

REVIEW ARTICLE

Islet stress, degradation and autoimmunity

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β -cell destruction in type 1 diabetes (T1D) results from the effect of inflammation and autoimmunity. In response to inflammatory signals, islet cells engage adaptive mechanisms to restore and maintain cellular homeostasis. Among these mechanisms, the unfolded protein response (UPR) leads to a reduction of the general protein translation rate, increased production of endoplasmic reticulum chaperones and the initiation of degradation by activation of the ER associated degradation pathway (ERAD) in which newly synthesized proteins are ubiquitinated and processed through the proteasome. This adaptive phase is also believed to play a critical role in the development of autoimmunity by the generation of neoantigens. While we have previously investigated the effect of stress on transcription, translation and post-translational events as possible source for neoantigens, the participation of the degradation machinery, yet crucial in the generation of antigenic peptides, remains to be investigated in the context of T1D pathology. In this review, we will describe the relation between the unfolded protein response and the Ubiquitin Proteasome System (UPS) and address the role of the cellular degradation machinery in the generation of antigens. Learning from tumour immunology, we propose how these processes may unmask β -cells by triggering the generation of aberrant peptides recognized by the immune cells.

KEYWORDS

autoimmunity, β -cell, ER stress, neoantigen, proteasome

1 | INTRODUCTION

Type 1 diabetes (T1D) is an autoimmune disease characterized by destruction of pancreatic β -cells mediated by autoreactive T-cells. During insulinitis, proinflammatory cytokines released by infiltrating immune cells disturb the endoplasmic reticulum homeostasis leading to ER stress.¹ In response to these environmental changes, β -cells initiate an unfolded protein response (UPR)^{2,3} by activation of ER sensors (i.e., IRE1a, PERK and ATF6) and induction of stress factors (ATF3, ATF4, XBP1 splicing). Activation of these pathways promotes cellular repair by reducing protein translation, promoting degradation of misfolded proteins and by activating chaperone protein synthesis to restore ER equilibrium.

This adaptive phase is believed to trigger the development of autoimmunity. Pioneer work from Eizirik *et al* has shown that the presence of inflammatory cytokines affects gene expression and leads

to increased alternative mRNA splicing events providing evidence for β -cell neo-autoantigens generation during insulinitis.⁴ The inflammatory milieu also impacts post-translational modification (PTM) processes: the calcium-dependent enzymes tissue transglutaminase 2 and peptidyl amine deaminase⁵ were shown to be activated in islets and dendritic cells leading to increase in deamidation and citrullination of islet-autoantigens, improving their potency to bind HLA molecules and their immunogenic properties.^{6–10} Such modifications have been demonstrated to increase visibility of β -cells to the immune system as seen by the increased T-cell response observed after chemical induction of ER stress in β -cells.¹¹ Recently, we demonstrated that translation of the insulin mRNA was also influenced by environmental modifications and that ER stress induction led to recruitment of ribosomes at alternative translation initiation sites and to translation of a highly immunogenic insulin-gene derived polypeptide targeted by T-cell autoreactivity in T1D patients.¹² Indeed, the rate of β -cell destruction increased upon inflammatory stress, while expression of

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defective ribosomal products (DRIPs) of insulin was increased by the calcium-dependent stressor thapsigargin, but not by calcium-independent tunicamycin. These translational errors, which are generated when converting genetic information into proteins as well as non-functional proteins resulting from alternative RNA splicing or PTM modifications, are believed to undergo a rapid degradation after synthesis and to constitute a subset of peptides rapidly loaded on MHC class I molecules.¹³

“The inflammatory milieu in type 1 diabetic islets triggers transcriptional, translational and post-translational changes in β -cells leading to the generation of neoepitopes and exposure to the immune system.”

In this process, the ubiquitin proteasome system (UPS) plays a key role but little is known about the regulatory mechanisms involved, and how this degradation mechanism may participate in the development of autoimmunity. In this review, we describe the connection between the UPR and UPS in the generation of antigen class I peptides and its possible role in the generation of β -cell neoantigens. Finally, we will propose possible strategies targeting the proteasome/immunoproteasome for the treatment of autoimmune diabetes.

