REVIEW

Going viral in the islet: mediators of SARS-CoV-2 entry beyond ACE2

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Abstract

Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Following initial infection of airway epithelia, SARS-CoV-2 invades a wide range of cells in multiple organs, including pancreatic islet cells. Diabetes is now recognised as a risk factor for severe COVID-19 outcomes, including hospitalisation and death. Additionally, COVID-19 is associated with a higher risk of new-onset diabetes and metabolic complications of diabetes. One mechanism by which these deleterious outcomes may occur is via the destruction of insulin-producing islet β cells, either directly by SARS-CoV-2 entry into β cells or indirectly due to inflammation and fibrosis in the surrounding microenvironment. While the canonical pathway of viral entry via angiotensin-converting enzyme 2 (ACE2) has been established as a major route of SARS-CoV-2 infection in the lung, it may not be solely responsible for viral entry into the endocrine pancreas. This is likely due to the divergent expression of viral entry factors among different tissues. For example, expression of ACE2 has not been unequivocally demonstrated in β cells. Thus, it is important to understand how other proteins known to be highly expressed in pancreatic endocrine cells may be involved in SARS-CoV-2 entry, with the view that these could be targeted to prevent the demise of the β cell in COVID-19. To that end, this review discusses alternate receptors of SARS-CoV-2 (CD147 and GRP78), as well as mediators (furin, TMPRSS2, cathepsin L, ADAM17, neuropilin-1, and heparan sulphate) that may facilitate SARS-CoV-2 entry into pancreatic islets independent of or in conjunction with ACE2.

Introduction

COVID-19 and diabetes

In 2019, 9.3% of the world’s population was estimated to have diabetes, a number that is predicted to increase by 25% within the next 10 years (Saeedi et al. 2019). At the same time, despite the availability of effective vaccines, SARS-CoV-2 infections are predicted to continue around the world (Telenti et al. 2021). These two pandemics are closely linked, and each condition may contribute to the global burden of the other. Prevalent diabetes is an independent risk factor for hospitalisation and death among individuals with COVID-19 (Wander et al. 2021). Conversely, a positive test for SARS-CoV-2 is associated with a 40% higher risk of new-onset diabetes and increased use of glucose-lowering medications, including insulin (Al-Aly et al. 2021, Wander et al. 2022, Xie & Al-Aly 2022).
Moreover, an excess burden of metabolic complications commonly associated with diabetes is observed within months of COVID-19 infection. These sequelae include disorders of lipid metabolism and obesity (Al-Aly et al. 2021). Such outcomes are exacerbated in hospitalised individuals with COVID-19, where risks are highest in those admitted to intensive care (Al-Aly et al. 2021).

While islet cell autoantibodies are rarely detected, new-onset diabetes after COVID-19 appears to be frequently complicated by diabetic ketoacidosis (DKA) (Mista et al. 2021). New-onset diabetes that presents with DKA has been recognised as ketosis-prone and is marked by significant impairment of insulin secretion and insulin action at presentation (Vellanki & Umpierrez 2017). Together, these lines of evidence suggest that early β-cell injury may contribute to the pathogenesis of new-onset diabetes after COVID-19; however, mechanisms contributing to such β-cell injury, including the critical pathways mediating viral entry, remain poorly characterised.

**SARS-CoV-2 enters human pancreatic endocrine cells**

Studies investigating viral entry into islet α and β cells have done so using either SARS-CoV-2 itself or a pseudovirus where the envelope glycoprotein is replaced by the SARS-CoV-2 spike protein. In human islets, SARS-CoV-2 was shown to enter α and β cells ex vivo (Yang et al. 2020, Müller et al. 2021), with viral infiltrates detected mostly in insulin-positive β cells ( Wu et al. 2021). Although considered functionally immature, human pluripotent stem cell–derived α and β cells were permissive to SARS-CoV-2 pseudovirus infection in vitro and in vivo when xenografted into mice (Yang et al. 2020). Further, immunohistochemical and molecular analyses showed SARS-CoV-2 infiltration in the pancreas from humans with COVID-19, particularly in β cells, sometimes more so than in other pancreatic endocrine cells (Müller et al. 2021, Steenblock et al. 2021, Tang et al. 2021a, Wu et al. 2021). In sum, there is convincing evidence that SARS-CoV-2 directly infects the islet.

Following infection, SARS-CoV-2 causes morphological, transcriptional, and functional derangements in islet cells (Mine et al. 2021). For example, dilatation and vacuolisation of the endoplasmic reticulum (ER)–Golgi apparatus complex was evident in SARS-CoV-2-infected islet cells (Müller et al. 2021). At the transcriptional level, loss of β-cell identity was observed, wherein α- and acinar cell markers were increased and insulin was decreased in SARS-CoV-2–vs mock-infected β cells (Müller et al. 2021, Tang et al. 2021a). Insulin protein was also decreased in SARS-CoV-2-infected β cells (Tang et al. 2021a), as was number of insulin secretory granules, C-peptide and insulin immunoreactivity, and glucose-stimulated insulin secretion (Müller et al. 2021). It is suggested these perturbations may be mediated by ER stress (Müller et al. 2021). SARS-CoV-2 infection of islets also upregulated several interferon-stimulated genes (Müller et al. 2021), indicative of an inflammatory response that may exacerbate cellular damage.

**ACE2-mediated SARS-CoV-2 entry**

Angiotensin-converting enzyme 2 (ACE2) is the canonical receptor of SARS-CoV-2. Following the binding of SARS-CoV-2 to ACE2, there are two routes for viral entry into host cells: direct membrane fusion and endocytosis.

