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REVIEW

Not so sweet and simple: impacts of SARS-CoV-2 on the β cell

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ABSTRACT

The link between COVID-19 infection and diabetes has been explored in several studies since the start of the pandemic, with associations between comorbid diabetes and poorer prognosis in patients infected with the virus and reports of diabetic ketoacidosis occurring with COVID-19 infection. As such, significant interest has been generated surrounding mechanisms by which the virus may exert effects on the pancreatic β cells. In this review, we consider possible routes by which SARS-CoV-2 may impact β cells. Specifically, we outline data that either support or argue against the idea of direct infection and injury of β cells by SARS-CoV-2. We also discuss β cell damage due to a "bystander" effect in which infection with the virus leads to damage to surrounding tissues that are essential for β cell survival and function, such as the pancreatic microvasculature and exocrine tissue. Studies elucidating the provocation of a cytokine storm following COVID-19 infection and potential impacts of systemic inflammation and increases in insulin resistance on β cells are also reviewed. Finally, we summarize the existing clinical data surrounding diabetes incidence since the start of the COVID-19 pandemic.

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Introduction

Since its first recognized case in December 2019, SARS-CoV-2, the virus responsible for the novel coronavirus disease 2019 (COVID-19), has generated an immense global health burden.[1,2] Although our understanding of the virus and its short- and long-term impacts continue to evolve, one association that emerged early on in the pandemic was that comorbid diabetes is linked to a poorer prognosis in patients with COVID-19 infection.[3–5] Studies have aimed to elucidate the interplay between COVID-19 infection and preexisting diabetes as well as newly developed hyperglycemia. Preexisting diabetes has been shown to be an independent risk factor for admission into the Intensive Care Unit (ICU) and associated with a higher risk of death as compared to nondiabetic patients with COVID-19 infections.[6–8] Furthermore, there is a high prevalence of ketosis and ketoacidosis in patients infected with COVID-19.[9] New cases of hyperglycemic states and diabetes development have also been described following infection with the virus.[10,11] This relationship likely reflects in part the effect of critical illness on systemic inflammation, insulin resistance and insulin secretion in general, and studies have also shown that COVID-19 infection can induce insulin resistance or even reduce insulin secretion in infected patients.[10,12,13] Recent evidence has also elucidated that COVID-19 infection may be capable of causing direct damage to the pancreas, which in conjunction with insulin resistance, could exacerbate hyperglycemia in diabetic individuals or lead to new hyperglycemia and diabetes in previously nondiabetic individuals.[14,15]

The β cells within the pancreatic islets of Langerhans are responsible for insulin production and are therefore a crucial regulator of systemic glucose metabolism. Thus, understanding the effects of COVID-19 on the islet and on β cells themselves is critical to gain a better understanding about the interplay between COVID-19 infection and diabetes. Given this, recent reports have specifically begun to tackle the mechanisms of the virus's effect on the pancreatic β cells, including the

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question of whether or not the virus directly targets and infects the β cell. In this review, we outline studies supporting or refuting this idea, other potential mechanistic etiologies of COVID-19-related impacts on the β cell, and existing clinical data surrounding diabetes incidence since the start of the COVID-19 pandemic.

Potential mechanisms of β cell Injury in COVID-19 Infection

Given the associations of COVID-19 infection with diabetes and diabetic ketoacidosis, a logical next step is the investigation of mechanistic etiologies of COVID-19-induced islet injury. This could theoretically occur via direct infection of β cells, effects on β cells due to infection of surrounding cells, or systemic effects of COVID-19 with either direct or indirect impacts on the β cell (Figure 1).

Can COVID-19 potentially directly infect and enter islets?

Reports of increased hyperglycemia with COVID, links to ketoacidosis, and cases of newly identified diabetes have begged the question: can SARS-CoV-2 directly enter and infect islets and specifically β cells? Evidence of viral entry into β cells has been shown with other viruses such as enteroviruses, coxsackie, cytomegalovirus, and varicella zoster virus.[16,17] The mechanism by which the SARS-CoV-2 virus infects cells has been studied in multiple cell types. The SARS-CoV-2 virus has viral spike glycoproteins present on its surface that bind via the receptor binding domain (RBD) to the human angiotensin-converting enzyme 2 (ACE-2) receptors on the host cell.[18,19] The virus is then primed by human proteases.[18,19] Here, to allow for membrane fusion, the S protein is proteolytically activated by host cell proteases, including transmembrane serine protease 2 (TMPRSS2), cathepsins, TMPRSS4, the proprotein convertase

