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Immunoproteasome Activation During Early Antiviral Response in Mouse Pancreatic β-cells: New Insights into Auto-antigen Generation in Type I Diabetes?

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Abstract

Type 1 diabetes results from autoimmune destruction of the insulin producing pancreatic β-cells. The immunoproteasome, a version of the proteasome that collaborates with the 11S/PA28 activator to generate immunogenic peptides for presentation by MHC class I molecules, has long been implicated in the onset of the disease, but little is known about immunoproteasome function and regulation in pancreatic β-cells. Interesting insight into these issues comes from a recent analysis of the immunoproteasome expressed in pancreatic β-cells during early antiviral defenses mediated by interferon β (IFNβ), a type I IFN implicated in the induction of the diabetic state in human and animal models. Using mouse islets and the MIN6 insulinoma cell line, Freudenburg et al. found that IFNβ stimulates expression of the immunoproteasome and the 11S/PA28 activator in a manner fundamentally similar to the classic immuno-inducer IFNγ, with similar timing of mRNA accumulation and decline; similar transcriptional activation mediated primarily by the IRF1 and similar mRNA and protein levels. Furthermore, neither IFNβ nor IFNγ altered the expression of regular proteolytic subunits or prevented their incorporation into proteolytic cores. As a result, immunoproteasomes had stochastic combinations of immune and regular proteolytic sites, an arrangement that would likely increase the probability with which unique immunogenic peptides are produced. However, immunoproteasomes were activated by the 11S/PA28 only under conditions of ATP depletion. A mechanism that prevents the activation of immunoproteasome at high ATP levels has not been reported before and could have a major regulatory significance, as it could suppress the generation of immunogenic peptides as cell accumulate immunoproteasome and 11S/PA28, and activate antigen processing only when ATP levels drop. We discuss implications of these new findings on the link between early antiviral response and the onset of type 1 diabetes.

Keywords: Immunoproteasome; Autoantigen; Type 1 diabetes; Pancreatic β-cells; IFNβ; IFNγ; MIN6 cells; Mouse islets

Classic View of the Regular and Immune Versions of the Proteasome

The immunoproteasome is a version of the proteasome that specializes in the production of immunogenic peptides for presentation by MHC class I molecules [1-3]. The regular proteasome is expressed in somatic cells and degrades most cellular proteins, but only a fraction of the resulting product peptides is immunogenic (Figure 1A). The immunoproteasome is constitutively expressed in cells of the immune system and accumulates in somatic cells mainly after stimulation by IFNγ (Figure 1B). Both versions of the proteasome consist of structurally similar 20S proteolytic cores that differ only in their proteolytic subunits. In the case of complete subunit replacement, the regular β1, β2, and β5 proteolytic subunits in the proteasome (Figure 1A, subunits in yellow) are replaced with their corresponding immune versions β1i (also known as LMP2), β2i (MECL1), and β5i (LMP7) in the immunoproteasome (Figure 1B, subunits in black). However, many somatic cells initially accumulate the 20S proteolytic cores with only partially replaced subunits (not shown in Figure 1). Mice lacking all three of the immune subunits have 50% fewer epitopes presented by MHC class I molecules and reject wild-type cells [4], demonstrating that the immune subunits play a major role in antigen generation *in vivo*. However, this role likely reflects both a change in the pattern of protein cleavage by the altered 20S proteolytic core [5-11] and regulation by the 11S/PA28 activator (referred henceforth as the 11S) [12-15]. The interferon-inducible nature of the 11S activator suggests that it functions primarily with the immunoproteasome, but its role is elusive. On the one hand, the 11S activator opens the gate to the 20S proteolytic core, thereby preparing it for substrate entry and product release, essentially like the constitutive 19S activator. On the other hand, only the 19S recruits polyubiquitinated protein substrates and, in an ATP hydrolysis-dependent manner, facilitates substrate unfolding necessary for entry into the proteolytic 20S core. It has been suggested that 11S promotes the release of peptides generated from polyubiquitinated substrates recruited by the 19S activator in the context of hybrid 11S-20S-19S particles [16,17] (Figure 1B, left) and/or promotes ubiquitin-independent proteolysis [18] (Figure 1B, right). The accumulation of the immunoproteasome and 11S particles is typically coordinated with the induction of the MHC class I molecules and other components necessary for antigen presentation (Figure 1C) [19].

