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Narrowing in on the anti-β cell-specific T cells: looking 'where the action is'

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Abstract

Purpose of the review—By necessity, the vast majority of information we have on autoreactive T cells in human type 1 diabetes (T1D) has come from the study of peripheral blood of donors with T1D. It is not clear how representative the peripheral autoreactive T-cell repertoire is of the autoreactive T cells infiltrating the islets in T1D. We will summarize and discuss what is known of the immunohistopathology of insulitis, the T-cell receptor repertoire expressed by islet-infiltrating T cells, and the autoreactivity and function of islet-infiltrating T cells in T1D.

Recent findings—Recovery and analysis of live, islet-infiltrating T cells from the islets of cadaveric donors with T1D revealed a broad repertoire and proinflammatory phenotype of $CD4^+$ T-cell autoreactivity to peptide targets from islet proteins, including proinsulin, as well as $CD4^+$ T-cell reactivity to a number of posttranslationally modified peptides, including peptides with citrullinations and hybrid insulin peptide fusions. Islet-infiltrating $CD8^+$ T cells were also derived and required further isolation and characterization.

Summary—The recovery of live, islet-infiltrating T cells from donors with T1D, reactive with a broad range of known targets and posttranslationally modified peptides, allows for the specific functional analysis of islet-infiltrating T cells for the development of antigen-specific immunotherapies.

Keywords

autoreactivity; human type 1 diabetes; islet-infiltrating T cells

INTRODUCTION

Our understanding of the targets of autoreactive $CD4^+$ and $CD8^+$ T cells in individuals at risk for or with type 1 diabetes (T1D) has come, by necessity, from the study of these cells in peripheral blood. T-cell targets in human T1D for $CD4^+$ T cells include peptides of glutamic acid decarboxylase (GAD65) [1], proinsulin, insulin, islet antigen 2, chromogranin A [2], zinc transporter 8 [3], and others (reviewed in [4,5]). Targets of autoreactive $CD8^+$ T

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Conflicts of interest

There are no conflicts of interest.

cells in the periphery include peptides from (prepro)insulin, islet antigen 2, islet-specific glucose-6-phosphatase catalytic subunit-related protein, GAD65, islet amyloid polypeptide precursor protein (IAPP), and zinc transporter 8 [3–10]. More recently, a new class of autoreactivity has been identified in T1D: autoreactivity to modified autoantigens [11,12▪▪, 13–15,16▪▪,17–19,20▪▪]. Human autoreactive T cells that recognize and respond to epitopes containing posttranslational modifications such as a vicinal disulfide bond in insulin [13], citrullination or deamidation [11,14,15,16▪▪,17–19,20▪▪], or hybrid insulin peptide fusions of proinsulin C-peptide with islet amyloid polypeptide or neuropeptide-Y [12▪▪] have been described and characterized.

It has become clear that islets can remain functional several years after clinical diagnosis of T1D [21]. Insulin-expressing islets are still apparent several years postdiagnosis [22▪▪] and functional auto-immune responses are still detected long after diagnosis [23,24]. The gold standard in preventive and therapeutic strategies for T1D-involving islet replacement or regeneration is to suppress, tolerize, or anergize the autoreactive T-cell response while leaving the immune system intact to respond to foreign antigens (i.e., infections), and to avoid the use of global immunosuppression, with its attendant toxicities. However, it remains an open question if the peripheral autoreactive T-cell repertoire is representative of the islet-infiltrating T-cell repertoire. In this review, we will focus on what is known concerning the phenotype and function of the islet-infiltrating cells in T1D.

KEY POINTS

- Heterogeneous nature of insulitis in human T1D observed in histological studies of pancreata from donors with T1D and in analyses of live, islet-infiltrating T cells.
- Broad repertoire of islet-infiltrating T-cell autoreactivity included recognition of known islet-derived peptides and as well as posttranslationally modified peptides.
- Islet-infiltrating T cells were proinflammatory.