2 | DEGRADATION AND REGULATORY MECHANISMS

Insulin is synthesized by pancreatic β -cells as proinsulin that co-translationally translocates into the ER lumen. After signal sequence cleavage and folding into its native conformation, proinsulin exits the ER and traffics via the Golgi system to secretory granules. The high demand in insulin, estimated to be up to a million molecules synthesized per minute and per cell¹⁴ (which is consistent with the high amount of insulin mRNA present in each β -cell¹⁵), requires efficient folding of proinsulin molecules in the ER lumen of pancreatic β -cells and targeting to the insulin secretory granules, while proper glucose regulation involves a severe quality control of the secretory pathway. Under stress, the ER associated degradation pathway (ERAD) triggers degradation of proteins that failed to achieve their functional conformation. Recognition of improper folded proteins involves chaperone proteins (BiP, HsP70) recognized by ERAD-associated ligase gp78, HRD1, TEB4, TRC8 or TMEM129. In this process, proteins dislocation and retro-translocation to the cytosol, rather than the secretory pathway, is facilitated by Derlin proteins and the dislocation complex is targeted to the proteasome by ATP dependent ubiquitin-binding factors (Valosin-Containing Protein p97). The close relation between the UPR and the Ubiquitin Proteasome System (UPS) in response to stress suggests that environmental changes may impair the degradation machinery. Dysregulation of this system and inappropriate degradation was shown to lead to the accumulation of cytoplasm aggregates and to the development of neurodegenerative diseases (eg, Huntington, Alzheimer or Parkinson disease¹⁶). Recently, accumulation of vesicles containing proinsulin in β -cells from islet autoantibody positive pancreas donors, suggesting a defect in proinsulin conversion or an

accumulation of immature vesicles caused by an increase in insulin demand and/or a dysfunction in vesicular trafficking.¹⁷ In a similar process, accumulation of islet amyloid polypeptide protein (IAPP) and the formation of amyloid fibrils has been associated with disease progression in type 1 diabetes as seen by the increased CD8 T-cell response to pIAPP in T1D patients.^{18–21} Moreover, downregulation of derlin1, Hrd1 or p97 were shown to reduce proinsulin degradation,²² suggesting that these factors may participate to the generation of stress-induced proinsulin-derived peptides (Figure 1).

In this degradation process, the proteasome plays a central role. This multiproteic complex, expressed in all cell types, is composed of a 20S proteolytic core and two 19S regulatory subunits acting as caps of the core and forming the final 26S holo-structure. The crystal structures of the human 20S core and the 26S complex were unravelled at high resolution.^{23,24} The proteolytic core forms a barrel of four stacked rings of seven subunits each. The two outer rings include the α subunits, and the two inner rings the β subunits. Amongst the β subunits, three different proteolytic activities are encountered. The β 1, β 2, β 5 have caspase, trypsin, chymotrypsin-like functions, respectively. The active residue relies on the N-terminal threonine of the enzymatically active subunits exposed in the luminal part of the barrel. The regulatory 19S multi subunit complex acts as a check point for the entrance of proteins in the 20S barrel, by recognizing the ubiquitinated substrate, removing the ubiquitin chain and delivering the unfolded protein into the proteolytic core. Structurally, it can be stratified into lid and base. The base consists of six ATPases of the AAA family, responsible for unfolding and linearizing the substrate, at ATP expense, and three ubiquitin receptors in charge of substrate recognition. The lid comprises of nine subunits, Rpn3,5 to 9,11,12 and 15, some with undefined roles. Rpn11 is known for its ability to remove and promote recycling of ubiquitin chains. Evidence is provided that Rpn6 supports the stabilization of the complex through interactions with the α subunits. The degradation ability of the proteolytic subunits combined with the regulative role of the 19S complex is important for the maintenance of cellular proteostasis and for the definition of the MHC class I ligandome.

Similarly to transcription and translation, protein degradation is not a static process and can be regulated in response to extracellular signals to ensure homeostasis, survival or apoptosis. Upon cytokine treatment or after induction of oxidative stress, the β catalytic subunits of the proteasome are substituted by their induced counterparts β 1i/LMP2, β 2i/MECL-1, β 5i/LMP7, to generate the immunoproteasome (Figure 2). Strikingly, while immature dendritic cells present an equal proteasome/immunoproteasome ratio, significantly low amounts of standard proteasome are detected upon maturation,²⁵ suggesting the importance of the immunoproteasome in the antigen presentation machinery.