**Host cell membrane fusion pathway**

SARS-CoV-2 contains a transmembrane spike glycoprotein comprising the S1 and S2 subunits. S1 binds to the host cell receptor, and S2 mediates fusion of the viral and host cell membranes. As illustrated in Fig. 1, SARS-CoV-2 entry begins with binding of the spike protein to ACE2 (Hoffmann et al. 2020a). SARS-CoV-2-spikemust be sequentially cleaved along the S1/S2 junction, usually by furin (Hoffmann et al. 2020b), and at a second site (S2‘) by host serine proteases, typically transmembrane serine protease 2 (TMPRSS2) (Hoffmann et al. 2020a) (Fig. 1). This exposes hydrophobic amino acid residues in processed S2 that embed themselves into the host cell membrane, further facilitating fusion of the viral envelope and host cell membrane (Huang et al. 2020) (Fig. 1).

**Alternative endocytosis pathway**

Alternatively, SARS-CoV-2-spikem binding to ACE2 is followed by the uptake of virions into endosomes. Cathepsin L (CTSL), a pH-sensitive endosomal protease, primes the spike by cleaving it into smaller fragments after the initial furin-mediated S1/S2 cleavage (Zhao et al. 2021). The viral envelope then fuses with the endosomal membrane, releasing viral machinery and genetic material. Although the membrane fusion pathway is 100–1000 times more efficient than the endocytosis pathway (as measured for SARS-CoV) (Matsuyama et al. 2005), the mode of viral entry among different cells is dependent on protease expression (Padmanabhan et al. 2020).

**ACE2 expression in α and β cells**

While the literature is largely in agreement that SARS-CoV-2 enters the islet, the mechanism of viral entry is still being
debated. A major point of uncertainty is whether ACE2 is expressed in α and β cells (El-Huneidi et al. 2021). Data in this regard have derived mostly from RNA sequencing (RNA-seq) and immunohistochemistry analyses.

RNA sequencing
Two studies demonstrated ACE2 expression in α and β cells, one via single-cell RNA-seq (scRNA-seq) on adult human islets (Yang et al. 2020) and the other by analysing existing scRNA-seq data sets (Lazartigues et al. 2020). However, most of the data from a total of 11 bulk and single-cell RNA-seq data sets show little ACE2 expression in α or β cells (Coate et al. 2020, Kusmartseva et al. 2020, Lee et al. 2020, Liu et al. 2020, Qadir et al. 2021, Wu et al. 2021) and quantitatively much lower expression than key genes enriched in α (e.g. GCG, ARX, and IRX2) and β (e.g. INS, PDX1, and MAFA) cells (Coate et al. 2020).

Immunohistochemistry
Among studies showing positive ACE2 staining in islets, there is general agreement that β cells express more ACE2 than α cells. In fact, β cells had the highest frequency of ACE2 staining among pancreatic endocrine cell types (Müller et al. 2021). One paper described islet ACE2 immunofluorescence as weak and diffuse, arising from a subset of cells that were identified to be β cells and not α cells (Fignani et al. 2020). Further, ACE2 in β cells was predominantly found in insulin secretory granules, with some expression on the plasma membrane (Fignani et al. 2020). Interestingly, a study that utilised various antibodies raised against different regions of ACE2 found that the prevalent ACE2 isoform in β cells is short ACE2 (Fignani et al. 2020). Given that short ACE2 lacks the amino acid residues required to bind SARS-CoV-2-sp (Onabajo et al. 2020), it is unlikely to be responsible for viral entry. Finally, two larger cohort studies utilising multiple anti-ACE2 antibodies on pancreatic sections from female and male donors with and without SARS-CoV-2 infection and a range of ages and BMIs found that pancreatic endocrine cells exhibited little/no ACE2 expression (Coate et al. 2020, Kusmartseva et al. 2020). Despite extensive validation, the commonly used anti-ACE2 antibodies produce vastly different staining patterns among various studies (Yang et al. 2010, 2020, Brar et al. 2017, Coate et al. 2020, Fignani et al. 2020, Hikmet et al. 2020, Kusmartseva et al. 2020, Lazartigues et al. 2020, Müller et al. 2021, Qadir et al. 2021, Steenblock et al. 2021, Wu et al. 2021). These differences could not be explained by β-cell maturity level or technical aspects, like antibody dilution (Coate et al. 2020).

What may be contributing to the variability in islet ACE2 expression across studies of human donors? One factor may be differences in clinical characteristics such as age, sex, and the presence of SARS-CoV-2 infection. Another important factor, particularly in SARS-CoV-2-positive donors, is the presence of pro-inflammatory cytokines, which can upregulate β-cell/islet ACE2 expression (Fignani et al. 2020). Since cytokine levels change over the course of COVID-19 disease, the timing of sample collection in
relation to onset of illness in SARS-CoV-2 positive donors is a critical consideration for assessing ACE2 expression.

The cytokine milieu to which islets are exposed may influence the ability of ACE2 to mediate SARS-CoV-2 entry. In Fig. 2, potential scenarios are presented for SARS-CoV-2 entry into islet cells in the setting of high vs low ACE2 expression, taking into account the roles of other viral entry mediators. When ACE2 is sufficiently expressed, cofactors and co-receptors may assist ACE2-mediated entry, and alternative receptors may work in parallel to increase the rate of viral entry. If islet ACE2 levels remain too low to permit viral entry, alternative receptors may be primarily responsible for SARS-CoV-2 entry into islet cells. Additionally, viral invasion of the islet may induce a local inflammatory response (Müller et al. 2021). This may affect expression of entry mediators (Chu et al. 2018, Cantuti-Castelvetri et al. 2020), potentially altering the mode of subsequent infection by virions into the same or surrounding host cells so that it differs from that of the original infection.

