814558116>.
- Fenneman AC, et al. Gut microbiota and metabolites in the pathogenesis of endocrine disease. *Biochem Soc Trans*. 2020;48(3):915–31. <https://doi.org/10.1042/BST20190686>.
- Jean-Baptiste VSE, et al. Type 1 diabetes and type 1 interferonopathies: localization of a type 1 common thread of virus infection in the pancreas. *EBioMedicine*. 2017;22:10–7. <https://doi.org/10.1016/j.ebiom.2017.06.014>.
- Principi N, et al. Type 1 diabetes and viral infections: what is the relationship? *J Clin Virol*. 2017;96:26–31. <https://doi.org/10.1016/j.jcv.2017.09.003>.
- Hanna SJ, et al. Insights from single cell RNA sequencing into the immunology of type 1 diabetes-cell phenotypes and antigen specificity. *Front Immunol*. 2021;12: 751701. <https://doi.org/10.3389/fimmu.2021.751701>.
- Gagnerault MC, et al. Pancreatic lymph nodes are required for priming of beta cell reactive T cells in NOD mice. *J Exp Med*. 2002;196(3):369–77. <https://doi.org/10.1084/jem.20011353>.
- Xu Q, et al. Single-cell RNA transcriptome reveals the intra-tumoral heterogeneity and regulators underlying tumor progression in metastatic pancreatic ductal adenocarcinoma. *Cell Death Discov*. 2021;7(1):331. <https://doi.org/10.1038/s41420-021-00663-1>.
- Fasolino M, et al. Single-cell multi-omics analysis of human pancreatic islets reveals novel cellular states in type 1 diabetes. *Nat Metab*. 2022;4(2):284–99. <https://doi.org/10.1038/s42255-022-00531-x>.
- Osum KC, et al. Interferon-gamma drives programmed death-ligand 1 expression on islet beta cells to limit T cell function during autoimmune diabetes. *Sci Rep*. 2018;8(1):8295. <https://doi.org/10.1038/s41598-018-26471-9>.
- Warshauer JT, et al. A human mutation in STAT3 promotes type 1 diabetes through a defect in CD8+ T cell tolerance. *J Exp Med*. 2021;218(8): e20210759. <https://doi.org/10.1084/jem.20210759>.
- Wiedeman AE, et al. Autoreactive CD8+ T cell exhaustion distinguishes subjects with slow type 1 diabetes progression. *J Clin Invest*. 2020;130(1):480–90. <https://doi.org/10.1172/JCI126595>.
- Smeets S, et al. Insulinitis and lymphoid structures in the islets of Langerhans of a 66-year-old patient with long-standing type 1 diabetes. *Virchows Arch*. 2021;478(6):1209–14. <https://doi.org/10.1007/s00428-020-02915-4>.
- Penaranda C, et al. Prevention of diabetes by FTY720-mediated stabilization of peri-islet tertiary lymphoid organs. *Diabetologia*. 2010;59(6):1461–8. <https://doi.org/10.2337/db09-1129>.
- Lee Y, et al. Recruitment and activation of naive T cells in the islets by lymphotoxin beta receptor-dependent tertiary lymphoid structure. *Immunity*. 2006;25(3):499–509. <https://doi.org/10.1016/j.immuni.2006.06.016>.
- Korpos E, et al. Identification and characterisation of tertiary lymphoid organs in human type 1 diabetes. *Diabetologia*. 2021;64(7):1626–41. <https://doi.org/10.1007/s00125-021-05453-z>.
- Pearl-Yafe M, et al. Does physiological beta cell turnover initiate autoimmune diabetes in the regional lymph nodes? *Autoimmun Rev*. 2006;5(5):338–43. <https://doi.org/10.1016/j.autrev.2006.02.005>.
- Wan X, et al. Pancreatic islets communicate with lymphoid tissues via exocytosis of insulin peptides. *Nature*. 2018;560(7716):107–11. <https://doi.org/10.1038/s41586-018-0341-6>.
- Zirpel H, Roep BO. Islet-resident dendritic cells and macrophages in type 1 diabetes: in search of Bigfoot's print. *Front Endocrinol*. 2021;12: 666795. <https://doi.org/10.3389/fendo.2021.666795>.
- Peng R, et al. Defective maturation of myeloid dendritic cell (DC) in NOD mice is controlled by IDD10/17/18. *Ann NY Acad Sci*. 2003;1005:184–6. <https://doi.org/10.1196/annals.1288.023>.