The constitution of the proteasome and immunoproteasome is variable, highly regulated and cell-specific. Therefore, different subunits can be incorporated in the same 20S core to generate intermediate proteasome forms. APCs can, for example, express β 1- β 2- β 5i and β 1i- β 2- β 5i influencing the nature of peptides generated, similar variants have also been described in liver, kidney, colon and heart tissues as well as in a variety of tumours, including melanoma, non-small cell lung carcinoma (NSLC) and myeloma.²⁶ Cortical thymic epithelial cells (cTEC),

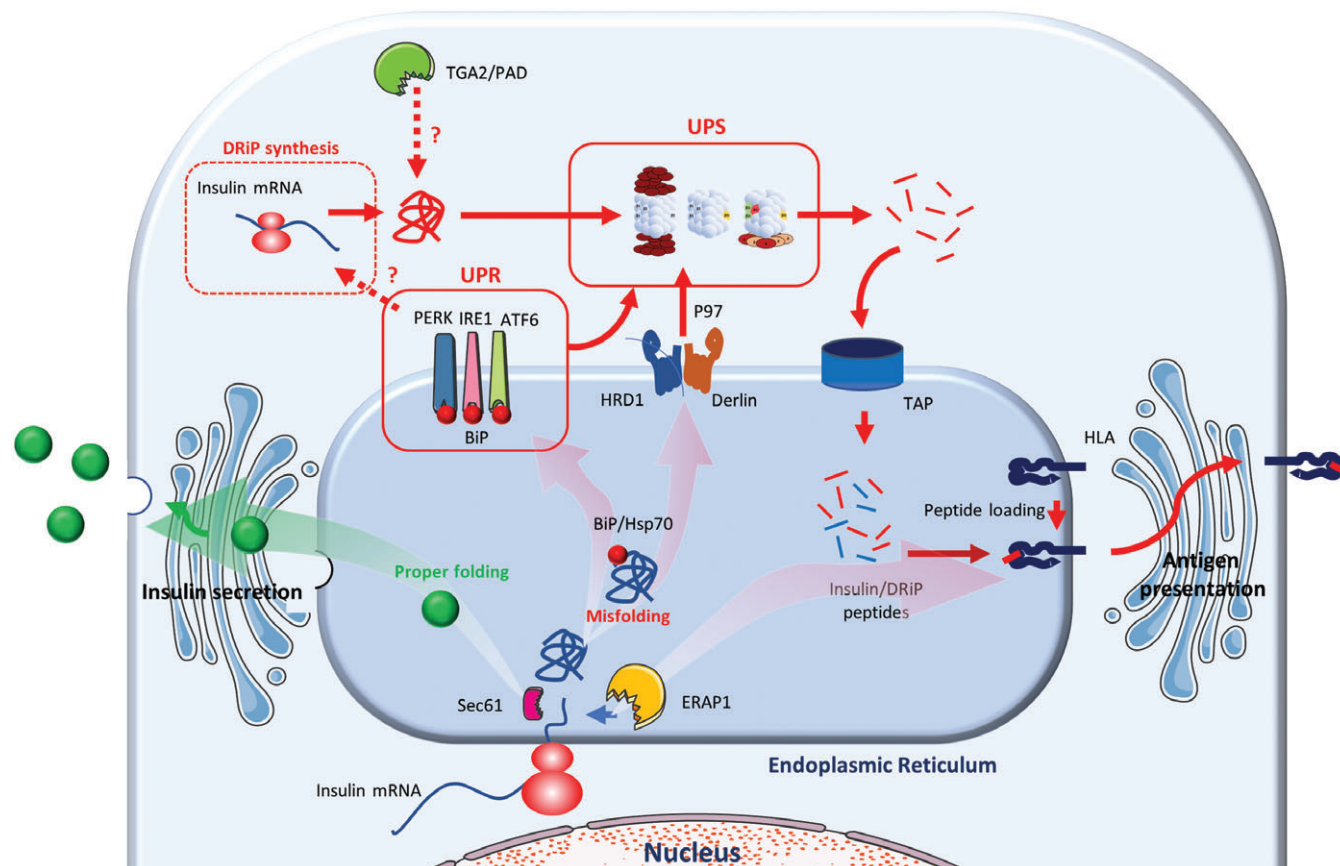


FIGURE 1 Schematic representation of preproinsulin processing. In normal environment, following cleavage of the signal peptide, proinsulin is targeted to the secretory pathway and further matured in insulin and C-peptide in secretory granules (green arrow). Under stress (red arrows), translation, post-translational modifications, UPR and UPS contribute independently or synergistically to the generation of T1D autoantigenic peptides

that participate to the expression of self-antigens during T-lymphocyte development and to the establishment of the T-cell repertoire, express another class of proteasome,²⁷ the thymoproteasome (Figure 2). In this conformation, the $\beta 5i$ subunit of the immunoproteasome is substituted by the $\beta 5t$ and is complexed with $\beta 1i$ and $\beta 2i$ in the 20S core, resulting in a poor chymotrypsin-like activity.²⁶ This diversity illustrates the complexity of the mechanisms underlying central and peripheral tolerance and the critical role played by the proteasome in the development of autoimmunity.

Moreover, the half-life of the immunoproteasome was found to be approximately 5 times lower (27 h) than of the constitutive one (133 h), while its speed of assembly was 4 times higher (21 min vs. 82 min).^{28,29} These values reflect the adaptive role held by the immunoproteasome. Inflammation is a common denominator in autoimmune diseases and the proteasomal composition has been shown to be regulated by inflammatory cytokines and IFN- γ mediated oxidative stress.