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EDITORIAL

SARS-CoV-2 induced post-translational protein modifications: A trigger for developing autoimmune diabetes?

Abstract

Emerging evidence indicates a bi-directional relationship between SARS-CoV-2 and diabetes. The possibility exists that SARS-CoV-2 could induce diabetes, but it is not yet clear whether this might be a fulminant-type diabetes, autoimmune diabetes, or a new-onset transient hyperglycaemia. This viewpoint discusses mechanisms by which SARS-CoV-2 might trigger type 1 diabetes mellitus (T1DM). Specifically, we looked at the role of post-translational protein modifications (PTMs) and the generation of neoepitopes as a potential mechanism in the induction of islet autoimmunity, and the pathways via which coronavirus infections might exacerbate the formation of PTMs and, in so doing, provoke the onset of T1DM.

KEYWORDS

Autoimmune diabetes, SARS-CoV-2, PTMs, oxidative stress

1 | INTRODUCTION

A growing body of evidence supports a diabetogenic effect of COVID-19. The SARS-CoV-2 virus has been linked to dysglycaemia in existing diabetes,[1] the development of new-onset diabetes[2] and an increase in severe diabetic complications, namely diabetic ketoacidosis.[3] It is, as yet, unclear whether SARS-CoV-2 might also precipitate an autoimmune type 1 diabetes mellitus (T1DM): studies in the UK[4] and Germany[5] have found an excess of T1DM cases during the pandemic whilst, in Italy, one study reported 20% fewer annual cases.[6] Nevertheless, the possibility that SARS-CoV-2 might trigger T1DM in genetically susceptible individuals should be examined, given the known association between respiratory viral infections, including coronaviruses, and the development of islet autoimmunity.[7] Such an exploration is further warranted in light of evidence that individuals with COVID-19 have relatively increased autoantibody reactivities,[8] and the publication of case-reports drawing a link between COVID-19 and the onset of autoimmune conditions, including Guillain-Bare Syndrome, Graves disease, and SLE.[9]

2 | POST-TRANSLATIONAL PROTEIN MODIFICATIONS AND ISLET AUTOIMMUNITY

Post-translational protein modifications (PTMs) are essential for normal cellular functioning. However, such modifications can also enable a breaking of central tolerance through the generation of neoepitopes that provide novel determinants able to activate T-cells. This phenomenon is well recognised in the pathogenesis of autoimmune conditions including rheumatoid arthritis (RA)[10] and coeliac disease.[11] Several antibodies to post-translationally modified islet peptides have now been identified[12] (Table 1). Indeed, antibodies to post-translationally modified insulin are not only more abundant than those to native insulin in newly diagnosed T1DM patients[13] but are also more sensitive and specific biomarkers of disease progression when compared to standard islet autoantibodies.[14] As such, their potential importance in the pathogenesis of T1DM is increasingly recognised.

Several mechanisms by which SARS-CoV-2 might trigger islet autoimmunity have been suggested, including molecular mimicry and prolonged presentation of β-cell epitopes secondary to over-expression of HLA Class I.[24] At present, less attention has been paid as to how viral infections might trigger T1DM by driving increased activity in pathways enhancing post-translational modifications and the production of neo-epitopes. We suggest mechanisms by which viral infections generally, and SARS-CoV-2 in particular, may enhance the formation of neoepitopes and, in doing so, trigger islet autoimmunity in genetically susceptible individuals. These mechanisms relate to (a) islet inflammation and oxidative stress (b) initiation of endoplasmic reticulum (ER) stress and (c) aberrant NETosis (see Figure 1 for a summary of potential mechanisms discussed). It is worth noting that ex vivo evidence of transcriptional changes within β-cells in response to SAR-CoV-2 infection[25] suggests post-transcriptional protein modifications, such as defective ribosomal gene products, may also be a source of neoepitopes generated by COVID-19.

TABLE 1 Neoepitopes identified in autoimmune diabetes

Type of PTM	Modification	Antigen	Reference
Enzymatic	Citrullination	GAD65	McGinty et al. (2014)[15]
		GRP8	Rondas et al. (2015)[16]; Buitinga et al. (2018)[17]
		IAPP	Marre et al. (2018)[18]
	Deamidation	IA-2	McLaughlin et al. (2016)[19]; Acevedo-Calado et al. (2017)[20]; Marre et al. (2018)[18]
		Proinsulin	Van Lummel et al. (2014)[21]
		GAD65	McGinty et al. (2014)[15]
Nonenzymatic	Oxidation	Insulin	Mannering et al. (2005)[22]; Strollo et al. (2015)[13]; Strollo et al. (2017)
	Carbonylation	P4Hb	Yang et al. (2016)[23]

Abbreviation: PTM, post-translational protein modifications.