The following sections outline the roles of different viral entry mediators, which may function as, or alongside, SARS-CoV-2 receptors (Fig. 3). Thus far, many studies of SARS-CoV-2 entry have utilised lung tissue or related models; this review aims to contextualise data on entry mediators to the endocrine pancreas.

Alternative SARS-CoV-2 receptors

Cluster of differentiation 147

Function

The cluster of differentiation 147 (CD147), also known as basigin or extracellular matrix metalloprotease inducer, is a widely expressed member of the immunoglobulin superfamily. It is predominantly membrane-bound and highly glycosylated. It is thought to be involved in cell-cell recognition, although its role in inducing extracellular matrix metalloproteases is most extensively studied. CD147 was found to be increased in pulmonary fibrosis (Guillot et al. 2006), and inhibition of CD147 reduced the differentiation of fibroblasts to myofibroblasts (Ulrich & Pillat 2020). This raises questions about its possible role in islet fibrosis, especially with COVID-19.

Expression in the islet

CD147 is very highly expressed in α, β, and γ cells (Uhlén et al. 2015, Segerstolpe et al. 2016), though one study reported it absent in islets (Zhao et al. 2001). Though not reported in islets, CD147 is upregulated in various cell types upon ER (Grass & Toole 2015) and oxidative (Ke et al. 2012) stress, which occur in patients with COVID-19 (Rosa-Fernandes et al. 2021).

Role in SARS-CoV-2 infection

The ability of CD147 to serve as an alternative SARS-CoV-2 receptor is exemplified by the demonstration that cells otherwise not susceptible to viral infection allowed SARS-CoV-2 (and pseudovirus) entry upon CD147 expression (Wang et al. 2020). Additionally, humanised CD147 mice infected with SARS-CoV-2 had detectable viral loads in their lungs, unlike virus-infected WT mice (Wang et al. 2020, Geng et al. 2021). Some (Wang et al. 2020, Geng et al. 2021), but not all (Ragotte et al. 2021, Shilts et al. 2021), studies provide evidence that SARS-CoV-2-spike binds to CD147. CD147-SARS-CoV-2-spike binding is also supported by molecular modelling data (Helal et al. 2020) and evidence of CD147 and SARS-CoV-2-spike colocalisation in lung and kidney tissue from donors with COVID-19 (Wang et al. 2020). Further, pseudovirus infection of CD147-expressing cells could be neutralised by addition of the extracellular...
domain of CD147, suggesting it competes with membrane-bound CD147 for spike binding (Wang et al. 2020). In intervention studies, CD147 overexpression increased SARS-CoV-2 infection (Wang et al. 2020), whereas CD147 blockade/knockdown had the opposite effect in most (Wang et al. 2020, Fenizia et al. 2021, Geng et al. 2021) but not all (Ragotte et al. 2021) studies.

CD147 facilitates SARS-CoV-2 entry into host cells via endocytosis (Wang et al. 2020). It does not bind ACE2 (Wang et al. 2020); however, CD147 silencing reduced ACE2
protein levels (Fenizia et al. 2021), suggesting an interaction between the two proteins that is yet to be understood. After SARS-CoV-2 infection, CD147 was upregulated in human airway epithelial cells (Fenizia et al. 2021) – a response that may exacerbate virus entry.

Concluding remarks
CD147 may serve as an alternative SARS-CoV-2 receptor (Fig. 3A) – as opposed to a cofactor/co-receptor, especially as it promotes SARS-CoV-2 infection under conditions in which (human) ACE2 receptor is not expressed. It may also act via other mechanisms to permit viral infection. CD147 is worthy of study in the mediation of SARS-CoV-2 entry in islet cells with and without high ACE2 expression.

Glucose regulatory protein 78
Function
Glucose regulatory protein 78 (GRP78) is an ER-localised chaperone that regulates ER signalling molecules to ensure proper protein folding. During ER stress, the unfolded protein response upregulates GRP78. However, GRP78 recycling from the Golgi system to the ER becomes saturated and GRP78 is missorted to the cell surface. Thus, cell surface GRP78 (csGRP78) can act as a receptor and may be involved in signal transduction (Ibrahim et al. 2019). Although ER and plasma membrane GRP78 are most commonly studied, GRP78 also exists as mitochondrial, cytosolic, secreted, and nuclear proteins (Ni et al. 2011).

Expression in the islet
GRP78 is expressed in all pancreatic cell types, including β cells (Uhlén et al. 2015, Segerstolpe et al. 2016). Some (Allagnat et al. 2012, Brozzi et al. 2015), but not all (Cardozo et al. 2005, Pirot et al. 2006, Åkerfeldt et al. 2008) studies show GRP78 to be upregulated in β cells under cytokine stress. Further, GRP78 is increased in diabetic mouse models (Laybutt et al. 2007, Wang et al. 2009) and islets from donors with type 2 diabetes (T2D) compared to non-diabetic donors (Laybutt et al. 2007, Hull et al. 2009). However, some studies find no significant increase in islet GRP78 levels in T2D donors (Marchetti et al. 2007, Marselli et al. 2020).

Role in SARS-CoV-2 infection
GRP78 may promote viral infection by serving as an alternative receptor and/or stabilising spike-receptor binding ((Ha et al. 2020); Fig. 3A). In silico modelling predicted that GRP78 docks to the receptor-binding domain of SARS-CoV-2-spike (Ibrahim et al. 2020), which has the same GRP78 recognition site as other human and bat coronaviruses (Elifiky 2020). GRP78 binds MERS-CoV-spike in vitro, and blockade/knockdown of GRP78 reduced MERS-CoV infection (Chu et al. 2018). Additionally, GRP78 overexpression increased, but was not sufficient for, MERS-CoV entry into cells (Chu et al. 2018). Similar to studies of MERS-CoV, GRP78 can bind SARS-CoV-2 and ACE2 in vitro at the ER and cell surface of kidney epithelial cells (Carlos et al. 2021). Specifically depleting or blocking csGRP78 decreased SARS-CoV-2 and pseudovirus entry (Carlos et al. 2021). Hence, csGRP78 may bind SARS-CoV-2-spike and act as an alternative receptor, cofactor, or co-receptor, working in place of or in conjunction with ACE2 to facilitate viral entry.