³⁰ Inflammatory cytokines (IFN γ) can phosphorylate α subunits and affect 19S capped proteasomes assembly^{31,32} and impact proteolytic properties. Similarly, IFN γ has been shown to induce the expression of the α and β subunits of the PA28 cap. Three α and four β subunits interact to form the PA28 $\alpha\beta$ heptameric ring. PA28 $\alpha\beta$ (11S) is mainly associated with 20S core containing the inducible catalytic subunits, via the outer α subunits of the barrel. PA28 $\alpha\beta$ acts in an ATP independent manner and is described to influence the generation of some peptides. Hybrid proteasomes

composed of one 19S and one 11S were also described. Alternatively, also the presence of free 20S cores was described when coping with oxidative stress.³³ In oxidative conditions, uncapped proteasomes were proven to be more resistant than the 26S holo-complexes.²⁸

Stress can also dictate the formation of the 20S core of the immunoproteasome in 3 different levels: during transcription, assembly and post-translation. Increased transcription of the βi subunits upon IFN γ exposure is attributed to the action of IRF1 and STAT1 transcription factors.^{34,35} Finally, the amino acids coating the substrate-binding regions of the $\beta 1$, $\beta 5$ and $\beta 1i$, $\beta 5i$, respectively, were proposed to be responsible for the distinct catalytic properties. Also, incorporation of $\beta 5i$ was shown to enhance the activity of $\beta 1i$ and $\beta 2i$ via structural interactions.²⁶ As a result, the peptide pool generated by the standard, the immunoproteasome and the intermediate forms was proposed to be quantitatively and qualitatively different. While the participation of the proteasome component still remains to be investigated in diabetes, IFN γ was shown to trigger an increase expression of the βi in human islets,^{36,37} demonstrating the potential generation of inflammation-specific epitopes and a possible subsequent CTL response.

