SARS-CoV-2 and pancreas: a potential pathological interaction?

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The widespread extrapulmonary complications of COVID-19 have gained momentum; the pancreas is another major target for SARS-CoV-2. Here, we take a closer look into a potential pathological interaction. We provide an overview of current knowledge and understanding of SARS-CoV-2 infection of the pancreas with the special focus on pancreatic islets and propose direct, indirect and systemic mechanisms for pancreas injury as result of the COVID-19-diabetes fatal bidirectional relationship.

COVID-19 caused by SARS-CoV-2 resulted in a widespread global morbidity and mortality and poses a serious threat on public health as a result of detrimental defects on pulmonary, immune, endocrine and homeostatic regulation. Although at the beginning of the outbreak, COVID-19 appeared as pulmonary disease targeting lung alveolar epithelial cells, we now know of many other target cells and organs as well as extrapulmonary complications caused by SARS-CoV-2 with long-term devastating consequences of heterogenous symptoms ("*Long COVID*"), especially critical for metabolic diseases and pathological impacts on metabolically active tissues such as fat, liver and pancreas at the cellular level.

COVID-19 and metabolic derangement: a two-way road

Not only an immune but a metabolic network at both systemic and cellular levels is instrumental for the host to cope with pathogens. SARS-CoV-2 highjacks the cellular machinery to replicate. On its way, it can alter both host metabolic responses and adaptive mechanisms, which are primarily in place to defend the infection, but through massive inflammatory responses, they can trigger chronic collateral damage to metabolic health and result in multi-organ failure. The important bidirectional relationship between COVID-19 and the patients' metabolic status has been observed in numerous studies, such that (1) patients with obesity, type 2 diabetes (T2D) and the metabolic syndrome suffer from more severe and critical COVID-19 disease and (2) COVID-19 induces severe metabolic complications in pre-existing diabetes and is even associated with persistent insulin resistance, new-onset hyperglycemia and β-cell dysfunction and subsequently diabetes at an alarming rate [1-3], as observed from several other viral infections, although not as apparently and severely [4].

Substantial epidemiological, clinical and retrospective data showed obesity and T2D, two frequently co-existing chronic metabolic disorders, as risk factors for severe COVID-19 and higher mortality [1]. The strong association between higher COVID-19 mortality and

obesity indicates that obesity shifts COVID-19 towards fatal disease with adverse outcomes in all populations. Underlying disease mechanisms are complex and multifactorial, similar to the metabolic syndrome itself, including abnormally elevated glucose metabolism and resultant intensified viral replication, impaired innate and/or adaptive immune responses, endothelial dysfunction, insulin resistance and last but not least the excessive chronic systemic inflammation, referred to as “cytokine storm” in COVID-19 [1]. All these mechanisms show shared pathophysiology to some degree and are likely to act synergistically in exacerbating the severity of SARS-CoV-2 infection and compromising metabolic health, which is supported by the observation that tight glycemic control could minimize the severity of the disease and that glucose levels directly correlate with COVID-19 severity [1].

Reciprocally, metabolic disturbance has frequently been observed during COVID-19 disease with alert glucose metabolism, ketoacidosis and hyperglycemia in patients with and without pre-existing diabetes, or development of new-onset diabetes as well as pancreas injury [1-3]. Besides endocrine disorders, several reports indicate SARS-CoV-2 associated acute pancreatitis and exocrine derangements [5].

For many years, clinical, epidemiological, pathological and *in vitro* studies have implicated enteroviruses as initiators of autoimmunity and β-cell failure in genetically susceptible individuals [4]. Enteroviruses could trigger β-cell autoimmunity or exert direct cytotoxicity on pancreatic β-cells [4]. Whether also SARS-CoV-2 is a diabetogenic virus initiating (1) direct destruction of β-cells, whether SARS-CoV-2 (2) causes pleiotropic alterations of glucose metabolism that could trigger diabetes or whether (3) the exocrine pancreas is the major SARS-CoV-2 target and would then secondarily lead to disturbance within the endocrine pancreas is under debate (see Fig.1).