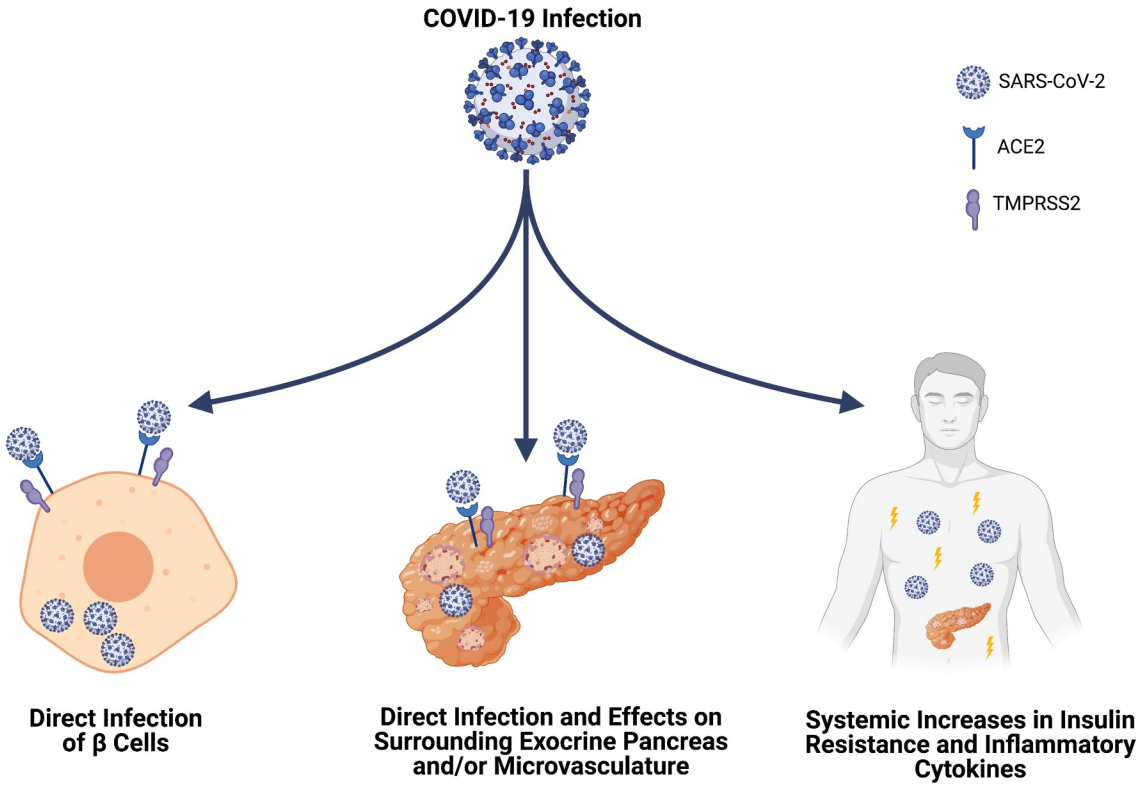

Figure 1. Potential mechanisms of beta cell injury due to COVID-19 Infection. Beta cell injury could theoretically occur via direct infection of β cells, bystander effects on β cells due to infection of surrounding cells, or systemic effects of COVID-19 with either direct or indirect impacts on the β cell.

furin and others.[18,20–23] Although TMPRSS2 appears to be most critical for SARS-CoV-2 Spike protein priming,[20] furin pre-activation may be able to reduce the virus' dependence on other target cell proteases.-[18] Because the interaction between SARS-CoV-2 and the ACE-2 receptor is considered key for SARS-CoV-2 entry into the host cell, cellular ACE-2 expression has frequently been used as a marker of potential susceptibility to direct infection, often in combination with expression of TMPRSS2 and other co-activating proteases.[24]

Interest in islet ACE2 actually predates the COVID-19 pandemic, with several rodent studies published in the early 21st century suggesting a role of ACE2 in islets and β cell functionality. Acinar and islet ACE2 localization have been observed using immunohistochemistry in rat pancreata.[25] A 2008 study also reported ACE2 expression in mouse acinar and islet tissue using immunohistochemistry, and showed that whole-body knockout of ACE2 resulted in impaired glucose tolerance and decreased first-phase insulin secretion.[26] Adenoviral-based ACE2 overexpression within the pancreas reduced β cell apoptosis and improved β cell mass and glucose tolerance in *db/db* mice.[27] However, studies testing the expression of these receptors in human islets and β cells have shown conflicting results, especially regarding islet ACE2 expression.

The case for the presence of machinery required for SARS-CoV-2 infection of human β cells

A case study published in 2010 described ACE2 immunohistochemical staining in frozen autopsy specimens from a single donor infected with SARS-CoV-1, showing a clear signal in pancreatic islets, but very weak staining for ACE2 in surrounding exocrine tissues (Table 1).[28] No analyses were performed to further elucidate the identity of the positive islet cells. Since then, several bulk RNA sequencing analyses have identified ACE2 transcript expression in islets[33–36] and single-cell RNA sequencing of dispersed human islets also showed evidence of A*CE2* or *TMPRSS2* expression in acinar cells, ductal cells, β cells, α cells, mesenchymal cells, and endothelial cells.[30] Testing has also demonstrated that furin mRNA and protein are expressed by human pancreatic islets.[37] Interestingly, combined analysis of islet microarray and RNA sequencing suggested that while *ACE2* expression in β cells is lower than that of *TMPRSS* in islets from nondiabetic donors, *ACE2* was upregulated in islets from donors with T2D.[36]