- Welzen-Coppens JM, et al. Reduced numbers of dendritic cells with a tolerogenic phenotype in the prediabetic pancreas of NOD mice. *J Leukoc Biol*. 2012;92(6):1207–13. <https://doi.org/10.1189/jlb.0312168>.
- Yin N, et al. Functional specialization of islet dendritic cell subsets. *J Immunol*. 2012;188(10):4921–30. <https://doi.org/10.4049/jimmunol.1103725>.
- Turley S, et al. 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(10):1527–37. <https://doi.org/10.1084/jem.20030966>.
- Ferris ST, et al. A minor subset of Batf3-dependent antigen-presenting cells in islets of Langerhans is essential for the development of autoimmune diabetes. *Immunity*. 2014;41(4):657–69. <https://doi.org/10.1016/j.immuni.2014.09.012>.
- Melli K, et al. Amplification of autoimmune response through induction of dendritic cell maturation in inflamed tissues. *J Immunol*. 2009;182(5):2590–600. <https://doi.org/10.4049/jimmunol.0803543>.
- Silveira PA, et al. The preferential ability of B lymphocytes to act as diabetogenic APC in NOD mice depends on expression of self-antigen-specific immunoglobulin receptors. *Eur J Immunol*. 2002;32(12):3657–66. [https://doi.org/10.1002/1521-4141\(200212\)32:12%3c3657::AID-IMMU3657%3e3.0.CO;2-E](https://doi.org/10.1002/1521-4141(200212)32:12%3c3657::AID-IMMU3657%3e3.0.CO;2-E).

37. Noorchashm H, et al. B-cells are required for the initiation of insulinitis and sialitis in nonobese diabetic mice. *Diabetes*. 1997;46(6):941–6. <https://doi.org/10.2337/diab.46.6.941>.
38. Haase C, et al. Local activation of dendritic cells leads to insulinitis and development of insulin-dependent diabetes in transgenic mice expressing CD154 on the pancreatic beta-cells. *Diabetes*. 2004;53(10):2588–95. <https://doi.org/10.2337/diabetes.53.10.2588>.
39. Yin N, et al. Lymphangiogenesis is required for pancreatic islet inflammation and diabetes. *PLoS ONE*. 2011;6(11): e28023. <https://doi.org/10.1371/journal.pone.0028023>.
40. Hirono S, et al. Identification of the lymphatic drainage pathways from the pancreatic head guided by indocyanine green fluorescence imaging during pancreaticoduodenectomy. *Dig Surg*. 2012;29(2):132–9. <https://doi.org/10.1159/000337306>.
41. Cesmebasi A, et al. The surgical anatomy of the lymphatic system of the pancreas. *Clin Anat*. 2015;28(4):527–37. <https://doi.org/10.1002/ca.22461>.
42. Pane JA, et al. Rotavirus acceleration of murine type 1 diabetes is associated with a T helper 1-dependent specific serum antibody response and virus effects in regional lymph nodes. *Diabetologia*. 2013;56(3):573–82. <https://doi.org/10.1007/s00125-012-2798-4>.
43. Pane JA, et al. Rotavirus acceleration of murine type 1 diabetes is associated with increased MHC class I-restricted antigen presentation by B cells and elevated proinflammatory cytokine expression by T cells. *Virus Res*. 2014;179:73–84. <https://doi.org/10.1016/j.virusres.2013.11.009>.
44. Brown DW, Welsh RM, Like AA. Infection of peripancreatic lymph nodes but not islets precedes Kilham rat virus-induced diabetes in BB/Wor rats. *J Virol*. 1993;67(10):5873–8. <https://doi.org/10.1128/JVI.67.10.5873-5878.1993>.
45. Wolter TR, et al. DNA microarray analysis for the identification of innate immune pathways implicated in virus-induced autoimmune diabetes. *Clin Immunol*. 2009;132(1):103–15. <https://doi.org/10.1016/j.clim.2009.02.007>.
46. Smith KA, Efstathiou S, Cooke A. Murine gammaherpesvirus-68 infection alters self-antigen presentation and type 1 diabetes onset in NOD mice. *J Immunol*. 2007;179(11):7325–33. <https://doi.org/10.4049/jimmunol.179.11.7325>.
47. Turley SJ, et al. Endocrine self and gut non-self intersect in the pancreatic lymph nodes. *Proc Natl Acad Sci USA*. 2005;102(49):17729–33. <https://doi.org/10.1073/pnas.0509006102>.
48. Barbeau WE, Bassaganya-Riera J, Hontecillas R. Putting the pieces of the puzzle together—a series of hypotheses on the etiology and pathogenesis of type 1 diabetes. *Med Hypotheses*. 2007;68(3):607–19. <https://doi.org/10.1016/j.mehy.2006.07.052>.