SARS-CoV-2 entry receptors in pancreatic cells: twilight zone

Angiotensin-converting enzyme 2 (ACE2), an integral component of the renin-angiotensin-aldosterone system (RAAS), is the key entry receptor for SARS-CoV-2. In principle, SARS-CoV-2 cell entry requires engagement of the virus' spike subunit with ACE2 following priming of the spike protein by the SARS-CoV-2 entry-associated cellular proteases TMPRSS2 or CTSL. Cellular co-expression of ACE2 and TMPRSS2 has been considered critical for efficient SARS-CoV-2 uptake. Entering through the respiratory tract, SARS-CoV-2 initially destroys ACE2 receptor expressing alveolar cells. However, ACE2 is widely expressed in other organs like the heart, kidney, gut and pancreas [6]. Multiple independent labs investigated whether the canonical SARS-CoV-2 cell entry machinery is present in human pancreatic cells, which resulted in many studies which did find pancreatic islets' ACE2 or TMPRSS2 expression [5, 7-14], whiler other studies did not [6, 15, 16] (Table 1). A more consistent result among different groups is the substantial ACE2 expression in pancreatic ductal cells and in the microvasculature. In particular, this has been confirmed by extensive profiling using multiple complementary approaches from single-cell RNA-seq to fluorescence *in situ* hybridization and IHC [6, 15, 16], together with extremely low to undetectable expression levels in islets from donors with and without diabetes. In contrast, others demonstrated that primary human or stem cell-derived β-cells presented a considerable expression, albeit heterogeneously, of ACE2 or TMPRSS2 (Table 1) [5, 7-14]. To add more to the controversy, conflicting data were also obtained in regards to a potential correlation of ACE2 or TMPRSS2 expression with BMI [8, 9, 15, 16] or with diabetes [8, 15]. While Taneera *et al*. [8] reported significant upregulation of ACE2 in diabetic donors, Coate *et al*. [15] and We *et al.* [13] challenged such finding by showing no differences in ACE2 expression between non-diabetic donors and individuals with T2D. The rationale behind such analysis is that potential upregulation of islet ACE2 expression in diabetes may foster SARS-CoV-2's entry and subsequent

replication, compromising a natural cellular defence response which would accelerate excessive local inflammation and β-cell demise.

Differences in methodological or technical approaches such as tissue processing and preservation, imprecise reagents or biological variations including heterogeneity in islet and pancreas organ donors and low-sample sizes as well as rapid data evaluation and publication turnover may explain the discrepancy in results. Islets are well-known for unspecifically picking-up immunoglobulins which had led to several misinterpretations. It is important to note that none of the studies which had validated ACE2 antibody specificity obtained samples from ACE2 loss-of-function systems, i.e. from ACE2-KO mice or virally-depleted ACE2 in isolated islets, which could critically be useful in the future to resolve the discrepancy.

As ACE2 is needed for canonical SARS-CoV-2 cell entry, most of the initial aforementioned studies set ACE2 together with the protease TMPRSS2, although dispensible [14], as primary indicators for SARS-CoV-2 entry into pancreatic islets and as a proof-of-concept for islet tropism towards SARS-CoV-2. However, other SARS-CoV-2 entry factors, such as neuropilin 1 (NRP1), the transferrin receptor TFRC, the pro-protein convertase FURIN, another protease CTSL and dipeptidyl peptidase-4 (DPP4), which is used as entry receptor by MERS-CoV, are expressed in pancreatic islets, with particularly high expression of NRP1 and TFRC in human β-cells [12-14], which may explain some tropism of SARS-CoV-2 within islets for β-cells in COVID-19 patients [14].

In addition to such previous expression studies, further in-depth analyses are still needed to identify the functional SARS-CoV-2 cellular entry and alternative receptors or mechanisms (like HMGB1, cellular heparan sulfate or circulating soluble ACE2 [17]) as there is productive SARS-CoV-2 infection throughout the pancreas *in vivo* in COVID-19 patients as well as *ex vivo* in infected human islets.

SARS-CoV-2 infection of pancreatic cells

Our understanding of the mechanisms and consequences of a SARS-CoV-2 infection has evolved rapidly since the onset of the pandemic; its pathogenic drivers, target cell type(s) including SARS-CoV-2 infection and tropism in various endocrine islet cell models.