ISLET-INFILTRATING T CELLS: HISTOLOGICAL AND IMMUNOHISTOLOGICAL DETECTION

Cellular infiltrate in the islets in individuals with T1D has been known for decades [25]. The histological study of two major collections, from several decades ago, of pancreata from individuals with T1D has informed investigators of the characteristics of insulitis [26–31] and examination of these valuable tissues continues [32]. The Network of Pancreatic Organ Donors with Diabetes (nPOD, http://www.jdrfnpod.org/) was established to recover, archive, and characterize, and then distribute pancreas and other tissues from donors with T1D to investigators [33,34]. In a meta-analysis of immunohistochemistry studies of pancreata from donors with T1D, insulitis was described as at least 15 $CD45^+$ cells/islet and insulitis as infiltration in less than 10% islets, though greater numbers of islet-infiltrating $CD45^+$ lymphocytes have been detected in some nPOD sample pancreata from donors with T1D

[22■■,35]. It is now accepted that insulitis is heterogeneous; adjacent pancreatic lobes can differ in the presence or absence of insulitis, and infiltrated islets can exist in close proximity to non-infiltrated, normal-appearing islets [36,37■].

In addition to islet-infiltrating T cells, macrophages and B cells have been detected in insulitis [37■,38,39,40■]. Cellular heterogeneity of B cells infiltrating the islets can be correlated with β-cell destruction, insulin-containing islets, and age of onset of T1D [38,40■]. Interestingly, the hyperexpression of Human Leukocyte Antigen (HLA) Class I by pancreatic β cells [26,39,41,42■,43] is associated with T1D, but can be heterogeneous in expression; this was seen as well even in those individuals with serum autoantibody, but without a clinical diagnosis of T1D [44]. However, the role of hyperexpression of HLA class I in T1D pathology remains under investigation.

DETECTION OF SPECIFIC AUTOREACTIVE T CELLS *IN SITU*: CONFIRMATION OF AUTOREACTIVE CD8$^+$ T CELLS IN ISLETS

Although the visualization of T-cell subsets in insulitis in sectioned pancreas has been described for decades, the direct visualization of autoantigen-specific T cells *in situ* has only been accomplished within the last 5 years. In a landmark technical and scientific advance, Coppieters *et al.* [41] used defined autoantigenic peptide-loaded HLA-A2 tetramers [10] to stain pancreas sections from donors with T1D and detected CD8$^+$ T-cell reactivity *in situ* in islets from individuals with T1D (disease duration of up to 8 years), but not in islets from control donors. Both single and multiple CD8$^+$ T-cell autoreactivities were observed within single islets [41]. This is the first direct visualization of autoreactive CD8$^+$ T cells in human insulitis.

T-CELL RECEPTOR REPERTOIRE OF AUTOREACTIVE T CELLS: SIGNS OF OLIGOCLONAL EXPANSION

Autoantigen-driven clonal expansion of T cells is considered a hallmark of the pathophysiology of T1D. Examination of pancreata from donors with T1D for expanded T-cell receptor (TCR) clonotypes has yielded mixed, but encouraging results. Three reports found specific TCR β-chain expansions [45–47] from pancreata from donors with T1D, whereas another study found restricted TCR α-chain usage from the pancreata from eight donors with T1D [48]. These results indicate that multiple T cells autoreactive with the same epitope can be found in pancreata from donors with T1D.