SARS-CoV-2 infection induces ER stress in host cells (Köseler et al. 2020, Rosa-Fernandes et al. 2021), likely due to an inability to accommodate the drastic increase in protein production that occurs during viral replication (Aoe 2020). Hence, coronavirus infection typically upregulates GRP78 (Chan et al. 2006, Chu et al. 2018). In line with this, serum GRP78 levels are increased in COVID-19 patients compared to both COVID-19-negative pneumonia patients and healthy volunteers (Sabirli et al. 2021). Under conditions of ER stress, other SARS-CoV-2 entry factors can also modulate GRP78 levels. For example, CD147 mediates ER stress-induced GRP78 upregulation (Tang et al. 2012). Thus, CD147 upregulation following ER stress may exacerbate GRP78 mediation of SARS-CoV-2 entry in a feed-forward fashion. Additionally, GRP78 can impact cellular ACE2 levels. GRP78 knockdown decreased cell surface ACE2 (csACE2) (but not total ACE2) independent of ER stress, suggesting that GRP78 may be involved in ACE2 trafficking (Carlos et al. 2021). Thus, via several interactions with other entry mediators, GRP78 can influence SARS-CoV-2 entry.

Concluding remarks
GRP78 can promote SARS-CoV-2 entry via multiple mechanisms, which may be due in part to its relationship with other viral entry factors. Apart from ER and cell surface GRP78, other GRP78 forms may also support viral infection, necessitating study of their interactions with SARS-CoV-2 and viral entry mediators. Given its high expression in islets, GRP78 is a prime candidate for initiating and/or mediating viral invasion of pancreatic endocrine cells.
Cofactors and co-receptors in SARS-CoV-2 entry

Several proteins outlined below work alongside ACE2 to mediate SARS-CoV-2 entry in various cell types. Cofactors furin, TMPRSS2, and CTSL are proteases that prime SARS-CoV-2-spike for entry; ADAM17 exerts proteolytic activity on ACE2; co-receptors neuropilin-1 (NRP1) and heparan sulfate (HS) assist SARS-CoV-2-spike-ACE2 binding. According to existing literature, the primary role of these mediators is to increase the efficiency of ACE2-mediated viral entry. This may make ACE2 a significant contributor to SARS-CoV-2 infection in β cells. Similarly, what represents a gap in current knowledge is that these mediators may also work alongside alternative receptors to permit viral entry into islet cells.

Furin

Function
Furin is a transmembrane endoprotease that cleaves a diverse range of proproteins prior to secretion, as a part of their maturation process (Thomas 2002). It is not only localised to the trans-Golgi network but also cycles between the cell surface and endosomes (Shapiro et al. 1997, Thomas 2002). In islet cells, furin controls proliferation and differentiation (Kayo et al. 1996), as well as secretory granule acidification (Louagie et al. 2008), the latter being critical for β-cell granule maturation and proinsulin-to-insulin conversion.

Expression in the islet
Furin has moderate-to-high expression in human pancreatic endocrine cells (Uhlén et al. 2015, Brouwers et al. 2021), including β cells (Sawada et al. 2000, Segerstolpe et al. 2016, Tang et al. 2021a), as shown by RNA-seq and immunohistochemistry.

Role in SARS-CoV-2 infection
As a proprotein convertase, furin cleaves and activates many viral proteins (including SARS-CoV-2 proteins (Murgolo et al. 2021)), thereby facilitating viral entry into host cells (Figs 1 and 3B). This cleavage may occur during viral production in an infected cell or on virus entry into a host cell (Hoffmann et al. 2020b, Shang et al. 2020, Papa et al. 2021). SARS-CoV-2-spike contains a polybasic furin cleavage site (FCS) at the S1/S2 junction (Coutard et al. 2020), which is absent in other SARS coronaviruses (Coutard et al. 2020). Studies in other viruses show that insertion of a similar polybasic FCS increases virulence (Claas et al. 1998). Interestingly, the structure of the FCS suggests that SARS-CoV-2 uses molecular mimicry to hijack host cell machinery for viral entry (Anand et al. 2020). In vitro studies showed that furin knockout decreased viral production 100-fold (Papa et al. 2021). Mutants of SARS-CoV-2-spike resistant to S1/S2 cleavage had significantly reduced entry into TMPRSS2-positive but not TMPRSS2-negative cells, suggesting that furin-mediated cleavage may only be relevant in cells where the membrane fusion pathway predominates over the endocytosis pathway (Hoffmann et al. 2020b). Cells with diminished furin activity still exhibited SARS-CoV-2-spike cleavage, albeit at much reduced levels (Papa et al. 2021). This is likely attributed to less efficient spike processing by other cellular proteases, such as trypsin, matriptase, and proprotein convertase 1 (Jaimes et al. 2020) – of which the last is highly expressed in β cells and thus may be relevant for viral entry in islets.

Concluding remarks
The unique furin cleavage site in SARS-CoV-2 makes viral envelope-host cell membrane fusion more efficient. Since furin is highly expressed in islets, studies are needed to confirm furin-mediated S1/S2 cleavage in islet cells. Moreover, other islet proteases/proprotein convertases may work alongside furin to cleave the SARS-CoV-2-spike protein at the S1/S2 junction (Jaimes et al. 2020, Tang et al. 2021b), potentially increasing the rate of viral entry.