49. Girdhar K, et al. A gut microbial peptide and molecular mimicry in the pathogenesis of type 1 diabetes. *Proc Natl Acad Sci USA*. 2022;119(31): e2120028119. <https://doi.org/10.1073/pnas.2120028119>.
50. Ho J, et al. Effect of prebiotic on microbiota, intestinal permeability, and glycaemic control in children with type 1 diabetes. *J Clin Endocrinol Metab*. 2019;104(10):4427–40. <https://doi.org/10.1210/je.2019-00481>.
51. Vatanen T, et al. The human gut microbiome in early-onset type 1 diabetes from the TEDDY study. *Nature*. 2018;562(7728):589–94. <https://doi.org/10.1038/s41586-018-0620-2>.
52. Costa FR, et al. Gut microbiota translocation to the pancreatic lymph nodes triggers NOD2 activation and contributes to T1D onset. *J Exp Med*. 2016;213(7):1223–39. <https://doi.org/10.1084/jem.20150744>.
53. Yuan X, et al. Functional and metabolic alterations of gut microbiota in children with new-onset type 1 diabetes. *Nat Commun*. 2022;13(1):6356. <https://doi.org/10.1038/s41467-022-33656-4>.
54. de Groot P, et al. Faecal microbiota transplantation halts progression of human new-onset type 1 diabetes in a randomised controlled trial. *Gut*. 2021;70(1):92–105. <https://doi.org/10.1136/gutjnl-2020-322630>.
55. Del Chierico F, et al. Pathophysiology of type 1 diabetes and gut microbiota role. *Int J Mol Sci*. 2022;23(23):14650. <https://doi.org/10.3390/ijms232314650>.
56. Jaakkola I, Jalkanen S, Hanninen A. Diabetogenic T cells are primed both in pancreatic and gut-associated lymph nodes in NOD mice. *Eur J Immunol*. 2003;33(12):3255–64. <https://doi.org/10.1002/eji.200324405>.
57. Xu B, Cook RE, Michie SA. Alpha4beta7 integrin/MAdCAM-1 adhesion pathway is crucial for B cell migration into pancreatic lymph nodes in nonobese diabetic mice. *J Autoimmun*. 2010;35(2):124–9. <https://doi.org/10.1016/j.jaut.2010.04.002>.
58. Fabien N, et al. Pancreatic lymph nodes are early targets of T cells during adoptive transfer of diabetes in NOD mice. *J Autoimmun*. 1995;8(3):323–34. <https://doi.org/10.1006/jaut.1994.0025>.
59. Hoglund P, et al. Initiation of autoimmune diabetes by developmentally regulated presentation of islet cell antigens in the pancreatic lymph nodes. *J Exp Med*. 1999;189(2):331–9. <https://doi.org/10.1084/jem.189.2.331>.
60. Willcox A, et al. Germinal centre frequency is decreased in pancreatic lymph nodes from individuals with recent-onset type 1 diabetes. *Diabetologia*. 2017;60(7):1294–303. <https://doi.org/10.1007/s00125-017-4221-7>.
61. Bogdani M, et al. Hyaluronan and hyaluronan-binding proteins accumulate in both human type 1 diabetic islets and lymphoid tissues and associate with inflammatory cells in insulinitis. *Diabetes*. 2014;63(8):2727–43. <https://doi.org/10.2337/db13-1658>.
62. Regnault B, et al. Early over expression of messenger RNA for multiple genes, including insulin, in the pancreatic lymph nodes of NOD mice is associated with islet autoimmunity. *BMC Med Genom*. 2009;2:63. <https://doi.org/10.1186/1755-8794-2-63>.
63. Gonzalez Badillo F, et al. Tissue-engineered stromal reticula to study lymph node fibroblastic reticular cells in type 1 diabetes. *Cell Mol Biotech*. 2020;13(5):419–34. <https://doi.org/10.1007/s12195-020-00627-y>.
64. Postigo-Fernandez J, Farber DL, Creusot RJ. Phenotypic alterations in pancreatic lymph node stromal cells from human donors with type 1 diabetes and NOD mice. *Diabetologia*. 2019;62(11):2040–51. <https://doi.org/10.1007/s00125-019-04984-w>.
65. Yip L, et al. Inflammation and hyperglycemia mediate Deaf1 splicing in the pancreatic lymph nodes via distinct pathways during type 1 diabetes. *Diabetes*. 2015;64(2):604–17. <https://doi.org/10.2337/db14-0803>.