DIRECT RECOVERY OF LIVE ISLET-INFILTRATING T CELLS: A NEW ERA

The technology for islet isolation from human pancreata, especially from juveniles with conditions like pancreatitis [49], has allowed for the isolation of islets from individuals with T1D and the recovery of live islet-infiltrating T cells directly from islets. In a pioneering study, Pathiraja *et al.*[50■■] isolated islets from a male tissue donor who was diagnosed with T1D at the age of 16 years, with a disease duration of 3 years and expressed high-risk HLA alleles (HLA-A*01 : 01, 02 : 01; B*08 : 01, 51 : 01; DRB1*03 : 01, 04 : 04; DQA1*03 : 01, DQB1*03 : 02; DQA1*05 : 01, DQB1*02 : 01). The investigators were able to visualize

islet-infiltrating T cells *ex vivo* from dispersed islets by flow cytometry and generated 53 T-cells clones from these islets. Interestingly, 26% of the clones recognized overlapping peptides in the C-peptide of proinsulin presented in the context of DQ8 and DQ2–DQ8 transdimer (DQ8*trans*) [50▪▪].

Recently, two networks of investigators have recovered the pancreata from multiple tissue donors with T1D. These donors were recovered through the highly collaborative and coordinated efforts of Vanderbilt University and from nPOD via collaborative agreements with the National Disease Research Interchange (NDRI) and the International Institute for the Advancement of Medicine (IIAM). Islets were isolated from these cases and then distributed to a number of β-cell biology and immunology laboratories, including ours. We received islets from nine donors with T1D (disease duration 2–20 years), from seven donors without T1D, and from two donors with type 2 diabetes.

Islets (~500–1000 islet equivalents; IEQ) were received within 2–5 days after donor brain death and were of variable purity (10–80%). Lymphocytic infiltrate in pancreatic acinar tissue has been reported from pancreata such as these [51,52]. Thus, to increase our odds of isolating islet-infiltrating, autoreactive T cells, we handpicked the islets from each donor upon receipt and isolated the T cells by using two compatible methods. The first method utilized flow cytometry and cell sorting to capture the *ex vivo* frequency of islet-infiltrating $CD4^+$ and $CD8^+$ T cells. In total, 100 IEQ were dispersed using a brief enzyme treatment, and then stained with a viability dye and lymphocyte markers (CD45, CD3, CD19, CD4, and CD8) immediately after handpick-ing islets. We chose to apply broad markers for T cells as we wanted to be able to detect and isolate any T cells that had infiltrated the islets. From flow cytometry, an average of 221 ± 471 $CD4^+$ T cells and 155 ± 210 $CD8^+$ T cells were detected (average CD4:CD8 ratio; 1.4 : 1) from the islets from donors with T1D, whereas three $CD4^+$ and 31 $CD8^+$ T cells were detected from islets of only one donor without T1D. $CD4^+$ and $CD8^+$ T cells were also sorted onto irradiated feeders with polyclonal T-cell stimulation and cytokines for *in vitro* expansion.

In the course of handpicking islets, we noted that carryover of cells other than islets was unavoidable in some instances. We then employed a second method to directly visualize cellular growth from individual islets. We plated 100 handpicked IEQ on a gel matrix with polyclonal T-cell stimulation and cytokines for further expansion. Direct cellular outgrowth from individual islets was visible in 5–10 days and we observed outgrowth from an average of 26% of the islets from the donors with T1D, but only 0.6% of the islets from donors without T1D (represented by one donor) or with type 2 diabetes. These results were expected based on studies of pancreata from individuals without T1D [22▪▪]. From the average number of recovered islets from the donors with T1D, we examined 0.56% of these islets.

We isolated a large panel of 236 $CD4^+$ and $CD8^+$ T-cell lines and clones from nine tissue donors with T1D by using these two methods [53▪▪]. In total, 111 of the lines and clones were $CD4^+$ T cells and 23 were $CD8^+$ T cells. We have noted a growth bias of $CD4^+$ T cells over $CD8^+$ T cells. Interestingly, 102 lines grown from individual islets were mixtures of $CD4^+$ and $CD8^+$ T cells. These lines must be sorted to expand $CD4^+$ and $CD8^+$ T-cell lines

separately for further characterization. Only ~0.5% of cells from three pure CD8β$^+$ T-cell lines from the islets of one donor with T1D were reactive with a pool of HLA-A2 pentamers loaded with peptides from insulin B chain, islet antigen 2, and islet-specific glucose-6-phosphatase catalytic subunit-related protein [10]. The remaining CD8β$^+$ T cells in these lines are still uncharacterized, highlighting the need for single cell cloning and further characterization of the CD8$^+$ T-cell lines. Although the information with regard to the autoreactivity of islet-infiltrating CD8$^+$ T cell is sparse to date [41,53▪▪], analysis of these islet-infiltrating CD8$^+$ T cells will yield important information concerning the functional phenotype of these cells.