Transmembrane serine protease 2

Function
TMPRSS2 is a membrane-anchored serine protease first characterised as being androgen-regulated in the prostate. It not only plays an important role in prostate cancer development and progression (Chen et al. 2010) but has also been associated with various processes including digestion, tissue remodelling, blood coagulation, fertility, and inflammation.

Expression in the islet
TMPRSS2 expression in islets remains somewhat unclear. Moderate-to-high TMPRSS2 expression in human islets was found by immunostaining (Steenblock et al. 2021) and corroborated by RNA-seq and microarray analysis (Taneera et al. 2020); however, specific cell type was not reported in these studies. Another study showed TMPRSS2 to be highly expressed in a subset of pancreatic endocrine cells enriched in α, β, and δ cells (Uhlén et al. 2015), while yet another...
found TMPRSS2 expression in α but not β cells (Segerstolpe et al. 2016). In contrast, scRNA-seq of primary human islets found low TMPRSS2 levels in α and β cells (Yang et al. 2020). Integrated analysis of six RNA-seq databases found that less than 1.5% of α and β cells expressed TMPRSS2 (Coate et al. 2020). However, another analysis of five scRNA-seq data sets found that 17% of α and 5.7% of β cells expressed TMPRSS2 (Kusmartseva et al. 2020). Overall, TMPRSS2 expression seems to be moderately low in α and β cells but likely high enough in other islet cell types to contribute to moderate/high expression in the islet as a whole.

**Role in SARS-CoV-2 infection**

Once SARS-CoV-2-spike is bound to ACE2, TMPRSS2 cleaves the spike protein at the S2' site to facilitate SARS-CoV-2 entry into cells via the membrane fusion pathway (Hoffmann et al. 2020a) (Fig. 1). TMPRSS2 can also cleave ACE2 at its cytoplasmic tail, increasing viral uptake through the CTSL/endocytosis pathway (Heurich et al. 2014). TMPRSS2 inhibitors were shown to hamper SARS-CoV-2 viral entry. Camostat mesylate, which inhibits serine proteases like TMPRSS2, reduced SARS-CoV-2-spike entry into TMPRSS2-positive cells but not TMPRSS2-negative cells (Hoffmann et al. 2020a). Interestingly, camostat mesylate combined with E-64d (CTSL inhibitor) prohibited viral entry into TMPRSS2-positive cells, whereas E-64d alone did not (Hoffmann et al. 2020a). Overall, this suggests that TMPRSS2 expression can compensate for low CTSL activity in SARS-CoV-2-spike processing (Hoffmann et al. 2020a). In cells with low/no TMPRSS2 expression, other TMPRSS2-related proteases may cleave SARS-CoV-2-spike (Hoffmann et al. 2021). One such protease is TMPRSS4, whose gene expression is highly correlated with ACE2 in lung cells, even more so than TMPRSS2 (Wruck & Adjaye 2020). TMPRSS4 has been shown to promote SARS-CoV-2 entry in intestinal enterocytes by cleaving SARS-CoV-2-spike (Zang et al. 2020). Within the pancreas, TMPRSS4 expression seems to be limited to exocrine and endotheilial cell types (Segerstolpe et al. 2016, Coate et al. 2020, Kusmartseva et al. 2020, Lee et al. 2020), though it is upregulated in pancreatic cancer cells (Katopodis et al. 2021). It is therefore possible that TMPRSS4 is involved in mediating SARS-CoV-2 infection in both the exocrine and endocrine pancreas, the latter via islet endothelial cell interactions.

**Concluding remarks**

The proteolytic activity of TMPRSS2 is important for SARS-CoV-2 viral entry in many cell types, though it is unknown whether this holds true if the entry receptor is not ACE2. Although TMPRSS2 expression in α and β cells may be moderate/low, compensation by other cellular proteases like CTSL may explain the permissiveness of α and β cells to SARS-CoV-2 infection.

**Cathepsin L**

**Function**

CTSL is a lysosomal cysteine endoprotease, though it is also found in the nucleus and can be secreted. It plays a major role in degrading extracellular, cytoplasmic, and nuclear proteins and is involved in a diverse range of processes such as autophagy, apoptosis, cell-cycle regulation, bone resorption, antigen processing, and tumor invasion/metastasis (Yadati et al. 2020). It is required for the development of type 1 diabetes (T1D) in mouse models (Maehl et al. 2005) and regulates human and mouse islet cell proliferation (Lo et al. 2019).

**Expression in the islet**

CTSL is ubiquitously expressed (Dana & Pathak 2020), consistent with its wide range of cellular functions. It is present in all pancreatic cell types (Segerstolpe et al. 2016, Tang et al. 2021a) and is moderately expressed in pancreatic endocrine cells (Tang et al. 2021a), including α and β cells (Kusmartseva et al. 2020). β-cell CTSL expression in T2D donors was shown to be higher than in non-diabetic donors (Kusmartseva et al. 2020).

**Role in SARS-CoV-2 infection**

In a human hepatoma cell line (Huh7), CTSL levels were elevated following SARS-CoV-2 pseudovirus entry (Zhao et al. 2021), while CTSL overexpression per se increased pseudovirus entry (Zhao et al. 2021). Conversely, CTSL knockdown in Huh7 cells or pharmacological inhibition in humanised ACE2 mice using E64d decreased SARS-CoV-2 pseudovirus entry (Zhao et al. 2021). Similarly, inactivation of CTSL with ammonium chloride reduced SARS-CoV-2 entry, but inhibition was weaker in TMPRSS2-positive vs TMPRSS2-negative cells (Hoffmann et al. 2020a). This indicates that TMPRSS2 and CTSL can each compensate for inactivity of the other.