In human pluripotent stem cell-derived pancreatic endocrine cells, immunofluorescent staining for ACE2 protein was detected in both in insulin- and glucagon-positive cells.[30] The authors also reported the ability to detect ACE2 staining on primary human β cells and α cells from dispersed islets, although percent positivity was not quantified.[30] A larger analysis performing immunohistochemical staining on nondiabetic sections from the INNODIA EUnPOD biobank identified three pancreatic cell types staining positive for ACE2: (1) intensely staining pericytes in inter-acini septa; (2) scattered positive duct cells, and (3) subsets of islet cells, which were mostly insulin positive.[29] ACE2 mRNA was also detected in collagenase isolated human islets and microdissected human islets from frozen pancreatic sections. Here, between and within cases, insulin-positive cells were noted to exhibit heterogeneous signal intensity and staining patterns. Based on differences in antibody staining and immunoblots in the EndoC-bH1 cell line, the authors suggested that human β cells may express a short ACE2 isoform. Reminiscent of data based on islets from individuals with T2D, cytokine treatment of human islets resulted in significant upregulation of ACE2 transcripts, suggesting a link between inflammation and ACE2 expression levels in β cells.[36]

Another group recently reported positive immunofluorescent staining for both ACE2 and TMPRSS2 in islets in 5/5 pancreas sections tested.[31] Strong ACE2 expression was also detected in

Table 1. The case for direct SARS-CoV-2 infection of β cells: positive studies testing protein expression in human islet tissues.

Study	Findings
Yang et al. (2010) *Acta Diabetol*.[28] • Sample size: n = 1 • Antibody (Ab) utilized: not identified	• Immunohistochemical staining performed on frozen pancreas autopsy specimens from a single donor infected with SARS-CoV-1 showed strong staining for ACE2 in pancreatic islets, but very weak staining for ACE2 in exocrine tissues.
Fignani et al. (2020). *Front. Endocrinol*.[29] • Sample size: n = 7 adult nondiabetic donors for formalin-fixed paraffin-embedded (FFPE) sections from the INNODIA EUnPOD biobank; n = 5 donors for laser capture microdissection (LCM) from frozen tissue; 1 donor with long-standing T1D • Abs: ACE2: MAB933 (R&D); Ab15348 and Ab108252 (Abcam)	• ACE2 mRNA detected in collagenase isolated islets and LCM islets. • Immunohistochemistry (IHC) analysis on FFPE pancreatic sections identified three main ACE2+ cell types: pericytes in inter-acini septa (intensely staining); duct cells (scattered positive); and a subset of islet cells (diffuse signal), which were mostly insulin positive. Similar expression and distribution in infiltrated islets from donor with long-standing T1D. • β cell ACE2 expression occurred mostly in cytoplasm, occasionally in plasma membrane; high heterogeneity in localization and signal intensity between islets and between cases. • ACE2 islet-related signal not detected with the Ab108252 antibody (reacts with a peptide located in the N-terminal ACE2 protein sequence). • Proinflammatory cytokine treatment induced upregulated of ACE2 mRNA in EndoC-bH1 cells and isolated human islets and ACE2 protein in EndoC-bH1 cells.
Yang et al (2020) *Cell Stem Cell*.[30] • Sample size: 3 biological replicates for all experiments • Abs: ACE2: AF933 (R&D); Ab15348 (Abcam); SARSCoV-2 Spike: Mouse Anti-SARSCoV-2 Spike Antibody [2B3E5], listed as provided by Dr Tom Moran	• In human pluripotent stem cell–derived pancreatic endocrine cells, ACE2 expression was detected in insulin- and glucagon-positive cells, both in vitro and after implantation under the kidney capsule of SCID-beige mice. • Single-cell RNA seq of dispersed human islets showed *ACE2* and *TMPRSS2* expression in acinar cells, ductal cells, β cells, alpha cells, mesenchymal cells, and endothelial cells. • IHC showed ACE2 expession in primary human β cells and alpha cells. • Human islets were infected with SARS-CoV-2 virus and after 24 hours immunostaining for SARS-CoV-2 Spike protein was positive in both insulin-positive and glucagon-positive cells.
Muller et al. (2021) *Nat Metab*.[31] • Sample size: *n* = 5 FFPE pancreatic tissue sections; *n* = 4 human islets exposed to SARS-CoV-2 ex vivo; *n* = 4 tissue sections from individuals testing positive for SARS-CoV-2 • Abs: Ab15348 and ab92323 (Abcam); SARS-CoV-2 Spike: Rabbit Ant-SARSCoV-2 Spike Antibody (Abcam); SARS-CoV-2 nucleocapsid: Rabbit Ant-SARSCoV-2 Nucleocapsid (Novus Biologicals)	• Immunofluorescence staining for both ACE2 and TMPRSS2 was observed in islets in all pancreas sections. Strong ACE2 expression also detected in endothelial cells and a subpopulation of ductal cells, with faint expression in acinar cells. TMPRSS2 was detected in endocrine cells and in some ducts. • Insulin-positive cells were more likely to show costaining for ACE2 and TMPRSS2 than glucagon or somatostatin-positive cells. • Human islets were exposed to SARS-CoV-2, and expression of viral spike and nucleocapsid protein became readily detectable after 3 d. Most of the SARS-CoV-2-infected cells appeared to lack hormone expression but were still positive for PDX1 or NKX6.1. Electron microscopy showed that infected islet cells exhibited dilatation and vacuolization of the endoplasmic reticulum–Golgi apparatus complex, with vacuoles containing viral particles showing coronavirus morphology. Infected islets exhibited reduced GSIS. Transcriptional analysis of infected islets with bulk RNA-Seq showed upregulation of interferon-stimulated genes and downregulation of genes linked to β cell physiology. • Pancreatic histopathology in four patients with COVID infection showed the presence of SARS-CoV-2 nucleocapsid protein in some small ducts, in single or grouped acinar cells, and in clusters located close to islets. Although few cells within these clusters costained for nucleocapsid protein and insulin, 23–65% costained for NKX6.1.
Shaharuddin et al. (2021) medRxiv.org. 2021.[32] • Sample size: not reported. • Abs: SARS-CoV-2 Spike S1: 40150-R007 (SinoBiological)	• Immunofluorescent staining of FFPE human pancreas sections from individual(s) with COVID-19 showed staining for SARS-Cov-2 S1 spike protein that was "frequently concentrated in C-peptide positive islet clusters". S1 protein also detected in most of chemotrypsin positive and some clusters of amylase positive acinar cells. No colocalization with cytokeratin 19 + ductal cells detected.