From the 111 CD4$^+$ T-cell lines and clones from islets of the nine donors with T1D, 50 of these were tested for reactivity against a large panel of peptides previously shown to be recognized by peripheral T cells of individuals with T1D in the context of the risk alleles HLA DRB1*03 : 01, DRB1*04 : 01 or DQB1*03 : 02 (reviewed in [4,5]). Islet-infiltrating CD4$^+$ T cells were shown to have reactivity to peptides from GAD65, proinsulin, islet antigen 2, and reactivity to B-cell lines transduced with the open reading frames for proinsulin or chromogranin A. CD4$^+$ T-cell lines or clones were found to be reactive with posttranslationally modified peptides, including citrullinated peptides from glucose-regulated protein 78 [17] and IAPP. In addition, CD4$^+$ T-cell lines or clones were found to be reactive with hybrid insulin peptides [12▪▪] consisting of fusions of the C-peptide with insulin A chain, neuropeptide Y, and two peptides from IAPP. In early passages (1–3) of the CD4$^+$ T-cell lines and clones, peptide-specific cytokine secretion was measured by multiplexed Luminex (Austin, TX, USA) analysis. All lines and clones secreted interferon-γ IFNγ, with some additionally secreting tumor necrosis factor-α TNFα and/or interleukin (IL)-2 with variable secretion of IL-9, chemokine (C-C motif) ligand 20 (CCL20), or granulocyte macrophage colony-stimulating factor (GM-CSF). Two lines secreted IL-13 in a nonpeptide-specific manner. IL-4 and IL-5 were not detected from any autoreactive T-cell line or clone. Taken together, these data indicate an inflammatory islet microenvironment with a broad repertoire of CD4$^+$ and CD8$^+$ T-cell autoreactivity in the islets of donors with T1D.

It is important to note that no one method can give us a complete picture of the islet-infiltrating T-cell repertoire. Generally, it is not possible to assign an entire pancreas or even one tissue block for analysis by a single method as the requests for these tissues are numerous from β-cell biology and immunology laboratories; usually, even sectioning through an entire islet (through the z-axis) is not done routinely. When islets are isolated from the donors with T1D, tissue blocks (both for cryosectioning and for paraffin embedding and sectioning) are taken from spatially defined regions, whereas the rest of the pancreas is processed for islet isolation. Given the heterogeneous distribution of islets with insulitis, it is possible that the numbers of islets per case analyzed may not reflect the overall repertoire, but a subset of that repertoire. In addition, the islet-infiltrating repertoire is likely to be dependent on the disease duration with epitope spreading [54–56], the pattern of HLA expressed by each individual, and, possibly, other factors that remain to be discovered. It is also important to note that the methods used here [50▪▪,53▪▪] introduce growth bias. Nonetheless, these large banks of islet-infiltrating T cells are a resource for the T1D community. These methods in combination with other methods, such as phenotyping with

mass cytometry and single cell RNA sequencing, may serve to give us a more complete analysis of islet-infiltrating T cells in future studies. The analysis of islets recovered from donors with new onset T1D and from donors who are autoantibody positive, but without a clinical diagnosis of T1D, will yield important information concerning the autoreactivity and function of islet-infiltrating T cells prior to disease onset.