CTSL cleaves SARS-CoV-2-spike downstream of the S1/S2 site, at a region distinct from the TMPRSS2 cleavage site (Murgolo et al. 2021) (Fig. 3C). This action of CTSL enhances virus entry (Zhao et al. 2021). S1/S2 cleavage site mutations (ΔCS) only reduced SARS-CoV-2 entry in cells utilising the ACE2-TMPRSS2 pathway (Hoffmann et al. 2020b). SARS-CoV-2-ΔCS replicates faster than...
WT SARS-CoV-2 in cells lacking TMPRSS2 (Peacock et al. 2021, Zhu et al. 2021), reinforcing a role for CTSL in increased virulence under permissive conditions.

CTSL can be secreted, often under systemic or local inflammatory conditions (Gomes et al. 2020). In keeping with this, COVID-19 patients had elevated circulating CTSL levels compared to healthy volunteers, positively correlating with disease severity (Zhao et al. 2021). This suggests that circulating CTSL may exacerbate viral entry during inflammation that accompanies COVID-19.

Concluding remarks
SARS-CoV-2 infection and CTSL seem to have a bidirectional relationship, whereby viral infection increases CTSL levels and in turn, CTSL mediates SARS-CoV-2 entry. While largely studied in the context of ACE2, this protease may work similarly with alternative receptors and is a good candidate for mediating viral entry into pancreatic endocrine cells with little/no TMPRSS2 expression.

A disintegrin and metalloprotease 17
Function
A disintegrin and metalloprotease 17 (ADAM17), also known as TNF-α converting enzyme, is a membrane-anchored protein responsible for proteolysis of several cell surface proteins (including ACE2; Fig. 3A) to enable ‘shedding’ of their ectodomains.

Expression in the islet
ADAM17 is expressed in the islet, including β cells. A subset of pancreatic endocrine cells enriched in α, β, and δ cells shows moderate ADAM17 expression (Uhlén et al. 2015). Similarly, in α- and β-cell populations from non-diabetic and/or T2D donors, ADAM17 is expressed moderately (Segerstolpe et al. 2016, Coate et al. 2020). Furthermore, circulating ADAM17 levels were elevated in patients with COVID-19 (Palacios et al. 2021).

Role in SARS-CoV-2 infection
In the case of SARS-CoV, internalisation of the virus-ACE2 complex upregulates ADAM17 expression/activity (Haga et al. 2008), as does ER stress (Rzymski et al. 2012). GRP78, which is also upregulated under cytokine/ER stress, protects ADAM17 from inhibition by protein disulphide isomerases (Schäfer et al. 2017). The increase in ADAM17 activity enhances ACE2 ‘shedding’, which decreases sACE2 (Kuba et al. 2005). This may explain the higher soluble ACE2 (sACE2) levels in COVID-19 patients compared to healthy controls (van Lier et al. 2021). Thus, shedding may be a cellular defence mechanism, whereby spike-sACE2 binding is reduced (Zipeto et al. 2020). However, SARS-CoV-2-sACE2 can enter cells via endocytosis mediated by angiotensin II type 1 receptor (AT1R) or vasopressin receptor 1B (AVPR1B) (Yeung et al. 2021), though this has yet to be studied in pancreatic endocrine cells.

While not yet shown with SARS-CoV-2, ADAM17 inhibition significantly attenuated SARS-CoV entry in vitro and reduced viral titres in mice in vivo (Haga et al. 2010). This appears to conflict with the idea above, where ADAM17 upregulation would reduce viral entry into cells. One explanation is that ADAM17 upregulation may increase the cleavage and maturation of several cytokines (Schreiber et al. 2020, Zipeto et al. 2020), whose increased levels may upregulate mediators of SARS-CoV-2 entry.

Concluding remarks
While ADAM17 may be capable of either decreasing or increasing SARS-CoV-2 entry into cells, it is unclear which effect predominates. Schreiber et al. reviews ADAM17’s possible roles in COVID-19 infection, including entry and post-infection inflammation-mediated damage (Schreiber et al. 2020). In sum, more studies are needed to better understand how ADAM17 affects SARS-CoV-2 infection of the islet.

Neuropilin-1
Function
NR1 is a cell surface receptor involved in angiogenesis and organ development. It exists in two isoforms – one is truncated and secreted (sNR1P) and the other is a transmembrane protein (Gagnon et al. 2000). NR1P has a large extracellular domain that binds ligands in a host of signalling pathways associated with cell migration, growth, and development (Pellet-Many et al. 2008). While its cytoplasmic domain has no known signalling sequence, literature on NR1P’s capability to signal independently is divided (Pellet-Many et al. 2008). Interestingly, minor alleles of NR1P were associated with T1D in children (Hasan et al. 2010).

Expression in the islet
NR1P is highly expressed in islets (Tang et al. 2021a, Wu et al. 2021). Within the pancreas, its expression is mostly confined to islets, especially β cells (Hasan et al. 2010), but rarely α cells (Hasan et al. 2010, Wu et al. 2021). NR1P
was found to be upregulated in β cells from humans with COVID-19 (Wu et al. 2021).

**Role in SARS-CoV-2 infection**

Furin-mediated SARS-CoV-2-spike cleavage creates a C-terminal motif (known as CendR) on S1 to which NRP1 binds (Cantuti-Castelvetri et al. 2020, Daly et al. 2020, Li & Buck 2021). NRP1 stabilizes the C-terminus of S1, facilitating more efficient S1/S2 cleavage (Fig. 3B) and allowing S2 to mediate membrane fusion more rapidly (Li & Buck 2021). Specifically inhibiting binding between NRP1 and SARS-CoV-2-S1-CendR reduces viral infection in various cell types, including human islets (Cantuti-Castelvetri et al. 2020, Daly et al. 2020, Wu et al. 2021).