66. Yip L, et al. Reduced DEAF1 function during type 1 diabetes inhibits translation in lymph node stromal cells by suppressing Eif4g3. *J Mol Cell Biol*. 2013;5(2):99–110. <https://doi.org/10.1093/jmcb/mjs052>.
67. Yip L, et al. Deaf1 isoforms control the expression of genes encoding peripheral tissue antigens in the pancreatic lymph nodes during type 1 diabetes. *Nat Immunol*. 2009;10(9):1026–33. <https://doi.org/10.1038/ni.1773>.
68. Gardner JM, Anderson MS. The sickness unto deaf. *Nat Immunol*. 2009;10(9):934–6. <https://doi.org/10.1038/ni0909-934>.
69. Fuhlbrigge R, Yip L. Self-antigen expression in the peripheral immune system: roles in self-tolerance and type 1 diabetes pathogenesis. *Curr Diab Rep*. 2014;14(9):525. <https://doi.org/10.1007/s11892-014-0525-x>.
70. Clare-Salzler M, Mullen Y. Marked dendritic cell-T cell cluster formation in the pancreatic lymph node of the non-obese diabetic mouse. *Immunology*. 1992;76(3):478–84.
71. Hall HT, et al. Increased diabetes development and decreased function of CD4+CD25+ Treg in the absence of a functional DAP12 adaptor protein. *Eur J Immunol*. 2008;38(11):3191–9. <https://doi.org/10.1002/eji.200838259>.
72. Bloem SJ, Roep BO. The elusive role of B lymphocytes and islet autoantibodies in (human) type 1 diabetes. *Diabetologia*. 2017;60(7):1185–9. <https://doi.org/10.1007/s00125-017-4284-5>.
73. Catani M, et al. Isolation of human monoclonal autoantibodies derived from pancreatic lymph node and peripheral blood B cells of islet autoantibody-positive patients. *Diabetologia*. 2016;59(2):294–8. <https://doi.org/10.1007/s00125-015-3792-4>.
74. Marino E, et al. Marginal-zone B-cells of nonobese diabetic mice expand with diabetes onset, invade the pancreatic lymph nodes, and present autoantigen to diabetogenic T-cells. *Diabetes*. 2008;57(2):395–404. <https://doi.org/10.2337/db07-0589>.
75. Kim H, et al. Targeting transcriptional coregulator OCA-B/Pou2f1 blocks activated autoreactive T cells in the pancreas and type 1 diabetes. *J Exp Med*. 2021;218(3): e20200533. <https://doi.org/10.1084/jem.20200533>.
76. Hoyne GF, et al. Visualizing the role of Cbl-b in control of islet-reactive CD4 T cells and susceptibility to type 1 diabetes. *J Immunol*. 2011;186(4):2024–32. <https://doi.org/10.4049/jimmunol.1002296>.
77. Marrero I, et al. High-throughput sequencing reveals restricted TCR Vbeta usage and public TCRbeta clonotypes among pancreatic lymph node memory CD4(+) T cells and their involvement in

- autoimmune diabetes. *Mol Immunol*. 2016;74:82–95. <https://doi.org/10.1016/j.molimm.2016.04.013>.
78. Kent SC, et al. Expanded T cells from pancreatic lymph nodes of type 1 diabetic subjects recognize an insulin epitope. *Nature*. 2005;435(7039):224–8. <https://doi.org/10.1038/nature03625>.
 79. Marrero I, et al. T cell populations in the pancreatic lymph node naturally and consistently expand and contract in NOD mice as disease progresses. *Mol Immunol*. 2012;52(1):9–18. <https://doi.org/10.1016/j.molimm.2012.04.004>.
 80. Liu B, et al. A hybrid insulin epitope maintains high 2D affinity for diabetogenic T cells in the periphery. *Diabetes*. 2020;69(3):381–91. <https://doi.org/10.2337/db19-0399>.
 81. Seay HR, et al. Tissue distribution and clonal diversity of the T and B cell repertoire in type 1 diabetes. *JCI Insight*. 2016;1(20): e88242. <https://doi.org/10.1172/jci.insight.88242>.
 82. Suri A, et al. First signature of islet beta-cell-derived naturally processed peptides selected by diabetogenic class II MHC molecules. *J Immunol*. 2008;180(6):3849–56. <https://doi.org/10.4049/jimmunol.180.6.3849>.
 83. Tsai S, et al. Dendritic cell-dependent in vivo generation of autoregulatory T cells by anti-diabetogenic MHC class II. *J Immunol*. 2013;191(1):70–82. <https://doi.org/10.4049/jimmunol.1300168>.