CONCLUSION

Our current understanding of insulitis in humans with T1D has expanded from immunohistochemical characterization, including detection of specific autoreactive $CD8^+$ T cell *in situ*, and TCR repertoire analysis, to the more recent recovery and analysis of live, islet-infiltrating $CD4^+$ and $CD8^+$ T cells, directly from the site of action in the pancreas. A broad range of autoreactive $CD4^+$ and $CD8^+$ T cells, with reactivity to known islet-associated peptides and posttranslationally modified peptides, infiltrate the islets and have a proinflammatory phenotype, was identified. These studies will aid in the design of durable, antigen-specific therapies for those at risk for or with T1D.

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REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

1. Baekkeskov S, Aanstoot HJ, Christgau S, et al. Identification of the 64K autoantige in insulin-dependent diabetes as the GAGA-synthesizing enzyme glutamic acid decarboxylase. Nature. 1990; 347:151–156. [PubMed: 1697648]
2. Gottlieb PA, Delong T, Baker RL, et al. Chromogranin A is a T cell antigen in human type 1 diabetes. J Autoimmun. 2014; 50:38–41. [PubMed: 24239002]
3. Dang M, Rockell J, Wagner R, et al. Human type 1 diabetes is associated with T cell autoimmunity to zinc transporter 8. J Immunol. 2011; 186:6056–6063. [PubMed: 21471440]
4. Lieberman SM, DiLorenzo TP. A comprehensive guide to antibody and T-cell responses in type 1 diabetes. Tissue Antigens. 2003; 62:359–377. [PubMed: 14617043]
5. Di Lorenzo TP, Peakman M, Roep BO. Translational mini-review series on type 1 diabetes: systematic analysis of T cell epitopes in autoimmune diabetes. Clin Exper Immunol. 2007; 148:1–16. [PubMed: 17349009]
6. Rathmann S, Rajasalu T, Rosinger S, et al. Preproinsulin-specific CD8+ T cells secrete IFNgamma in human type 1 diabetes. Ann NY Acad Sci. 2004; 1037:22–25. [PubMed: 15699489]
7. Scotto M, Afonso G, Larger E, et al. Zinc transporter (ZnT)8(186–194) is an immunodominant CD8+ T cell epitope in HLA-A2+ type 1 diabetic patients. Diabetologia. 2012; 55:2026–2031. [PubMed: 22526607]