When infected with SARS-CoV-2, NRP1-negative ACE2-positive HeLa cells exhibited less viral entry than cells expressing both NRP1 and ACE2, but cells lacking ACE2 exhibited no viral entry (Daly et al. 2020). In human islets infected with SARS-CoV-2 ex vivo, scRNA-seq showed that ACE2- and NRP1-positive cells had more SARS-CoV-2 nucelocapsid transcripts than cells expressing either ACE2 or NRP1 (Tang et al. 2021a). ACE2-positive NRP1-negative cells had fewer transcripts than ACE2-negative NRP1-positive cells, and double-negative cells showed little/no infection (Tang et al. 2021a). Further, SARS-CoV-2-spike pseudovirus entry was independently facilitated by ACE2 but not TMPRSS2 or NRP1 (Cantuti-Castelvetri et al. 2020). Together, these data support a role for NRP1 as a co-receptor that potentiates ACE2-mediated SARS-CoV-2 infection (Kyrrou et al. 2021), while data on its ability to independently permit viral entry is limited.

**Concluding remarks**

NRP1 is highly expressed in β cells, where it is upregulated in patients with COVID-19 and may serve to exacerbate infection. Its inhibition in human islets reduced SARS-CoV-2 infection, making it a good therapeutic drug target. While NRP1 facilitates SARS-CoV-2 entry as a co-receptor with ACE2 in various cell types, it may function similarly with alternative receptors that are present in islets—this is an area of investigation that requires further study.

**Heparan sulfate**

**Function**

HS exists as a highly acidic, negatively charged, unbranched polysaccharide and is either conjugated to proteins (HS proteoglycans; HSPGs) or remains unconjugated. Present in all cell types, both HS and HSPGs act as cell-surface co-receptors, altering ligand binding affinity. They also maintain extracellular matrix structure, thereby playing a role in cell–cell adhesion and angiogenesis. In mouse islets, HS was shown to be important for β-cell maturation and insulin secretion (Takahashi et al. 2009), as well as β-cell survival and protection from autoimmunity and T1D (Ziolkowski et al. 2012).

**Expression in the islet**

HS exhibits high expression in β cells and islets (Ziolkowski et al. 2012). Mouse models of T2D had lower islet HS and HSPG levels than WT mice (Dhounchak et al. 2021).

**Role in SARS-CoV-2 infection**

HS binds SARS-CoV-2-spike (Clausen et al. 2020) through its receptor-binding domain (Clausen et al. 2020). This increases the proximity of SARS-CoV-2 to the host cell membrane. The heparan-binding site on SARS-CoV-2-spike is adjacent to, but distinct from, the ACE2-binding residues (Clausen et al. 2020). Hence, SARS-CoV-2-spike may bind both ACE2 and HS simultaneously (Clausen et al. 2020) (Fig. 3B). After binding to HS, the receptor-binding domain of SARS-CoV-2-spike undergoes a conformational change (Mycroft-West et al. 2020), which may increase the number of spike proteins bound to any one ACE2 receptor (Clausen et al. 2020).

Treatment with heparinases (Clausen et al. 2020) and disruption of genes involved in HSPG biosynthesis (Clausen et al. 2020, Zhang et al. 2020) markedly reduced SARS-CoV-2 viral infection rates, without impacting ACE2 levels (Clausen et al. 2020) or cell viability (Clausen et al. 2020, Zhang et al. 2020). HS-assisted viral entry requires the actin cytoskeleton, consistent with HS-assisted endocytosis (Zhang et al. 2020).

**Concluding remarks**

HS is highly expressed in β cells/islets, where it could mediate SARS-CoV-2 infection as a co-receptor. There is evidence that SARS-CoV-2 entry is enhanced by formation of the ACE2-SARS-CoV-2-HS complex. Since HS does not directly bind ACE2, it is a good candidate for studies investigating SARS-CoV-2 recruitment to the cell surface when alternative receptors are involved.

**Other mediators of interest**

Several other proteins may mediate SARS-CoV-2 entry in cells that do not utilise the ACE2/TMPRSS2 pathway. One such protein is the transferrin receptor (TFRC), a cell
surface receptor responsible for the endocytosis of iron. TFRC is highly expressed in islets and is present in β cells (Segerstolpe et al. 2016, Wu et al. 2021). TFRC directly binds SARS-CoV-2-spike (Tang et al. 2020), and blocking this binding significantly reduces viral entry (Tang et al. 2020). Since TFRC shuttles between the cytoplasm and plasma membrane, it may transport SARS-CoV-2 in a similar fashion (Tang et al. 2020).

Dipeptidyl peptidase-4 (DPP-4), along with being the MERS-CoV receptor, is a target for several T2D medications. While DPP-4 is expressed in the islet, there is evidence to both support (Bugliani et al. 2018, Steenblock et al. 2021) and refute (Omar et al. 2014, Segerstolpe et al. 2016) its expression in β cells. Some (Li et al. 2020, Vankadari & Wilce 2020), but not all (Cameron et al. 2021) in silico studies find DPP-4 to bind SARS-CoV-2-S1. Further, one in vitro study observed no DPP-4–SARS-CoV-2-spike binding (Xi et al. 2020). DPP-4 was found to be increased in airway samples from patients with COVID-19 (Amati et al. 2020, Maremanda et al. 2020), and a meta-analysis reported that its inhibition was associated with lower COVID-19 mortality (Yang et al. 2021), though this idea faces some resistance (Noh et al. 2021). Overall, more studies are needed to determine DPP-4’s role in SARS-CoV-2 entry.