 84. Bending D, et al. Highly purified Th17 cells from BDC2.5NOD mice convert into Th1-like cells in NOD/SCID recipient mice. *J Clin Invest*. 2009;119(3):565–72. <https://doi.org/10.1172/JCI37865>.
 85. Mintern JD, et al. Constitutive, but not inflammatory, cross-presentation is disabled in the pancreas of young mice. *Eur J Immunol*. 2002;32(4):1044–51. [https://doi.org/10.1002/1521-4141\(200204\)32:4%3c1044::AID-IMMU1044%3e3.0.CO;2-B](https://doi.org/10.1002/1521-4141(200204)32:4%3c1044::AID-IMMU1044%3e3.0.CO;2-B).
 86. Zhang Y, et al. TLR9 blockade inhibits activation of diabetogenic CD8+ T cells and delays autoimmune diabetes. *J Immunol*. 2010;184(10):5645–53. <https://doi.org/10.4049/jimmunol.0901814>.
 87. Kurts C, et al. Class I-restricted cross-presentation of exogenous self-antigens leads to deletion of autoreactive CD8(+) T cells. *J Exp Med*. 1997;186(2):239–45. <https://doi.org/10.1084/jem.186.2.239>.
 88. Hernandez J, et al. Phenotypic and functional analysis of CD8(+) T cells undergoing peripheral deletion in response to cross-presentation of self-antigen. *J Exp Med*. 2001;194(6):707–17. <https://doi.org/10.1084/jem.194.6.707>.
 89. Kurts C, et al. CD4+ T cell help impairs CD8+ T cell deletion induced by cross-presentation of self-antigens and favors autoimmunity. *J Exp Med*. 1997;186(12):2057–62. <https://doi.org/10.1084/jem.186.12.2057>.
 90. Grinberg-Bleyer Y, et al. Pathogenic T cells have a paradoxical protective effect in murine autoimmune diabetes by boosting Tregs. *J Clin Invest*. 2010;120(12):4558–68. <https://doi.org/10.1172/JCI42945>.
 91. Nti BK, et al. Treg cells in pancreatic lymph nodes: the possible role in diabetogenesis and beta cell regeneration in a T1D model. *Cell Mol Immunol*. 2012;9(6):455–63. <https://doi.org/10.1038/cmi.2012.36>.
 92. Vecchione A, et al. Reduced follicular regulatory T cells in spleen and pancreatic lymph nodes of patients with type 1 diabetes. *Diabetes*. 2021;70(12):2892–902. <https://doi.org/10.2337/db21-0091>.
 93. Mattner J, et al. Genetic and functional data identifying Cd101 as a type 1 diabetes (T1D) susceptibility gene in nonobese diabetic (NOD) mice. *PLoS Genet*. 2019;15(6): e1008178. <https://doi.org/10.1371/journal.pgen.1008178>.
 94. Ferraro A, et al. Expansion of Th17 cells and functional defects in T regulatory cells are key features of the pancreatic lymph nodes in patients with type 1 diabetes. *Diabetes*. 2011;60(11):2903–13. <https://doi.org/10.2337/db11-0090>.
 95. Green EA, et al. CD4+CD25+ T regulatory cells control anti-islet CD8+ T cells through TGF-beta-TGF-beta receptor interactions in type 1 diabetes. *Proc Natl Acad Sci USA*. 2003;100(19):10878–83. <https://doi.org/10.1073/pnas.1834400100>.
 96. Clough LE, et al. Release from regulatory T cell-mediated suppression during the onset of tissue-specific autoimmunity is associated with elevated IL-21. *J Immunol*. 2008;180(8):5393–401. <https://doi.org/10.4049/jimmunol.180.8.5393>.
 97. Van Belle TL, et al. Interleukin-21 receptor-mediated signals control autoreactive T cell infiltration in pancreatic islets. *Immunity*. 2012;36(6):1060–72. <https://doi.org/10.1016/j.immuni.2012.04.005>.
 98. Carlos D, et al. Mast cells control insulinitis and increase Treg cells to confer protection against STZ-induced type 1 diabetes in mice. *Eur J Immunol*. 2015;45(10):2873–85. <https://doi.org/10.1002/eji.201545498>.
 99. Chen YG, et al. Activated NKT cells inhibit autoimmune diabetes through tolerogenic recruitment of dendritic cells to pancreatic lymph nodes. *J Immunol*. 2005;174(3):1196–204. <https://doi.org/10.4049/jimmunol.174.3.1196>.