8. Toma A, Laika T, Haddouk S, et al. Recognition of human proinsulin leader sequence by class I-restricted T-cells in HLA-A*0201 transgenic mice and in human type 1 diabetes. Diabetes. 2009; 58:394–402. [PubMed: 19011169]
9. Xu X, Gu Y, Bian L, et al. Characterization of immune response to novel HLA-A2-restricted epitopes from zinc transporter 8 in type 1 diabetes. Vaccine. 2016; 34:854–862. [PubMed: 26541133]
10. Velthuis JH, Unger WW, Abreu JR, et al. Simultaneous detection of circulating autoreactive CD8+ T-cells specific for different islet cell-associated epitopes using combinatorial MHC multimers. Diabetes. 2010; 59:1721–1730. [PubMed: 20357361]
11. Delong T, Baker RL, He J, et al. Diabetogenic T-cell clones recognize an altered peptide of chromogranin A. Diabetes. 2012; 61:3239–3246. [PubMed: 22912420]
12■■. Delong T, Wiles TA, Baker RL, et al. Pathogenic CD4 T cells in type 1 diabetes recognize epitopes formed by peptide fusion. Science. 2016; 351:711–714. The study identified a new class of autoantigen targets in T1D: hybrid insulin peptides. [PubMed: 26912858]
13. Mannering SI, Harrison LC, Williamson NA, et al. The insulin A-chain epitope recognized by human T cells is posttranslationally modified. J Exp Med. 2005; 202:1191–1197. [PubMed: 16260488]
14. McGinty JW, Chow IT, Greenbaum C, et al. Recognition of posttranslationally modified GAD65 epitopes in subjects with type 1 diabetes. Diabetes. 2014; 63:3033–3040. [PubMed: 24705406]
15. McGinty JW, Marre ML, Bajzik V, et al. T cell epitopes and post-translationally modified epitopes in type 1 diabetes. Curr Diab Rep. 2015; 15:90. [PubMed: 26370701]
16■■. McLaughlin RJ, de Haan A, Zaldumbide A, et al. Human islets and dendritic cells generate posttranslationally modified islet autoantigens. Clin Exp Immunol. 2016; 185:133–140. The study showed that deamidation of islet autoantigens, isolated both from islets and dendritic cells, increased their binding affinity to high-risk HLA haplotypes. [PubMed: 26861694]
17. Rondas D, Crevecoeur I, D'Hertog W, et al. Citrullinated glucose-regulated protein 78 is an autoantigen in type 1 diabetes. Diabetes. 2015; 64:573–586. [PubMed: 25204978]
18. Strollo R, Vinci C, Arshad MH, et al. Antibodies to posttranslationally modified insulin in type 1 diabetes. Diabetologia. 2015; 58:2851–2860. [PubMed: 26350612]
19. van Lummel M, Duinkerken G, van Veelen PA, et al. Posttranslational modification of HLA-DQ binding islet autoantigens in type 1 diabetes. Diabetes. 2014; 63:237–247. [PubMed: 24089515]
20■■. van Lummel M, van Veelen PA, de Ru AH, et al. Discovery of a selective islet peptidome presented by the highest-risk HLA-DQ8trans molecule. Diabetes. 2016; 65:732–741. The report is the first to show naturally processed and presented autoantigen epitopes by high-risk DQ8trans. [PubMed: 26718497]
21. Liu EH, Digon BJ 3rd, Hirshberg B, et al. Pancreatic beta cell function persists in many patients with chronic type 1 diabetes, but is not dramatically improved by prolonged immunosuppression and euglycaemia from a beta cell allograft. Diabetologia. 2009; 52:1369–1380. [PubMed: 19418039]
22■■. Campbell-Thompson M, Fu A, Kaddis JS, et al. Insulitis and beta-cell mass in the natural history of type 1 diabetes. Diabetes. 2016; 65:719–731. The study is a comprehensive evaluation of insulitis in the pancreata from donors with T1D. [PubMed: 26581594]
23. Sibley RK, Sutherland DE, Goetz F, Michael AF. Recurrent diabetes mellitus in the pancreas iso- and allograft. A light and electron microscopic and immunohistochemical analysis of four cases. Lab Invest. 1985; 53:132–144. [PubMed: 3894793]
24. Vendrame F, Pileggi A, Laughlin E, et al. Recurrence of type 1 diabetes after simultaneous pancreas-kidney transplantation, despite immunosuppression, is associated with autoantibodies and pathogenic autoreactive CD4 T-cells. Diabetes. 2010; 59:947–957. [PubMed: 20086230]
25. Schmidt MB. Ueber die beziehung der langenhans'schen inseln des pankreas zum diabetes mellitus. München Med Wochenschr. 1902:51–54.
26. Foulis AK, Farquharson MA, Hardman R. Aberrant expression of class II major histocompatibility complex molecules by B cells and hyperexpression of class I major histocompatibility complex molecules by insulin containing islets in type 1 (insulin-dependent) diabetes mellitus. Diabetologia. 1987; 30:333–343. [PubMed: 3301484]