In addition, during disease progression, the local increase of nitric oxide and endoplasmic reticulum (ER) Ca²⁺ depletion in response to proinflammatory cytokines secreted by infiltrated autoreactive immune cells, are believed to be the main causes for ER stress and

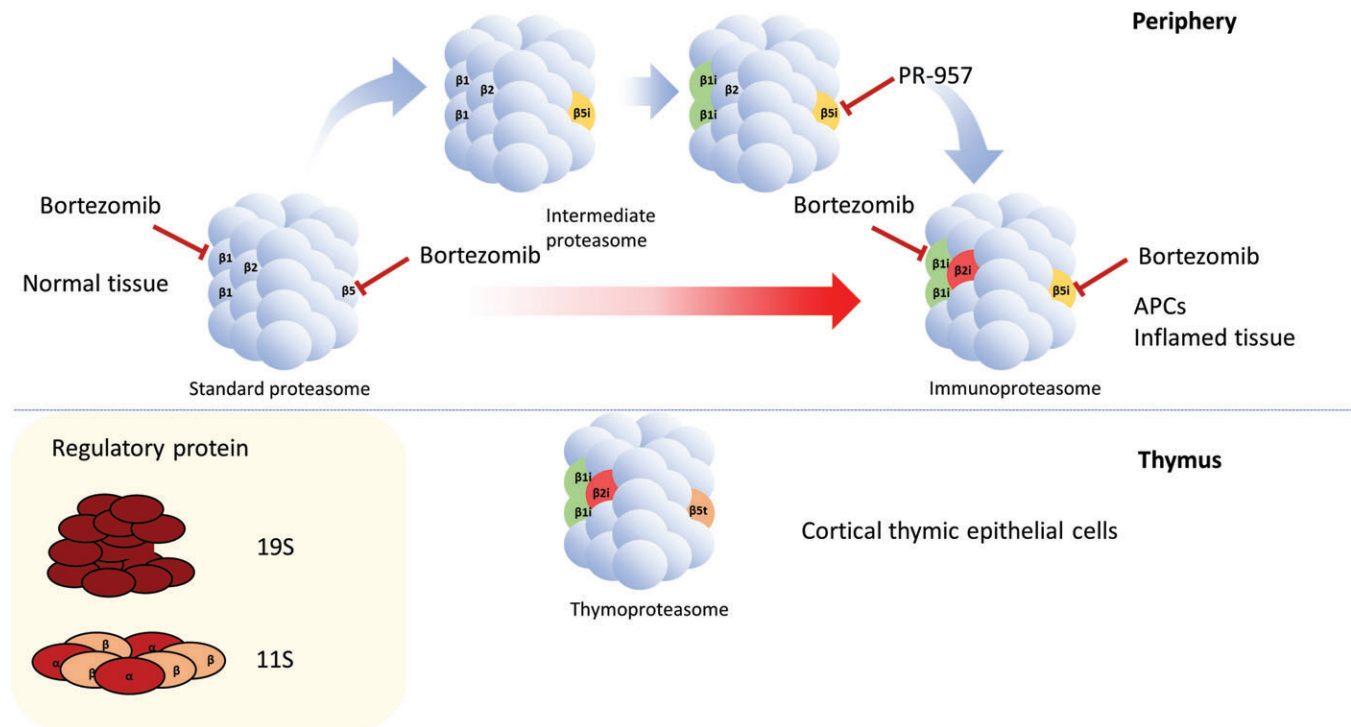


FIGURE 2 Scheme of the different types of 20S proteasomes. The β subunits of the standard proteasome ($\beta 1$, $\beta 2$ and $\beta 5$) are expressed in all cells. Upon stress, some or all β subunits are substituted by their induced counterparts ($\beta 1i$, $\beta 2i$ and $\beta 5i$), composing the intermediate and immunoproteasome. APCs express immunoproteasomes constantly. Cortical thymic epithelial cells express a unique type of $\beta 5$ subunit, indicated as $\beta 5t$. The 20S cores can assemble, on 1 or both ends, with a variety of regulatory proteins, among them 19S and 11S were implicated in the antigen presentation process. Targets of the proteasome inhibitors Bortezomib and PR-957 are indicated

β -cell failure. This oxidative stress triggers expression of βi subunits via the activation of the cAMP responsive element protein (CREB) by nitric oxide, suggesting that such mechanisms may occur during T1D pathology.³⁴

In diabetic conditions, the glycaemic environment was also shown to affect the proteasomal composition and activity of a cell but the results remain controversial. In human islets kept on high glucose for 24 hours an increase in trypsin activity was observed.³⁷ Controversially, in another study, the rat β -cell line INS-1E exposed to high glucose and the pancreatic islets from the rat diabetic model GK, showed a decrease in all three catalytic activities of the proteasome and in increase in ER stress markers and apoptosis. Similar results were obtained in human islets grown in high glucose for 14 days.³⁸ These discrepancies may rely on the technical variations (differences in timing and glucose levels), but further investigations are required to elucidate the role of glucose in proteasome and immunoproteasome function, since these proteolytic complexes are determinative for antigen presentation. These studies may give indications of the autoantigen origin in type 1 diabetes.