endothelial cells and a subpopulation of ductal cells, with ACE2 only faintly expressed in acinar cells. TMPRSS2 was also detected in endocrine cells and in some ducts, with insulin-positive cells more likely to show costaining for ACE2 and TMPRSS2 than glucagon or somatostatin-positive cells.[31]

The case against presence of machinery required for direct SARS-CoV-2 infection of human β cells

In contrast to the above studies, several manuscripts have also been published that point away from large-scale β cell expression of the machinery necessary for direct infection by SARS-CoV-2

Table 2. The case against direct SARS-CoV-2 infection of β cells: negative studies testing protein expression in human islet tissues.

Study	Findings
Brar et al. (2017) *Diabetes*.[38] • Sample size: not reported • Antibodies (Abs): ACE2: Rabbit polyclonal anti-ACE2, catalog number not included (Santa Cruz Biotechnology)	• Immunofluorescent staining of frozen OCT-embedded, formalin-fixed human pancreas from nondiabetic subjects (purchased from Zyagen) showed islet ACE2 staining that colocalized with glucagon and somatostatin, but not insulin or pancreatic polypeptide.
Hikmet et al. (2020) *Mol Syst Biol*.[39] • Sample size: n = 10 formalin-fixed paraffin-embedded (FFPE) pancreatic sections • Abs: ACE2: MAB93 (R&D) and HPA000288 (Atlas Antibodies)	• Immunohistochemical (IHC) staining of pancreatic sections only showed ACE2 positivity in interlobular ducts and endothelial cells/pericytes, but not islets, acinar glandular cells, or intercalated or intralobular ducts.
Coate et al. (2020). *Cell Metab*.[40] • Sample size: Intact isolated human islet cryosections *n* = 3; FFPE pancreatic tissue sections: n-5 juvenile, *n* = 14 adult nondiabetic *n* = 12 with T2D and *n* = 11 with T1D; *n* = 7 COVID-19 patient FFPE pancreatic tissue sections (3/7 with diabetes) • Abs: ACE2: MAB933 and AF933 (R&D); HPA000288 (Atlas Antibodies), ab15348 (Abcam); TMPRSS2: HPA035787 (Sigma)	• Analysis of existing bulk RNA sequencing datasets where human islet α and β cells were enriched by fluorescence-activated cell sorting showed that, compared to expression of key islet-enriched genes, median expression of ACE2 and TMPRSS2 mRNA was drastically (84% and 92% lower than α and β cell-enriched transcripts, respectively) • Aggregate analysis of four single-cell RNA-seq datasets of human pancreatic cells showed that <1.5% of β cells expressed ACE2 or TMPRSS2 and no β cells co-expressed ACE2 and TMPRSS2. Other suggested effector proteases were more highly expressed but <1.3% of β cells co-expressed ACE2 with SARS-CoV-2 effector proteases, such as Cathepsin L (CTSL), ADAM metallopeptidase domain 17, furin, and TMPRSS4. • Immunofluorescent staining of islet cryosections did not detect ACE2 in insulin-positive or glucagon-positive cells, but ACE2 signal was prominent in surrounding microvasculature. • Immunofluorescent staining of FFPE pancreatic sections from nondiabetic and diabetic donors showed that ACE2 did not co-localize with markers of α or β cells but was detected within microvascular structures. TMPRSS2 protein was not detected within islets, but was detected in exocrine tissue structure resembling intercalated and larger ducts. • Pancreatic tissue sections from individuals with COVID-19 did not show signs of pancreatitis, interstitial edema, inflammatory infiltrate, hemorrhage, or necrosis.
Kusmarteva et al. (2020) *Cell Metab*.[41] • Sample size: SmFISH: 7 donors; Histologic analyses: 36 SARS-CoV-2 negative donors without diabetes; 3 SARS-CoV-2-positive donors • Abs: MAB933 and AF933 (R&D); Ab15348 and Ab108252 (Abcam); SARSCoV-2 Coronavirus Nucleocapsid protein: MA-1-7404 (clone B46F) (Invitrogen)	• Combined islet single-cell RNA sequencing data from five public repositories showed that ACE2 mRNA was expressed in <2% of endocrine, endothelial, and select innate immune cells. ACE2 was detectable in 4–5% of acinar cells and ductal cells in nondiabetic donors and ~8.07% of acinar and ductal cells in T2D donors. TMPRSS2 mRNA was detectable in 5.46% of β cells but only 0.10% of β cells were positive for ACE2 and TMPRSS2 and <1% of donors expressed ACE2 in combination with other tested proteases. • ACE2 and TMPRSS2 single molecular fluorescent in situ hybridization (smFISH) signal was observed in ducts, acinar tissue, and endothelial cells, but to a much lesser extent in islet endocrine cells, while other proteases tested were observed in endocrine tissues. • Chromogen-based IHC in FFPE pancreatic sections showed increasing percentage of ACE2+ staining with increasing age until later adulthood, followed by a decline after 50 y of age, as well as a positive correlation between ACE2+ area and body mass index. • Immunofluorescent staining for ACE2 with insulin and glucagon showed ACE2 expression in the pancreatic ductal epithelium and microvasculature within acinar and islet regions, but no evidence of β or α cell expression. • Histopathological analysis performed on sections from 3 donors with COVID-19 infection at the time of death showed moderate ACE2 staining intensity in both endocrine and exocrine endothelium and low to moderate ACE2 staining in the ductal epithelium. Two-thirds cases showed little to no immunopositivity for SARS-CoV-2 nucleocapsid protein, but one case showed positive staining in some intralobular and interlobular ductal epithelial cells near an islet and widely scattered throughout the exocrine regions.