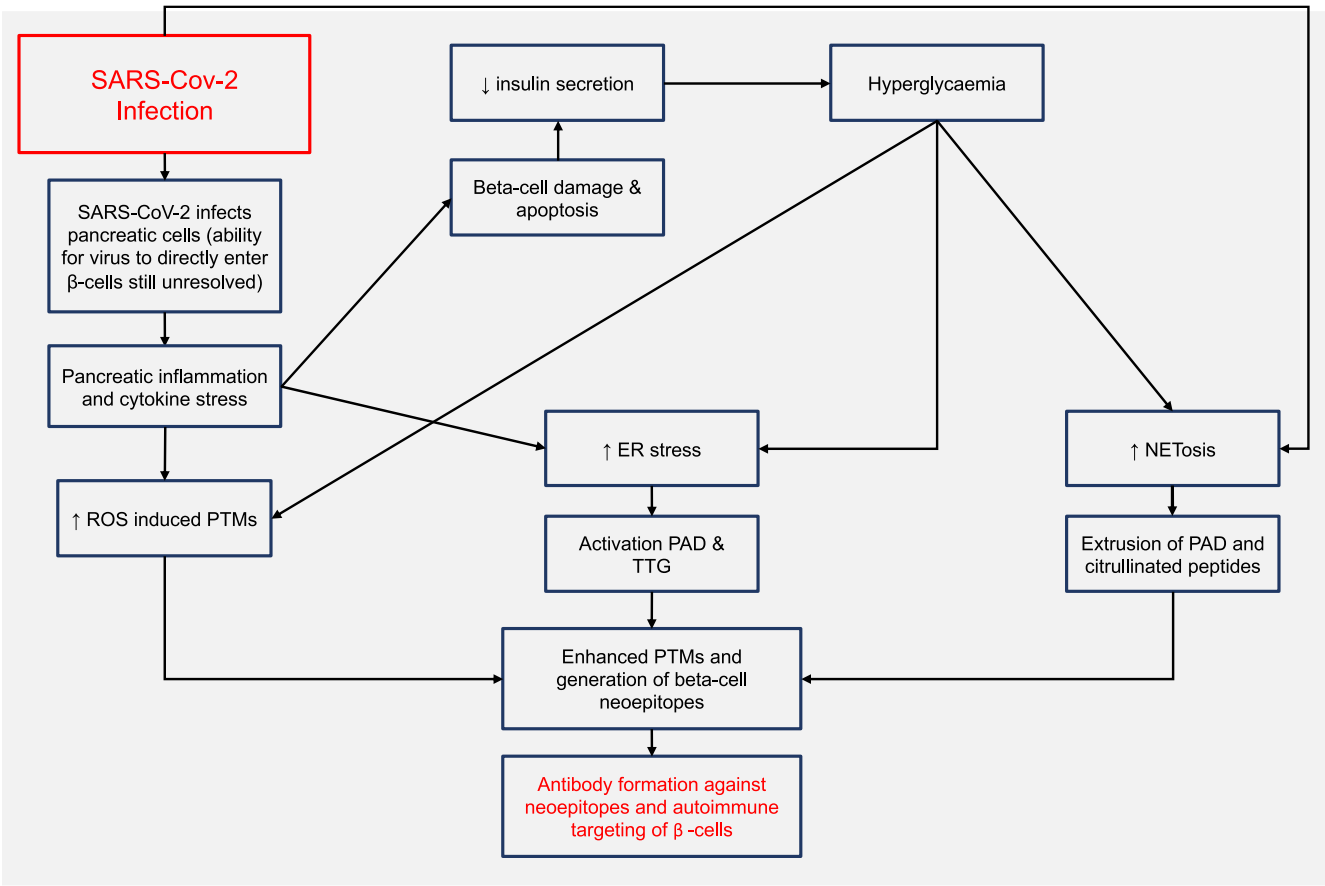

FIGURE 1 Potential pathways of neoepitope generation and autoantibody formation following SARS-CoV-2 infection. Figure produced by C. Chaplin and P. Pozzilli

2.1 | Islet inflammation and oxidative stress

It is now thought that severe disease in COVID-19 is the consequence of the body's own hyper-inflammatory response to the virus, which involves the secretion of a plethora of cytokines - the 'cytokine storm'. Whether SARS-CoV-2 can directly infect islet cells to induce inflammation is currently unresolved. Whilst cellular entry of SARS-CoV-2 is thought to be dependent upon ACE-II receptors[26] and expression of this receptor has been identified in pancreatic β-cells,[27] other studies suggest that β-cell expression may be of insufficient levels to enable viral entry and resulting β-cell damage.[28] Receptors such as Neuropilin-1, which are expressed at high levels by β-cells may, however, provide alternative means of facilitating SARS-CoV-2 entry.[29] In vivo evidence of SARS-CoV-2 infecting β-cells is still relatively limited, although a new study has identified SARs-CoV-2 antigens within NKX6.1-