27. Foulis AK, Farquharson MA, Meager A. Immunoreactive alpha-interferon in insulin-secreting beat cells in type 1 diabetes mellitus. Lancet. 1987; 2:1423–1427. [PubMed: 2891993]
28. Foulis AK, Liddle CN, Farquharson MA, et al. The histopathology of the pancreas in type 1 (insulin-dependent) diabetes mellitus: a 25-year review of deaths in patients under 20 years of age in the United Kingdom. Diabetologia. 1986; 29:267–274. [PubMed: 3522324]
29. Foulis AK, McGill M, Farquharson MA. Insulitis in type 1 (insulin-dependent) diabetes mellitus in man: macrophages, lymphocytes, and interferon-gamma containing cells. J Pathol. 1991; 165:97–103. [PubMed: 1744803]
30. Gepts W. Pathologic anatomy of the pancreas in juvenile diabetes mellitus. Diabetes. 1965; 14:619–633. [PubMed: 5318831]
31. Gepts W, De Mey J. Islet cell survival determined by morphology. An immunocytochemical study of the islets of Langerhans in juvenile diabetes mellitus. Diabetes. 1978; 27(Suppl 1):251–261. [PubMed: 75815]
32. Willcox A, Richardson SJ, Bone AJ, et al. Analysis of islet inflammation in human type 1 diabetes. Clin Exp Immunol. 2009; 155:173–181. [PubMed: 19128359]
33. Campbell-Thompson M, Wasserfall C, Kaddis J, et al. Network for Pancreatic Organ Donors with Diabetes (nPOD): developing a tissue biobank for type 1 diabetes. Diabetes Metab Res Rev. 2012; 28:608–617. [PubMed: 22585677]
34. Pugliese A, Yang M, Kusmarteva I, et al. The Juvenile Diabetes Research Foundation Network for Pancreatic Organ Donors with Diabetes (nPOD) program: goals, operational model and emerging findings. Pediatr Diabetes. 2014; 15:1–9.
35. Campbell-Thompson ML, Atkinson MA, Butler AE, et al. The diagnosis of insulitis in human type 1 diabetes. Diabetologia. 2013; 56:2541–2543. [PubMed: 24006089]
36. Rowe PA, Campbell-Thompson ML, Schatz DA, Atkinson MA. The pancreas in human type 1 diabetes. Semin Immunopathol. 2011; 33:29–43. [PubMed: 20495921]
37■. Krogvold L, Wiberg A, Edwin B, et al. Insulitis and characterisation of infiltrating T cells in surgical pancreatic tail resections from patients at onset of type 1 diabetes. Diabetologia. 2016; 59:492–501. The study demonstrates the components of insulitis from pancreatic biopsies from patients at the onset of T1D. [PubMed: 26602422]
38. Arif S, Leete P, Nguyen V, et al. Blood and islet phenotypes indicate immunological heterogeneity in type 1 diabetes. Diabetes. 2014; 63:3835–3845. [PubMed: 24939426]
39. Itoh N, Hanafusa T, Miyazaki A, et al. Mononuclear cell infiltration and its relation to the expression of major histocompatibility complex antigens and adhesion molecules in pancreas biopsy specimens from newly diagnosed insulin-dependent diabetes mellitus patients. J Clin Invest. 1993; 92:2313–2322. [PubMed: 8227346]
40■. Leete P, Willcox A, Krogvold L, et al. Differential insulitic profiles determine the extent of beta-cell destruction and the age at onset of type 1 diabetes. Diabetes. 2016; 65:1362–1369. Insulitis was differentiated into two groups: CD20Hi with a diagnosis of T1D before the age of 7 years and CD20lo with a diagnosis after the age of 13 years and also retaining more insulin-containing islets than in the former group. [PubMed: 26858360]
41. Coppieters KT, Dotta F, Amirian N, 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; 209:51–60. [PubMed: 22213807]
42■. Richardson SJ, Rodriguez-Calvo T, Gerling IC, et al. Islet cell hyperexpression of HLA Class I antigens: a defining feature in type 1 diabetes. Diabetologia. 2016; 59:2448–2458. First reported over 30 years ago, this study now confirms that β-cells hyperexpress HLA ***class*** I in human T1D. [PubMed: 27506584]
43. Skog O, Korsgren S, Wiberg A, et al. Expression of human leukocyte antigen class I in endocrine and exocrine pancreatic tissue at onset of type 1 diabetes. Am J Pathol. 2015; 185:129–138. [PubMed: 25524212]
44. Rodriguez-Calvo T, Suwandi JS, Amirian N, et al. Heterogeneity and lobularity of pancreatic pathology in type 1 diabetes during the prediabetic phase. J Histochem Cytochem. 2015; 63:626–636. [PubMed: 26216138]