(Table 2). Prior to the current COVID-19 pandemic, one analysis focused on impacts of renin-angiotensin signaling on β cell function showed that immunofluorescent staining of ACE2 in insulin-positive β cells was actually not detected in mouse or human pancreatic sections, and suggested that functional impacts of ACE2 on β cells might be mediated through paracrine mechanisms.[38] A broad review of immunohistochemistry datasets from different tissues showed positive ACE2 staining in interlobular duct cells and endothelial cells/pericytes but not in islet cells.[39]

Two independent groups of investigators recently published studies focused on using

multiple analyses in human donor tissues to test the idea of whether SARS-CoV-2 can directly infect the human islet, with mostly negative results.[40,41] An aggregated analysis of existing bulk RNA sequencing datasets where human islet α and β cells were enriched by fluorescence-activated cell sorting showed median expression levels of ACE2 mRNAs were reduced by 84% and 92% compared to expression of key α and β cell-enriched genes and analysis of single-cell sequencing datasets showed that <1.5% of β cells express ACE2.[40] mRNA Immunofluorescent staining of a small number of intact cryopreserved islets, as well as formalin-fixed paraffin-embedded sections identified no ACE2 within islet endocrine cells, although strong staining was present within the islet microvasculature.[40] Similarly an integration analysis of single-cell RNA sequencing data from human islets with findings from direct visualization of gene and protein expression using ACE2 single molecular in situ hybridization, immunohistochemistry, and immunofluorescence in human pancreas sections did not support widespread islet β cell infection via the ACE2 receptor.[41] Here, analysis of preexisting single-cell RNA sequencing datasets also suggested that ACE2 was expressed in <2% of islet endocrine cells, and immunofluorescent staining of cadaveric donor sections with and without diabetes showed ACE2 was highly expressed in the pancreatic ductal epithelium and the microvasculature within acinar and islet regions, but no evidence of β cells or α cells expressing ACE2 protein.[41]

Both of these studies also explored expression of co-activating proteases, as well as co-expression of these proteases with ACE2. Similar to ACE2, aggregate analysis of prior existing bulk RNA sequencing and single-cell sequencing showed relatively low β cell TMPRSS2 expression (<1.5–5.5% of cells).[40,41] Co-expression of ACE2 and TMPRSS2 within β cells was especially low (0–0.1% of β cells). Other effector proteases were more highly expressed in β cells, but co-expression with ACE2 was still relatively rare- <1–1.3% of β cells co-expressed ACE2 with SARS-CoV-2 effector proteases tested, including cathepsin L, ADAM metallopeptidase domain 17, furin, and TMPRSS4.[40,41] Similarly, TMPRSS2 single molecular fluorescent in situ hybridization signal was observed in pancreatic ducts, acinar tissue, and endothelial cells, but to a much lesser extent in islet endocrine cells, although other proteases tested were observed in endocrine tissues.[41] Immunofluorescent staining did not detect TMPRSS2 protein within islets, but did detect TMPRSS2-positive structures resembling intercalated and larger ducts in exocrine tissue.[40]