“Fundamental knowledge on the mechanisms underlying the β -cell adaptive and subsequent dysfunctional phases is critical to identify efficient novel therapeutic targets to decrease β -cell stress, improve viability/function and reduce β -cell visibility to the immune system.”

3 | PROTEASOME/IMMUNOPROTEASOME AND CELL LIGANDOME

The cell ligandome generated by the proteasome or immunoproteasome has been studied in detail in the case of viral infection and cancer. In tumour immunology, several MAGE antigenic peptides were shown to be derived from the immunoproteasome and the intermediate conformations and not by constitutive proteasome.^{26,39} Among these peptides, MAGE-A3(271-279) is uniquely cleaved by the $\beta 1$ - $\beta 2$ - $\beta 5i$ form, while MAGE-A10(254-262) and MAGE-C2(191-200) is cleaved by the $\beta 1i$ - $\beta 2$ - $\beta 5i$ form.^{39,40}

However, the different cleavage specificities between the constitutive and the induced catalytic subunits⁴¹ were challenged by a study by Mishto et al⁴² showing that peptidome differences relied more on peptide quantity rather than quality. Yet, these results based on digestions performed by isolated proteasomes and not on experiments related to antigen presentation and CTL activation illustrate the complexity of the antigen presentation pathway *in vivo*. Interestingly, while some peptides can be produced by both proteasomes, studies conducted on E1B(192-200) of *Listeria monocytogenes* demonstrate that proteolytic capacities of the immuno- and standard proteasomes may differ in speed, affecting also the overall antigen presentation kinetics and CTL response.⁴³ Moreover, the cellular localization of the immunoproteasome may also play a role. $\beta 1i$ and $\beta 5i$ were found to be in close proximity to the ER while the standard proteasomes were described to be homogeneously distributed in both nucleus and cytoplasm of the IFN γ -treated embryonic lung tissue cell line L132.⁴⁴

Considering that the ER contains all the components needed for antigen presentation after the action of the proteasome, the determined localization of the immunoproteasome may explain the increased levels of antigen presentation noticed, but further investigation is needed.

Like in tumours, the immunoproteasome may have a role in auto-immune disease. Indeed, we previously reported differences in processing preproinsulin by immuno- vs. constitutive proteasomes.^{45,46} Islet autoimmunity and β -cell destruction could be demonstrated by CD8 T-cells reactive with such proteasomal products, but also non-proteasomally derived preproinsulin epitopes. Interestingly, destruction of β -cells increased under high glucose conditions, pointing to hyperglycaemia and metabolic stress as environmental factors contributing to the demise of β -cells in T1D.^{45,47} Whether and how these factors influence proteasomal composition and antigen presentation of pancreatic β -cells is still unknown. In multiple sclerosis, where CD8 cytotoxic T-cells target epitopes of the self-derived protein myelin, PA28 $\alpha\beta$ and immunoproteasome subunits were higher in brain tissue of MS patients compared to healthy donors. Association of PA28 $\alpha\beta$ with 20S immunoproteasome resulted in higher production of the myelin antigenic epitope MBP111–119.^{48,49}

While ultimately, HLA polymorphism define the ligandome, the variety of peptides generated is very large and even more amplified by more unconventional events occurring during degradation. In fact, examples of hybrid peptides, generated from fragments of two peptides from the same protein (*cis*-splicing) or different proteins (*trans*-splicing), have been described for tumour antigenic epitopes. Thus, peptides presented in class I HLA may exhibit different sequence than the parental unprocessed sequence as seen for peptides derived from gp100, tyrosinase, FGF-5 or Sp100 in melanomas. Two different mechanisms for peptide splicing were described: transpeptidation and condensation. In the former, an acyl-enzyme intermediate is formed between the proteasome and the first peptide via an ester bond, this bond receives a nucleophilic attack from the N-terminal site of the second peptide. Condensation, describes the formation of peptide bond *de novo*.⁵⁰ While theoretically, both the constitutive and induced β subunits may perform transpeptidation or condensation, some SP100 derivatives are more pronounced in the presence of the immunoproteasome,⁵¹ and spliced gp100(47-52)-(40-42) shown to occur by condensation by the action of β 5i subunit.⁵⁰