 100. Kent SC, et al. Loss of IL-4 secretion from human type 1a diabetic pancreatic draining lymph node NKT cells. *J Immunol*. 2005;175(7):4458–64. <https://doi.org/10.4049/jimmunol.175.7.4458>.
 101. Waldner H, et al. The autoimmune diabetes locus Idd9 regulates development of type 1 diabetes by affecting the homing of islet-specific T cells. *J Immunol*. 2006;176(9):5455–62. <https://doi.org/10.4049/jimmunol.176.9.5455>.
 102. Whitener RL, et al. The type 1 diabetes-resistance locus Idd22 controls trafficking of autoreactive CTLs into the pancreatic islets of NOD mice. *J Immunol*. 2017;199(12):3991–4000. <https://doi.org/10.4049/jimmunol.1602037>.
 103. St Clair JR, et al. Contrast-enhanced ultrasound measurement of pancreatic blood flow dynamics predicts type 1 diabetes progression in preclinical models. *Nat Commun*. 2018;9(1):1742. <https://doi.org/10.1038/s41467-018-03953-y>.
 104. Aspod C, Rome S, Thivolet C. Early events in islets and pancreatic lymph nodes in autoimmune diabetes. *J Autoimmun*. 2004;23(1):27–35. <https://doi.org/10.1016/j.jaut.2004.03.007>.
 105. Villalta SA, et al. Inhibition of VEGFR-2 reverses type 1 diabetes in NOD mice by abrogating insulinitis and restoring islet function. *Diabetes*. 2013;62(8):2870–8. <https://doi.org/10.2337/db12-1619>.
 106. Sandor AM, Jacobelli J, Friedman RS. Immune cell trafficking to the islets during type 1 diabetes. *Clin Exp Immunol*. 2019;198(3):314–25. <https://doi.org/10.1111/cei.13353>.
 107. Hanninen A, et al. Mucosa-associated (beta 7-integrinhigh) lymphocytes accumulate early in the pancreas of NOD mice and show aberrant recirculation behavior. *Diabetes*. 1996;45(9):1173–80. <https://doi.org/10.2337/diab.45.9.1173>.
 108. Shan Z, et al. CCR7 directs the recruitment of T cells into inflamed pancreatic islets of nonobese diabetic (NOD) mice. *Immunol Res*. 2014;58(2–3):351–7. <https://doi.org/10.1007/s12026-014-8500-9>.
 109. Sandor AM, et al. CD11c(+) cells are gatekeepers for lymphocyte trafficking to infiltrated islets during type 1 diabetes. *Front Immunol*. 2019;10:99. <https://doi.org/10.3389/fimmu.2019.00099>.
 110. Nandedkar-Kulkarni N, et al. Insulin receptor-expressing T cells appear in individuals at risk for type 1 diabetes and can move into the pancreas in C57BL/6 transgenic mice. *J Immunol*. 2021;206(7):1443–53. <https://doi.org/10.4049/jimmunol.1900357>.
 111. Graham KL, et al. Autoreactive cytotoxic T lymphocytes acquire higher expression of cytotoxic effector markers in the islets of NOD mice after priming in pancreatic lymph nodes. *Am J Pathol*. 2011;178(6):2716–25. <https://doi.org/10.1016/j.ajpath.2011.02.015>.
 112. Diggins KE, et al. Exhausted-like CD8+ T cell phenotypes linked to C-peptide preservation in alefacept-treated T1D subjects. *JCI Insight*. 2021;6(3): e142680. <https://doi.org/10.1172/jci.insight.142680>.
 113. Gearty SV, et al. An autoimmune stem-like CD8 T cell population drives type 1 diabetes. *Nature*. 2022;602(7895):156–61. <https://doi.org/10.1038/s41586-021-04248-x>.
 114. Abdelsamed HA, et al. Beta cell-specific CD8(+) T cells maintain stem cell memory-associated epigenetic programs during type 1 diabetes. *Nat Immunol*. 2020;21(5):578–87. <https://doi.org/10.1038/s41590-020-0633-5>.
 115. Schenkel JM, et al. Conventional type I dendritic cells maintain a reservoir of proliferative tumor-antigen specific TCF-1(+) CD8(+) T cells in tumor-draining lymph nodes. *Immunity*. 2021;54(10):2338–2353.e2336. <https://doi.org/10.1016/j.immuni.2021.08.026>.
 116. Zhang ZL, et al. Lymphocyte subsets in thymus and peripheral lymphoid tissues of aging and diabetic NOD mice. *Autoimmunity*. 1994;17(1):41–8. <https://doi.org/10.3109/08916939409014657>.