Early striking observations show SARS-CoV-2 infection of hPSC-derived islet organoids, hPSC-derived islet xenografts and primary human α- and β-cells [7] and iPSC-derived pancreatic endocrine and exocrine cells [11] and human islets [10] resulting in cellular as well as transcriptional alterations of genes linked to β-cell function and upregulation of interferon (IFN)-mediated inflammatory signatures, markers of oxidative stress and cell death [7, 10, 11], the latter reminiscent of the transcriptional upregulation of cytokines and chemokines in lung autopsy samples from COVID-19 patients [7] as well as in SARS-CoV-2 active viral replication [10], which is blocked by the RNA polymerase inhibitor remdesivir, which had provoked hope for its use in therapy. Support for SARS-CoV-2-mediated islet damage is provided by notable morphological and functional alterations including reduced numbers of insulin-secretory granules in β-cells, loss of insulin gene transcription, impairment in insulin secretion and higher numbers of bi-hormonal insulin/glucagon-positive cells; all collectively reflecting a path towards β-cell degranulation, dedifferentiation and loss [10, 13, 14]. Indeed, SARS-CoV-2 infection of human islets leads to lower insulin gene expression in β-cells, while glucagon and other α-cell as well as acinar cell markers are upregulated in β-cells. Such loss in insulin as well as an unusual presense of trypsin/insulin double-positive cells were confirmed in autopsy samples from COVID-19 patients hinting towards SARS-CoV-2 induced β-cell transdifferentiation [14]. Facing the low capacity of β-cell turnover as well as rarely seen α-to-β-cell differentiation, it is unclear at this stage, whether such Janus-faced cells may ever go back to a fully functional β-cell.

Furthermore, through intrinsic alterations in the cellular inflammatory status, SARS-CoV-2 virus-mediated pancreatic damage may initiate recruitment of tissue-resident immune cells, which constitutes a path towards eventual β-cell autoimmunity. This merits future long-term epidemiological and clinical observations, since despite a high prevalence of mostly asymptomatic SARS-CoV-2 infection in children, there is so far no association between SARS-CoV-2 antibodies and type 1 diabetes (T1D) autoimmunity [18], which was confirmed this year by national registries.

Importantly, several independent groups attempted to identify and localize SARS-CoV-2 RNA or protein in pancreases from deceased COVID-19 patients, so far with relatively low-sample size. The virus nucleocapsid protein SARS-CoV-2-N was found in the exocrine-interlobular ductal compartment, often in close proximity to islets [10, 16], or in insulin-negative, β-cell marker NKX6.1-positive cells within islets, and rarely and occasionally, capsid/ insulin-double-positive cells were observed [10].

In contrast, several other studies [11-14] revealed the presence of SARS-CoV-2 protein in β-cells within the islets of autopsy pancreases from COVID-19 patients, albeit with heterogenous pattern and variations among pancreases, which indeed indicates β-cell infection during the course of disease; whether infection persists needs to be identified. Resulting islet damage is suggested by apoptotic or necroptotic cell death in islets from infected human islets or COVID-19 pancreases [12, 13], reminiscent of islets in T1D. Consistently, albeit assessed in only two COVID-19 patients, islets show immune cell infiltration with a general increase in inter-islet CD45-positive cells [12].

Altough histopathological observations from all these previous studies did not achieve consistent results and range from infected exocrine compartment close to the islets to clear infection of β-cells, which indicate -despite different methodology used in the studies- very heterogenous disease mechanisms; similar to what is seen in diabetes itself.

A noticable infection of the exocrine pancreas, in particular in ductal structures raises the question whether there is another causal link between SARS-CoV-2 and acute pancreatitis and even new-onset diabetes. However, examination of few pancreatic tissues from COVID-19 patients did not show pathological evidence of pancreatic inflammation [15]. From more comprehensive pancreatic histopathological analyses of stool and blood in association with enterovirus infection in the past, it became clear that an interplay of environmental factors, i.e. viral infection together with a strong genetic predisposition towards virus-associated pathways may finally result in autoimmunity and T1D, however, a single virus infection has not been sufficient neither to cause autoimmunity nor T1D [4] and thus, a definitive conclusion on the COVID-19-diabetes association from such few pancreas studies without the availability of respective genetic analyses and long-term assessments is currently impossible.