Other molecules involved in endocytosis, like PIKfyve (Baranov et al. 2020) and TPC2 (Grimm & Tang 2020), may be useful targets to reduce SARS-CoV-2 entry into cells. HDL scavenger receptor B type 1 has been shown to facilitate ACE2-mediated SARS-CoV-2 entry (Wei et al. 2020). Additionally, as previously mentioned, SARS-CoV-2-sACE2 can enter cells via receptor-mediated endocytosis using AT1R and/or AVPR1B. The relevance and impact of these proteins on viral entry in islet cells requires investigation.

Mediators as pharmacological targets

Several therapeutic agents have been proposed and/or approved for treating COVID-19. Here, we highlight two examples that specifically target SARS-CoV-2 entry mediators, and we contrast their potential utility in limiting COVID-19 infection of pancreatic endocrine cells.

Meplazumab is a humanised anti-CD147 MAB. In vitro studies demonstrated its efficacy in blocking SARS-CoV-2 replication in Vero E6 cells and SARS-CoV-2-pseudovirus entry in human T cells (Wang et al. 2020). Several clinical trials are underway to test its effectiveness against COVID-19 infection in humans. In one of these trials, meplazumab was found to reduce disease severity and accelerate recovery, especially in severe and critical COVID-19 cases (Bian et al. 2021). Given that CD147 is highly expressed in islet endocrine cells, meplazumab may prove useful in reducing the entry of SARS-CoV-2 and its deleterious effects in the islet.

Another drug of interest is nafamostat mesylate, which exhibits anti-inflammatory and anticoagulant properties and is currently approved to treat pancreatitis. Nafamostat mesylate is a serine protease inhibitor active against TMPRSS2 (Hempel et al. 2021). In a screen of 24 FDA-approved drugs, it was found to exhibit the greatest antiviral efficacy against SARS-CoV-2 in a human lung cell line whose susceptibility to virus entry is largely TMPRSS2-dependent (Ko et al. 2021). Nafamostat mesylate has also been shown to reduce SARS-CoV-2 infection in mouse models (Li et al. 2021). Despite these promising pre-clinical data, results in humans with COVID-19 infection have been discouraging. For example, there was no significant difference in time to clinical improvement (Zhuravel et al. 2021) or in-hospital outcomes (Inokuchi et al. 2021) between hospitalised patients with COVID-19 treated with vs without nafamostat. Another study reported no evidence of anti-inflammatory, anticoagulant, or antiviral activity with nafamostat (Quinn et al. 2022).

In considering these human data together with evidence that TMPRSS2 expression is moderately low in islet α and β cells, nafamostat mesylate may not be a good candidate for reducing entry of SARS-CoV-2 and its deleterious effects in the islet.

Conclusions

SARS-CoV-2 infects pancreatic endocrine cells, including β cells. The canonical ACE2-TMPRSS2 pathway (Fig. 1) has been extensively researched in airway epithelia, but it remains to be fully understood in the islet. Given that islet ACE2 and TMPRSS2 expression is uncertain, investigation into alternative mediators, including their interactions with one another, is needed to better understand the mechanisms underlying SARS-CoV-2 infection of islets.

Many of the mediators discussed exhibit moderate-to-high expressions in the islet. Given their islet expression patterns coupled with their role in other cells, we propose that furin, CTSL, GRP78, NRP1, CD147, and heparan sulphate are likely mediators of SARS-CoV-2 entry in the endocrine pancreas (Fig. 3). Data on CD147 suggest that it may be an alternative receptor for SARS-CoV-2 entry. GRP78 has been shown to mediate SARS-CoV-2 entry; however, more study is needed to deduce its dependence on other receptors. Furin, CTSL, NRP1, and heparan sulphate may assist ACE2 and/or the dominant SARS-CoV-2 entry pathway.
receptor in the islet to increase viral entry but are unlikely to facilitate viral entry independently. For ADAM17, more work is needed to resolve whether its upregulation after viral entry promotes or hinders infection by subsequent virions. Lastly, while TMPRSS2 mediates viral entry in other cell types, it does not seem to be adequately expressed in β cells for this function.

With respect to islet ACE2, its induction by pro-inflammatory cytokines (and ER stress) may make it an important contributor to islet SARS-CoV-2 entry (Fig. 2), including exacerbation of infection via feed-forward mechanisms. To better understand SARS-CoV-2 entry in the context of cytokine and ER stress, a potential avenue for further study is the ACE2/ADAM17/GRP78 interplay. In Fig. 4, we propose a model for how ACE2, GRP78, ADAM17 and CD147 may interact under conditions of ER stress, including the inflammatory response that accompanies COVID-19.

It is worth noting that SARS-CoV-2 has been shown to infiltrate α cells; therefore, more study is needed to understand how this may impact β-cell function, especially since α-to-β cell communication is required for maintaining normal insulin secretion and glycaemia. Finally, SARS-CoV-2-induced islet dysfunction likely occurs independent of direct viral entry, perhaps from inflammation and fibrosis in the islet microenvironment; however, the literature would suggest this may be in addition to the direct route of viral invasion.

In sum, systematic investigation of candidate SARS-CoV-2 entry mediators in the endocrine pancreas may inform therapeutic strategies to protect islets from the deleterious effects of COVID-19. Such strategies may need to be continually adapted to keep up with new variants of SARS-CoV-2, whose viral entry mechanisms may differ from previous variants.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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Author contribution statement
R R and S Z conceived the idea for the manuscript. R R, P L W and S Z wrote the manuscript. P L W, B B and S Z edited the manuscript.

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