 117. Kornete M, et al. Th1-like ICOS+ Foxp3+ Treg cells preferentially express CXCR3 and home to beta-islets during pre-diabetes in BDC2.5 NOD mice. *PLoS ONE*. 2015;10(5): e0126311. <https://doi.org/10.1371/journal.pone.0126311>.

118. Chen Z, et al. Where CD4+CD25+ T reg cells impinge on autoimmune diabetes. *J Exp Med*. 2005;202(10):1387–97. <https://doi.org/10.1084/jem.20051409>.
119. Green EA, Choi Y, Flavell RA. Pancreatic lymph node-derived CD4(+) CD25(+) Treg cells: highly potent regulators of diabetes that require TRANCE-RANK signals. *Immunity*. 2002;16(2):183–91. [https://doi.org/10.1016/s1074-7613\(02\)00279-0](https://doi.org/10.1016/s1074-7613(02)00279-0).
120. Sebastiani G, et al. Regulatory T-cells from pancreatic lymphnodes of patients with type-1 diabetes express increased levels of microRNA miR-125a-5p that limits CCR2 expression. *Sci Rep*. 2017;7(1):6897. <https://doi.org/10.1038/s41598-017-07172-1>.
121. Jacob N, et al. Butyrate induced Tregs are capable of migration from the GALT to the pancreas to restore immunological tolerance during type-1 diabetes. *Sci Rep*. 2020;10(1):19120. <https://doi.org/10.1038/s41598-020-76109-y>.
122. Tonkin DR, Haskins K. Regulatory T cells enter the pancreas during suppression of type 1 diabetes and inhibit effector T cells and macrophages in a TGF-beta-dependent manner. *Eur J Immunol*. 2009;39(5):1313–22. <https://doi.org/10.1002/eji.200838916>.
123. Liu T, et al. Combination of double negative T cells and anti-thymocyte serum reverses type 1 diabetes in NOD mice. *J Transl Med*. 2016;14:57. <https://doi.org/10.1186/s12967-016-0815-y>.
124. Jalili RB, et al. Fibroblast cell-based therapy for experimental autoimmune diabetes. *PLoS ONE*. 2016;11(1): e0146970. <https://doi.org/10.1371/journal.pone.0146970>.
125. Creusot RJ, et al. Lymphoid-tissue-specific homing of bone-marrow-derived dendritic cells. *Blood*. 2009;113(26):6638–47. <https://doi.org/10.1182/blood-2009-02-204321>.
126. Engman C, et al. Generation of antigen-specific Foxp3+ regulatory T-cells in vivo following administration of diabetes-reversing tolerogenic microspheres does not require provision of antigen in the formulation. *Clin Immunol*. 2015;160(1):103–23. <https://doi.org/10.1016/j.clim.2015.03.004>.
127. Creusot RJ, et al. Tissue-targeted therapy of autoimmune diabetes using dendritic cells transduced to express IL-4 in NOD mice. *Clin Immunol*. 2008;127(2):176–87. <https://doi.org/10.1016/j.clim.2007.12.009>.
128. Ferreira GB, et al. 1,25-Dihydroxyvitamin D3 promotes tolerogenic dendritic cells with functional migratory properties in NOD mice. *J Immunol*. 2014;192(9):4210–20. <https://doi.org/10.4049/jimmunol.1302350>.
129. Yossipof TE, et al. Tellurium compounds prevent and reverse type-1 diabetes in NOD mice by modulating alpha4beta7 integrin activity, IL-1beta, and T regulatory cells. *Front Immunol*. 2019;10:979. <https://doi.org/10.3389/fimmu.2019.00979>.
130. Jorns A, et al. Diabetes prevention by immunomodulatory FTY720 treatment in the LEW.1A1R1-iddm rat despite immune cell activation. *Endocrinology*. 2010;151(8):3555–65. <https://doi.org/10.1210/en.2010-0202>.
131. You S, et al. Therapeutic use of a selective S1P1 receptor modulator ponesimod in autoimmune diabetes. *PLoS ONE*. 2013;8(10): e77296. <https://doi.org/10.1371/journal.pone.0077296>.
132. Chang CL, et al. Cytopyloine, a polyacetylenic glucoside, prevents type 1 diabetes in nonobese diabetic mice. *J Immunol*. 2007;178(11):6984–93. <https://doi.org/10.4049/jimmunol.178.11.6984>.
133. Kerkvliet NI, et al. Activation of aryl hydrocarbon receptor by TCDD prevents diabetes in NOD mice and increases Foxp3+ T cells in pancreatic lymph nodes. *Immunotherapy*. 2009;1(4):539–47. <https://doi.org/10.2217/imt.09.24>.