Analyses of tissues from individuals testing positive for SARS-CoV-2

With conflicting data about islet and β cell expression of markers essential for COVID-19 entry and infection, studies directly testing for SARS-CoV-2 are important for understanding whether the virus directly infects islets. This has been approached in several ways. Several investigators have taken the approach of analyzing cadaveric donor sections from individuals with COVID-19 infections. One analysis of pancreatic sections from three individual donors that tested positive for SARS-CoV-2 around the time of death showed moderate ACE2 staining intensity in the endothelium within both endocrine and exocrine compartments and low to moderate ACE2 staining in the ductal epithelium.[41] Two cases showed little to no immunopositivity for SARS-CoV-2 nucleocapsid protein, but one case showed positive staining in some intralobular and interlobular ductal epithelial cells near an islet and widely scattered throughout the exocrine regions.[41] Hematoxylin and eosin staining from pancreatic tissue sections from seven individuals with COVID-19 (three with diabetes) did not show signs of pancreatitis, interstitial edema, inflammatory infiltrate, hemorrhage, or necrosis.[40] A recently posted preprint reported that pancreatic sections from at least one donor with COVID19 exhibited immunofluorescent staining for SARS-CoV-2 S1 spike protein that was “frequently concentrated in C-peptide-positive islet clusters”. S1 spike protein was also detected in most of chymotrypsin-positive and some clusters of amylase-positive acinar cells. However, no colocalization with cytokeratin 19 + ductal cells was detected.[32]

The potential for direct infection of human islets ex vivo has recently been demonstrated.[31] Here, isolated human islets exposed to SARS-

CoV-2 exhibited detectable expression of viral spike and nucleocapsid protein by d 3 post-exposure. Interestingly, most of the SARS-CoV-2-infected cells appeared to lack mature hormone expression, but were still positive for PDX1 or NKX6.1, raising the possibility that endocrine cells lose hormone positivity upon infection. Transmission electron microscopy showed that infected islet cells exhibited dilatation and vacuolization of the endoplasmic reticulum–Golgi apparatus complex, with vacuoles containing viral particles showing coronavirus morphology.[31] Infected islets exhibited reduced glucose-stimulated insulin secretion and transcriptional analysis of infected islets with bulk RNA-Seq showed upregulation of interferon-stimulated genes and downregulation of genes linked to β cell physiology. Consistent with these findings, investigators analyzed pancreatic histopathology from four patients with COVID infection, which showed the presence of SARS-CoV-2 nucleocapsid protein in clusters located close to islets, among which 23–65% of positive cells co-stained for NKX6.1, although co-staining for insulin was rare.[31]

Effects on β cells due to infection of surrounding cells

An alternative mechanism by which COVID-19 infection could lead to islet damage is by infection or damage of surrounding cells in the pancreatic tissue, resulting in a "bystander" effect in which the β cells are damaged. For example, studies suggest that pancreatitis can lead to both exocrine and endocrine cell damage and diabetes.[42–45] Since the beginning of the pandemic, associations have been drawn between COVID-19 infection and pancreatic injury and pancreatitis. Case reports have detailed the development of clinical pancreatitis following COVID-19 infection,[46–50] and several studies have suggested that elevations in amylase and lipase may be a fairly common feature of infection.[51,52] As noted above in studies focused on the islet, data on whether ACE2 is enhanced or decreased in pancreatic exocrine cells are conflicting, with some datasets suggesting an increase in ACE2 expression in the exocrine pancreas[40,53,54] and others suggesting relative depletion among the exocrine cells.[28,36] Thus, whether COVID-19-related pancreatic injury is related in part to direct infection is not clear. Additionally, the absence of signs of interstitial edema, inflammatory infiltrate, hemorrhage, or necrosis in pancreatic tissue sections from some individuals with COVID-19 points away from pancreatitis as a universal feature of infection.[40]

Another means by which COVID-19 could lead to damage in pancreatic tissue, including islets, is by damaging the pancreatic vasculature. COVID-19 infection is known to lead to a hypercoagulable state,[55,56] as endothelial injury caused by COVID-19 can lead to microvascular inflammation and thrombosis.[57] Endothelial damage has also been shown to activate immune cells and an inflammatory response in tissues, including pancreatitis.[58,59] Given data described above that machinery required for Sars-Cov-2 infection is commonly present in the islet microvasculature, the virus could also affect β cells via the microvasculature, potentially via micro-thrombotic lesions.[41]