positive β-cells from analysing pancreatic material of deceased COVID-19 patients.[25] However, even if SARS-CoV-2 is unable to enter β-cells directly, high expression of ACE-II receptors within pancreatic duct cells and the microvasculature[27,28] may still generate an inflammatory pancreatic environment in response to SARS-CoV-2 infection which, through the generation of a hypoxic environment, may indirectly stimulate inflammation within the endocrine pancreas. Reactive oxygen species (ROS) produced in response to cytokine stress can exacerbate protein modifications including oxidation, carbonylation, methylation and citrullination.[30] This oxidative stress may modify proteins directly or, indirectly, through the effect of ROS on downstream cellular pathways.[30] There is evidence that pancreatic inflammation can induce both enzymatic and non-enzymatic PTMs. Experiments exposing human islets to inflammatory cytokines (IL-1β, IFN-γ and TNF-alpha) found them to contain deamidated C-peptides[19] and citrullinated GRP78, a T1DM autoantigen.[16] Pancreatic inflammation can also generate oxidative modifications; human islets cultured with INF-γ, IL-1β and TNF-α have been found to contain elevated levels of carbonyl-modified P4Hb,[23] also a known T1DM autoantigen.[30] Given P4Hb's role in insulin folding, such a modification may lead to abnormal insulin production, hyperglycaemia and the generation of ER stress which, as described below, may also enhance PTMs. Raised levels of INF-γ, IL-1β and TNF-α, cytokines shown to induce PTMs in human islets, have all been found in SARS-CoV-2 positive individuals.[31] Additionally, as high glucose levels can stimulate the production of ROS via the action of NADPH oxidase,[32] the hyperglycaemic state induced by SARS-CoV-2 may serve as an additional source of oxidative stress and further amplify the formation of neoepitopes.

2.2 | Endoplasmic reticulum stress increases the activity of PTM enzymes

Endoplasmic Reticulum (ER) stress describes a state of increased pressure on the ER's role for protein folding. The body's need for insulin means that, physiologically, *β*-cells experience relatively elevated ER stress levels.[33] Evidence suggests excess ER stress could play a role in the development of diabetes: administering chaperone medications to counter ER stress can delay the onset of diabetes in NOD mice.[34] It has also been shown that excess ER stress can enhance neoepitope formation and precipitate immunogenicity.[35] The mechanism via which this occurs results from a cytosolic Ca^{2+} influx in response to ER stress, which leads to activation of calcium-dependent PTM enzymes including tissue-transglutaminase (tTG) and Peptidyl Arginine Deiminases (PAD).[35] Viral infections may exacerbate PTMs directly, through disrupting ER membranes which then leak calcium ions, or indirectly, through triggering inflammatory processes which generate ER stress. With regards to SARS-CoV-2, in the knowledge that hyperglycaemia and glucotoxicity may also generate ER stress,[35] it is possible that a SARS-CoV-2 associated dysglycaemia could contribute to increased ER stress, activation of tTG and PAD enzymes and the production of neoepitopes. Indeed, SARS-CoV2-2 has already been noted to influence the activity of PAD enzymes; a study analysing the transcriptome of human lung biopsy samples from SARS-CoV-2 positive individuals found altered expression of PAD4 and PAD2 enzymes.[36] Furthermore, a study mapping the interactions between SARS-CoV-2 and human proteins identified several interacting proteins associated with ER protein quality control, morphology and the ER stress response.[37]

2.3 | Aberrant NETosis and autoimmunity

NETosis is a feature of the innate immune system involving the production and release of Neutrophil extracellular traps (NETs) - web-like structures comprising histones and degenerative enzymes that act to bind pathogens. Enhanced NET formation has been implicated in the pathogenesis of several autoimmune conditions including RA[38] and, more recently, T1DM.[39] PAD4 enzymes are important in NET formation through catalysing histone citrullination and the induction of chromatin decondensation. It is thought that, in RA, enhanced NETosis may induce autoimmunity through externalising citrullinated proteins[38] and active PAD enzymes, the latter of which can then trigger citrullination of extracellular proteins.[40] Similar mechanisms might also explain the observed association between exaggerated NETosis and T1DM. However, unlike in RA, we are not aware of any identified autoantibodies in T1DM specific to citrullinated NET proteins.

There is some evidence of exaggerated NETosis in SARS-CoV-2 positive individuals.[9] In light of current evidence supporting links between enhanced NETosis, the generation of SARS-CoV-2 induced post-translational protein SARS-CoV-2 could play a role in initiating autoimmunity should not be dismissed. Furthermore, SARS-CoV-2 infection of the pancreatic islets could mediate significant cellular damage and *β*-cell apoptosis, resulting in a release of sequestered islet antigens. With concomitant NETosis, this could provide an opportunity for enhanced citrullination of *β*-cell antigens. It is also interesting to note that NETosis is increased under conditions of hyperglycaemia[39] providing another amplifying effect as to how COVID-19 induced dysglycaemia may lead to cellular conditions favourable to PTMs and the generation of neoepitopes.

3 | CONCLUSION

There is still much to be understood about the pathogenesis of both SARS-CoV-2 and T1DM in isolation. Nevertheless, current evidence suggesting SARS-CoV-2 may have the capacity to induce autoimmunity, and the observed bi-directional link between the virus and diabetes, suggest further research exploring a pathogenic link is warranted. This viewpoint hopes to highlight currently available evidence supporting a mechanistic link between viral infections, post-translational modifications and the initiation of an autoimmune diabetes.

CONFLICT OF INTEREST

No potential competing interests were reported by the authors.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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