Certain pathophysiological features, among them chronic inflammation with immune cell activation unite pancreatic diseases including pancreatitis and all forms of diabetes. Therefore, pathogenesis of the viral infection is dependent on the mechanism of action of the virus on target cells during the course of infection and viral persistence in target cells thereafter. More comprehensive studies in larger patient cohorts of different demographics, ethnicities, and geographic location with long-term clinical follow-ups are needed together with the exact cellular expression, frequency and specific pattern of infection and the virus-initiated cellular interplay with the immune system. At this point, we hypothesize an evolving SARS-CoV-2 targetted pancreas pathology in a direct, indirect and/or systemic manner (see Figure 1 for proposed model).

In concert, results obtained during the 2019-2021 pandemic represent (1) intriguing SARS-CoV-2 effects on the pancreas and (2) a possible fatal consequence of SARS-CoV-2 infection on endocrine islets and interconnected metabolic derangements. Detailed mechanistic knowledge is critically important to determine susceptibility of

pancreatic cells for SARS-CoV-2 in the context of COVID-19, as well as defence strategies to protect vulnerable cells in order to prevent severe SARS-CoV-2 disease outcome, including *Long COVID*.

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Competing interest statement

The authors declare no competing interests.

Table 1. Viral factors expressions as well as SARS-CoV-2 tropism in different pancreatic endocrine and exocrine cells extracted from recent literature.

Human pancreatic cell type	ACE2 expression	TMPRSS2 expression	Viral tropism
Endocrine β-cell	Expressed [5, 7-13] Extremely low or undetected [6, 15, 16]	Expressed [7, 8, 10, 12, 13] Extremely low or undetected [15, 16]	Entered and/or infected [7, 10-14] None [16]
Stem cell derived β-cell	Expressed [7, 11]	ND	Entered and/or infected [7, 11]
Endocrine α-cell	Expressed [7-10, 13] Extremely low or undetected [15, 16]	Expressed [7, 8, 10, 13] Extremely low or undetected [15]	Entered and/or infected [7, 13, 14]
Exocrine acinar cell	Expressed [5, 7, 10, 11, 15, 16]	Expressed [7, 11, 15, 16] Extremely low or undetected [10]	Entered and/or infected [11, 14]
Exocrine Ducts	Expressed [5-7, 9-11, 15, 16]	Expressed [7, 10, 15, 16]	Entered and/or infected [10, 11, 14, 16]
Microvasculature	Expressed [9, 12, 15, 16]		[12, 14]

ND; Not determined

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Figure legend

Figure 1. Proposed model of how SARS-CoV-2 targets islet β-cells. SARS-CoV-2 may interact with β-cells in islets through three different mechanisms. (I) *directly*: virus entry through several viral receptors in β-cells and their subsequent injury as a result of direct acute viral damage or long-term persistent presence of uncleared SARS-CoV-2. In both scenario, SARS-CoV-2 directly induces β-cell dysfunction and death or acts as initiator of β-cell autoimmunity. (II) *indirectly*: SARS-CoV-2 infects viral receptor-expressing pancreatic cells such as ductal or endothelial cells and pericytes within the microvasculature resulting in their structural and functional transformations leading to local inflammation and cytokine and chemokine release as well as generation of a pro-diabetic milieu which can perturb integrity of neighboring non-infected β-cells in a paracrine fashion and potentially leads to β-cell loss or dysfunction. (III) *systemically*: SARS-CoV-2 targets putative viral receptor-expressing cells in metabolic organs such as liver, fat and kidney causing loss of disease-tolerance mechanism, metabolic derangement and maladaptive functions. This can lead to systemic inflammation and the accumulation of pro-diabetic metabolites and ultimately damage β-cells constituting another possible mechanism of SARS-CoV-2 infection-related islet damage.

*Potential SARS-CoV-2 viral entry factors such as ACE2 (see table 1), NRP1, TFRC, FURIN, DPP4 and **associaed proteases such as TMPRSS2 (see table 1), CTSL. Created using smart servier medical art under https://creativecommons.org/licenses/by/3.0/.