45. Codina-Busqueta E, Scholz E, Munoz-Torres PM, et al. TCR bias of in vivo expanded T cells in pancreatic islets and spleen at the onset in human type 1 diabetes. J Immunol. 2011; 186:3787–3797. [PubMed: 21325620]

46. Conrad B, Weidmann E, Trucco G, et al. Evidence for superantigen involvement in insulin-dependent diabetes mellitus aetiology. Nature. 1994; 371:351–355. [PubMed: 8090207]

47. Somoza N, Vargas F, Roura-Mir C, et al. Pancreas in recent onset insulin-dependent diabetes mellitus. Changes in HLA, adhesion molecules and autoantigens, restricted T cell receptor V beta usage, and cytokine profile. J Immunol. 1994; 153:1360–1377. [PubMed: 7913115]

48. Yamagata K, Nakajima H, Tomita K, et al. Dominant TCR alpha-chain clonotypes and interferon-gamma are expressed in the pancreas of patients with recent-onset insulin-dependent diabetes mellitus. Diabetes Res Clin Pract. 1996; 34:37–46. [PubMed: 8968689]

49. Bottino R, Bertera S, Grupillo M, et al. Isolation of human islets for autologous islet transplantation in children and adolescents with chronic pancreatitis. J Transplant. 2012; 2012:642787. [PubMed: 22461976]

50■■. Pathiraja V, Kuehlich JP, Campbell PD, et al. Proinsulin-specific, HLA-DQ8, and HLA-DQ8-transdimer-restricted CD4+ T cells infiltrate islets in type 1 diabetes. Diabetes. 2015; 64:172–182. The first report of the isolation of T cells directly from the islets of a donor with T1D and demonstration of their autoreactivity to peptides of proinsulin in the context of DQ8 and DQ8*trans*. [PubMed: 25157096]

51. In't Veld P, De Munck N, Van Belle K, et al. Beta-cell replication is increased in donor organs from young patients after prolonged life support. Diabetes. 2010; 59:1702–1708. [PubMed: 20413508]

52. Rodriguez-Calvo T, Ekwall O, Amirian N, et al. Increased immune cell infiltration of the exocrine pancreas: a possible contribution to the pathogenesis of type 1 diabetes. Diabetes. 2014; 63:3880–3890. [PubMed: 24947367]

53■■. Babon JA, DeNicola ME, Blodgett DM, et al. Analysis of self-antigen specificity of islet-infiltrating T cells from human donors with type 1 diabetes. Nat Med. 2016; 22:1482–1487. Broad T-cell autoreactivity to known autoantigenic islet-derived peptides and postranslationally modified peptides was seen from a large bank of T cells grown or sorted directly from islets of nine donors with T1D. [PubMed: 27798614]

54. Tian J, Zekzer D, Lu Y, et al. B cells are crucial for determinant spreading of T cell autoimmunity among beta cell antigens in diabetes-prone nonobese diabetic mice. J Immunol. 2006; 176:2654–2661. [PubMed: 16456028]

55. Durinovic-Bello I, Schlosser M, Riedl M, et al. Pro- and anti-inflammatory cytokine production by autoimmune T cells against preproinsulin in HLA-DRB1*04, DQ8 Type 1 diabetes. Diabetologia. 2004; 47:439–450. [PubMed: 14745491]

56. Dai YD, Carayanniotis G, Sercarz E. Antigen processing by autoreactive B cells promotes determinant spreading. Cell Mol Immunol. 2005; 2:169–175. [PubMed: 16212883]