In autoimmune disease these processes are poorly investigated. Yet, DeLong *et al* illustrated the complexity of degradation and the diversity of the ligandome by describing immunoreactivity against hybrid peptides generated by a process of transpeptidation.⁵² CD4 T-cells isolated from insulitic lesions of type 1 diabetic patients were shown to react with such hybrid peptides, suggesting the potential relevance to islet autoimmunity and β -cell destruction in human disease.⁵³ Prediction and identification of these hybrid peptides is challenging but these may constitute an important component of the peptide ligandome. Initially, this identification was exclusively dependent on the isolation of the corresponding patients CTLs, usually present in low amounts. Recently, a novel tool for spliced peptide characterization, combining both experimental and computational data, was developed by Liepe *et al*. In brief, theoretical *m/z* ratios from

both spliced and linear peptides are compared with mass spectrometry data of *in vitro* digestions by the 20S proteasome. The occurrence of a specific peptide is then assured by tandem mass spectrometry.⁵⁴ Another limiting factor is the representation of only linear epitopes in the different protein databases. In a study aiming at investigating the prevalence of such peptides, a database containing numerous spliced peptides that may occur by *cis* splicing of epitopes separated by a maximum of 25 amino acids was developed. According to this *in silico* analysis, approximately one third of the HLA class I ligandome is qualitatively produced by proteasomal-catalyzed peptide splicing and these peptides comprise circa the 25% of the surface immunopeptidome. Importantly, spliced peptides are not produced by a random junction of epitopes, and their occurrence is highly dependent on protein sequence. However, these results are questioned by another study, supporting that only the 2% to 4% of the HLA class I ligands derive from spliced peptides. These ambiguous findings, highlight the necessity of a widely accepted methodology for spliced peptide identification.⁵⁵ Nevertheless, the relevance of spliced peptides in antigen presentation is also highlighted by their high affinity to MHC class I molecules and by their ability to activate CTL responses as extensively as linear antigenic peptides.^{50,56,57}

4 | CONCLUSION

The high degradation rate of the immunoproteasome when compared to the standard proteasome suggest that it is an important element of the cellular adaptive mechanism to inflammation but also possibly in the generation of aberrant polypeptide derived epitopes. Therefore, targeting the immunoproteasome to prevent autoimmunity may represent an interesting alternative to current therapy. In mouse models for inflammatory bowel disease, inhibition of the chymotrypsin-like activity of the β 5-subunit by Bortezomib was shown to reduce colitis.⁵⁸ Similarly, the selective inhibition of LMP7 by PR-957 (ONX0914) reduces inflammatory infiltration in a mouse model for rheumatoid arthritis.⁵⁹ In a recent clinical trial, Bortezomib administration reduces the numbers of peripheral blood and bone marrow plasma cells and ameliorates clinical manifestations of refractory systemic lupus erythematosus.⁶⁰ Yet, these beneficial effects are mainly mediated by a reduction of the I κ B degradation and the inhibition of Nf κ B regulated inflammatory mediators on effector cells (IFN γ , IL1 β , TNF α)^{34,59,61} and not on the prevention of the generation of neoantigen by the target cells. Worse still, the few studies investigating the role of the proteasome inhibitors on β -cell described an increase ER stress and an impaired islet function^{38,62} but a better understanding of these degradation processes appears essential for the identification of stress induced antigens and ultimately for the identification of new biomarkers of disease progression.

“Identification of new biomarkers of disease progression and establishment of a “diabetes immune signature” will provide a guide towards personalized medicine, defining the preferred immune modulation strategy in individual patients.”

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Conflict of interest

No potential conflicts of interest relative to this article are reported.

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