Systemic effects leading to islet stress and damage in COVID-19 infection

Pro-inflammatory cytokines play important roles in the pathogenesis of both diabetes and COVID-19 pathogenesis. Cytokines can drive β cell dysfunction, damage, and death in diabetes through both intrinsic cellular signaling pathways, as well as through augmentation of the islet immune cell response.[60–63] Similarly, pro-inflammatory cytokines have been shown to play a critical role in COVID-19 progression and severity.[64–67] Studies have shown an increase in cytokines including interleukin 1 β (IL-1β), interleukin 1 (IL-6), interleukin 12 (IL-12), Interferon gamma (IFN-γ), and tumor necrosis factor alpha (TNF-α) following COVID-19 infection.[65,66,68] Specifically, "cytokine storm", an unregulated production of pro-inflammatory cytokines at both the local and systemic levels, has been thought to be an important cause of mortality in COVID-19 infected patients.[69] This dramatic increase in cytokine levels induces a hyper-inflammatory state that leads to downstream tissue injury and multiorgan failure.[70,71]

Pancreatic injury leading to acute pancreatitis has been hypothesized to be caused in some COVID-19 patients in part by damage induced by pro-inflammatory cytokines.[52,72,73] Acutely, via local islet inflammation, this systemic proinflammatory state could also theoretically induce β cell death and impair β cell function.[74] Islet inflammation and activation of intrinsic β cell stress pathways have also been linked to altered antigen presentation.[75] Thus, in predisposed individuals, local islet inflammation linked to COVID-19 could theoretically result in increased islet autoimmunity.

Finally, systemic inflammation and critical illness are known to lead to increases in systemic insulin resistance and gluconeogenic stress hormones, which lead to increased demand on β cells.[76]Along these lines, stress hyperglycemia is a well-described phenomenon that occurs with critical illness due to multiple infectious and noninfectious etiologies.[77] A complicating factor is the use of medications (such as steroids) that also impact insulin resistance and secretion. Additionally, severe acute illness and hyperglycemia could uncover developing diabetes that had not yet reached the threshold of clinical presentation.

The impact of the COVID-19 pandemic on the incidence of diabetes during the pandemic

Diabetes is a risk factor for worsened outcomes with COVID infection,[6–8] and reviews of hospital admissions have identified cases of concurrent diabetic ketoacidosis (DKA) at the time of SARS-CoV-2 infection, especially in individuals with T2D.[78,79] Increases in DKA and associations of diabetes with poor outcomes could reflect known impacts of diabetes on severe systemic illness, and in the case of T2D, be confounded by relationships between COVID-19 severity and obesity.[5] However, if COVID-19 consistently results in large-scale damage to the pancreatic β cells via any of the mechanisms proposed above, then, in theory, epidemiologic data would identify increases in the incidence of diabetes associated with the current COVID-19 pandemic. Table 3 summarizes clinical datasets quantifying new onset diabetes during this period.

Overall, there is currently not supporting evidence of an increase in incidence of Type 1 diabetes cases associated with the current pandemic.[85–87] Data surrounding T2D have shown some effects on glycemic control at diagnosis, but this may also be related to lifestyle changes as a consequence of lockdown.[88–90] Identification of "new" diabetes also has the potential to be confounded by preexisting undiagnosed disease (especially in T2D, which can be insidious in onset).[91] Differing reports on rates and severity of DKA at the time of presentation may also reflect delays in seeking health care related to shutdowns in the context of different background rates of care access and DKA. Finally, an important consideration is that more subtle damage to the β cells could yield longer term increases in diabetes or autoimmunity over time, and so long-term follow-up studies will be important to more definitively address this question.

Concluding remarks

The impressive body of work that has already been generated surrounding COVID's effects on the islet may have generated more questions than answers. In aggregate, existing data seem to point away from direct infection of the β cells as a common occurrence in COVID-19 infection. Conflicting data regarding the protein expression of machinery required for direct infection in the islet and islet phenotypes in the context of infection highlight the challenges associated with antibody-based reagents to identify protein expression, and the need for careful reagent validation.[92] As suggested by findings from several groups, islet ACE2 expression and the islet phenotype associated with COVID-19 infection may be heterogenous among individuals or under certain conditions.-[29,36,41] Such heterogeneity could also explain some of the observed differences between studies, many of which utilized fairly small sample sizes (Tables 1 and 2). Thus, review of larger numbers of histologic samples from donors with COVID-19 infection is clearly needed to obtain the full picture of the spectrum of disease. Data showing the capability for direct islet

Table 3. Clinical datasets quantifying new onset diabetes since the pandemic.