134. Tian B, et al. Upregulating CD4+CD25+FOXP3+ regulatory T cells in pancreatic lymph nodes in diabetic NOD mice by adjuvant immunotherapy. *Transplantation*. 2009;87(2):198–206. <https://doi.org/10.1097/TP.0b013e3181933261>.
135. Subramanian L, et al. NKT cells stimulated by long fatty acyl chain sulfatides significantly reduce the incidence of type 1 diabetes in nonobese diabetic mice [corrected]. *PLoS ONE*. 2012;7(5): e37771. <https://doi.org/10.1371/journal.pone.0037771>.
136. Nevius E, Srivastava PK, Basu S. Oral ingestion of Capsaicin, the pungent component of chili pepper, enhances a discreet population of macrophages and confers protection from autoimmune diabetes. *Mucosal Immunol*. 2012;5(1):76–86. <https://doi.org/10.1038/mi.2011.50>.
137. Ploix C, et al. Protection against autoimmune diabetes with oral insulin is associated with the presence of IL-4 type 2 T-cells in the pancreas and pancreatic lymph nodes. *Diabetes*. 1998;47(1):39–44. <https://doi.org/10.2337/diab.47.1.39>.
138. Mbongue JC, et al. Tracking of an oral salmonella-based vaccine for type 1 diabetes in non-obese diabetic mice. *Front Immunol*. 2020;11:712. <https://doi.org/10.3389/fimmu.2020.00712>.
139. Phillips BE, et al. Arrest in the progression of type 1 diabetes at the mid-stage of insulinitis using an autoantigen-decorated all-trans retinoic acid and transforming growth factor beta-1 single microparticle formulation. *Front Immunol*. 2021;12: 586220. <https://doi.org/10.3389/fimmu.2021.586220>.
140. Karumuthil-Melethil S, et al. TLR2- and Dectin 1-associated innate immune response modulates T-cell response to pancreatic beta-cell antigen and prevents type 1 diabetes. *Diabetes*. 2015;64(4):1341–57. <https://doi.org/10.2337/db14-1145>.
141. Casas R, et al. Glutamic acid decarboxylase injection into lymph nodes: beta cell function and immune responses in recent onset type 1 diabetes patients. *Front Immunol*. 2020;11: 564921. <https://doi.org/10.3389/fimmu.2020.564921>.
142. Campbell-Thompson M, Rodriguez-Calvo T, Battaglia M. Abnormalities of the exocrine pancreas in type 1 diabetes. *Curr Diab Rep*. 2015;15(10):79. <https://doi.org/10.1007/s11892-015-0653-y>.
143. Wright JJ, et al. Decreased pancreatic acinar cell number in type 1 diabetes. *Diabetologia*. 2020;63(7):1418–23. <https://doi.org/10.1007/s00125-020-05155-y>.
144. Alexandre-Heymann L, et al. Structure and function of the exocrine pancreas in patients with type 1 diabetes. *Rev Endocr Metab Disord*. 2019;20(2):129–49. <https://doi.org/10.1007/s11154-019-09501-3>.
145. Penno MAS, et al. Changes in pancreatic exocrine function in young at-risk children followed to islet autoimmunity and type 1 diabetes in the ENDIA study. *Pediatr Diabetes*. 2020;21(6):945–9. <https://doi.org/10.1111/pedi.13056>.
146. Wan X, Thomas JW, Unanue ER. Class-switched anti-insulin antibodies originate from unconventional antigen presentation in multiple lymphoid sites. *J Exp Med*. 2016;213(6):967–78. <https://doi.org/10.1084/jem.20151869>.
147. Li R, et al. Bone marrow is a preferential homing site for autoreactive T-cells in type 1 diabetes. *Diabetes*. 2007;56(9):2251–9. <https://doi.org/10.2337/db07-0502>.
148. Guyot M, et al. Pancreatic nerve electrostimulation inhibits recent-onset autoimmune diabetes. *Nat Biotechnol*. 2019;37(12):1446–51. <https://doi.org/10.1038/s41587-019-0295-8>.
149. Foster TP, et al. Exocrine pancreas dysfunction in type 1 diabetes. *Endocr Pract*. 2020;26(12):1505–13. <https://doi.org/10.4158/EP-2020-0295>.
150. Brown H, et al. Lymph node sharing between pancreas, gut, and liver leads to immune crosstalk and regulation of pancreatic autoimmunity. *Immunity*. 2023. <https://doi.org/10.1016/j.immuni.2023.07.008>.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