Paper	Data Source	Country: Period and Population	Results
Lui et al. *J Diabetes Investig*. 2020.[78]	Reviewed territory-wide anonymized electronic health records (EHR) data from the Hong Kong Hospital Authority (18 hospitals)	• Hong Kong: 1/24-4/24/20 vs. 1/25-4/24/19 and 10/25-1/24/20 • All adults admitted with severe hyperglycemia, DKA and severe hypoglycemia; (mean age of 71 y)	• Majority with long-standing T2D • Compared to control periods, hospitalizations were significantly reduced for both severe hyperglycemia (5.8 /d vs. 8.2–8.8/d) and severe hypoglycemia (9.6/d vs. 11.9–12.7/d), but not different for DKA (1.1/d vs. 1.1–1.3/d).
Armeni et al. *Lancet Diabetes Endocrinol*. 2020.[79]	Retrospective case series from eligible patients from three north London hospitals	• UK: 3/1–3/30/20 • 35 Patients with COVID-19 and DKA (11/35; 31.4%), mixed DKA and HHS (13/35; 37.1%), HHS (2/35; 5.7%) or hyperglycemic ketosis (9/35; 25.7%)	• Median age 60 y • Known T2D in 28/35 (80%), known T1D in 5/35 (14%); only 2/35 (5.7%) with new presentation of diabetes
Lawrence et al. *Diabet. Med*. 2021.[80]	Retrospective review of newly diagnosed T1D cases and all pediatric ED visits at a tertiary center	• Australia: 3/30–5/20 vs. same period in 2015–2019 • 53 Children <18 y with the initial diagnosis of type 1 diabetes over a 6-y period	• New T1D diagnoses comparable in the pandemic period vs. pre-pandemic periods (11 in 2020 vs 6–10 in 2015–2019) • Frequency of DKA (venous pH <7.3) higher during the pandemic period (73% vs 26%) • Frequency of severe DKA (pH <7.1) was higher in the pandemic period vs. pre-pandemic periods (45% vs 5%) • No individuals reviewed were COVID-19 + . • ED visits overall reduced ~27% in 2020.
Tittel et al. *Diabetes Care*. 2020.[81]	Diabetes-Prospective Follow-up registry – 216 centers providing information about incident T1D	• Germany: 3/13–5/13 in each year between 2011 and 2020 • Pediatric T1D (< 18 y); 2020 incidence, based on 532 cases among 13.6 million subjects.	• T1D incidence per 100,000 patient-years increased from 16.4 in 2011 to 22.2 in 2019. • 2020 incidence (23.4, 95% CI: 21.5–25.5) did not differ significantly from the prediction based on prior years.
Unsworth et al. *Diabetes Care*. 2020.[82]	Retrospective review of multicenter regional data from five inpatient units comprising the North West London Pediatric Diabetes Network	• UK: 3/23/20– 6/4/20 • 30 Children (≤16 y) with new onset type 1 diabetes	• 3/5 Units with similar incident diabetes vs. prior 5 y; 2/5 with higher numbers: 10 over 2 months vs. 2 or 4 over 2 months in the prior 5 y. • 21/30 (70%) presented in DKA, with severe DKA in 11/21 (52%). • 5 Children with positive SARS-CoV-2 PCR or IgG: 3 presented with severe DKA (pH<7.1) and refractory hypokalemia • 3 Children with known T1D presented with DKA during the same period
Zubkiewicz-Kucharska et al. *Adv Clin Exp Med*. 2021.[83]	T1D pediatric registry from Center for Pediatric Endocrinology and Diabetology for Lower Silesia	• Poland: 1/1/00– 12/31/19 and 1/1/20–4/30/20 • All new cases of T1D in children aged 0–18 y	• T1D incidence increased over time from 2000 to 2019 • There were half as many cases of new T1D presenting in 3/20–4/20 vs. 3/19–4/19 • DKA in 31.75% of new cases in years 2000–2019 vs. 36.67% in the first 4 months of 2020, and 50% of those diagnosed in 3/20–4/20
Hippich et al. Med (N Y) 2020.[84]	Measured antibodies against SARS-CoV-2 for population-scale immune surveillance	• Bavaria, Germany: 1/20–7/20 • 11,884 Children (5,853 girls, 49.3%) enrolled in the Bavarian Fr1da study; 28 with newly detected islet autoantibodies	• 28 Children in the Fr1da study had newly detected islet autoantibodies and 12 developed clinical T1D • None of the children with new antibodies tested positive for SARS-CoV-2 antibodies. • No SARS-CoV-2Ab+ children developed T1D

infection and activation of interferon response ex vivo are suggestive,[30,31] but also must be interpreted with caution as they may not recapitulate in vivo susceptibility. Although large-scale ACE2 expression and direct infection may not be required for organ injury,[93] histologic findings in donors with active infection seem to point away from immune cell infiltration in pancreata as a common finding.[40,41] Finally, whether β cell effects occur directly or indirectly, the epidemiologic data to-date do not support consistent large-scale β cell injury acutely leading to diabetes. Moving forward, longer follow-up data on the changing incidence of diabetes will be critical. Additionally, longitudinal cohorts such as the global registry of patients with Covid-19-related diabetes (CoviDIAB) Project[10] will be key to

understanding differences in new onset diabetes associated with COVID-19 infection compared to more typical forms of diabetes.

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