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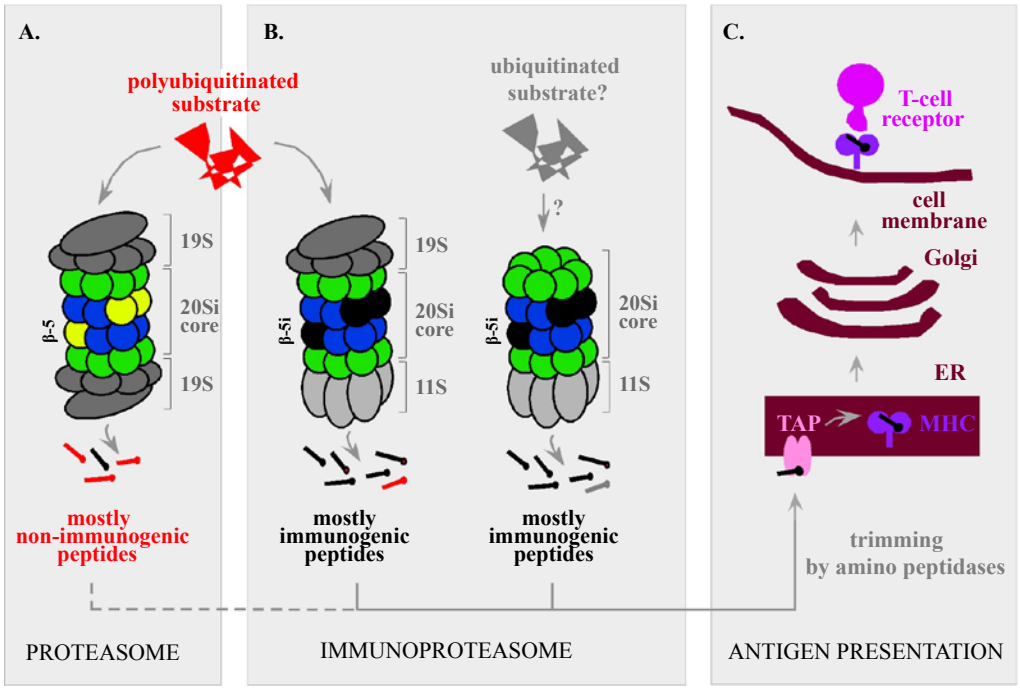

Figure 1: Classic view on the regular and immune versions of the proteasome.

(A). In somatic cells not exposed to IFNs, the proteasome collaborates with the 19S activator and degrades polyubiquitinated substrates to peptides that are poor precursors for T-cell ligands. **(B).** The immunoproteasome is more proficient in this role due to replacement of proteolytic subunits (yellow) with their immune versions (black) and collaboration with the 11S activator. **(C).** Key steps leading to antigen presentation by MHC class I molecules. See text for details.

The Emerging Issue of Early Immunoproteasome Activation by Type I IFNs and its Implications for the Onset of Type I Diabetes

Evidence was presented over a decade ago that mouse pancreatic β-cells express the immunoproteasome in response to IFNγ in a manner linked to a change in antigen presentation by MHC class I molecules and that this change is prevented by the proteasomal inhibitor MG132 [20]. These findings suggested a model in which a protein or proteins expressed in pancreatic β-cells and degraded by the immunoproteasome become the source of immunogenic peptides that then trigger the onset of autoimmunity in type I diabetes. However, while several β-cell specific antigens have subsequently been identified [21], few of them were shown to be generated by the immunoproteasome and/or appear to be clinically relevant. Part of the problem is that no study has analyzed the function and regulation of the immunoproteasome in pancreatic β-cells under conditions linked to disease onset, and that it is unclear whether the generation of unusual immunogenic peptides reflects an unusual pool of substrates recruited for proteolysis, abnormal function of the immunoproteasome, or a change in antigen presentation. Furthermore, the onset of autoimmunity in patients and animal models is increasingly linked to antiviral type I IFNs [22-29] that were initially not viewed as potent inducers of the immune response. This view was first challenged by the observation that type I IFNs induce early activation of $CD8^+$ T cells by an unknown antigen produced by the immunoproteasome and presented by MHC class I molecules in a chimpanzee model of acute HCV infection [30,31]. In a more recent study, Freudenburg et al. [32] proposed that activation of a similar process in pancreatic β-cells could shed light on the still enigmatic link between type I IFNs, viral infections, and the onset of autoimmunity in type I diabetes [33,34]. Key to this idea was the realization that type I IFNs would be secreted by virus-infected cells as part of the early antiviral response (Figure 2A, green), and could activate the generation and presentation of new antigens not only in the infected cell (Figure 2A, blue), but also in uninfected neighboring cells (Figure 2B, blue). While the nature of these antigens could be influenced by many factors, a prominent role would be played by mechanisms that regulate the early immunoproteasome. Analysis of these mechanisms by Freudenburg et al. [32] clarified some of the key aspects of early immunoproteasome activation, and strengthened the possibility that they could play an important role in the generation of abnormal self-antigens. We comment on these findings below.

IFNβ and IFNγ Induce Expression of the Immunoproteasome and 11S in a Similar Manner

Analysis by Freudenburg et al. was first to implicate the immunoproteasome in the early, IFNβ-mediated antiviral defenses of pancreatic β-cells [32]. However, to understand the significance of the IFNβ-mediated effects, the authors not only provided the usual evidence for the activation of immunoproteasome and 11S gene expression, but also determined how this expression compares with the activation of immunoproteasome and 11S gene expression by IFNγ, and to the expression of regular proteasome genes. This analysis produced two major findings. First, both IFNβ and IFNγ stimulated expression of the immunoproteasome and 11S genes in a fundamentally similar manner, although higher concentrations of IFNβ than of IFNγ were required for similar effects (50% of the maximal inducible protein levels were observed with 0.08 units/ml of IFNγ or 800 units/ml of IFNβ). The similarities included the timing of mRNAs accumulation and decline

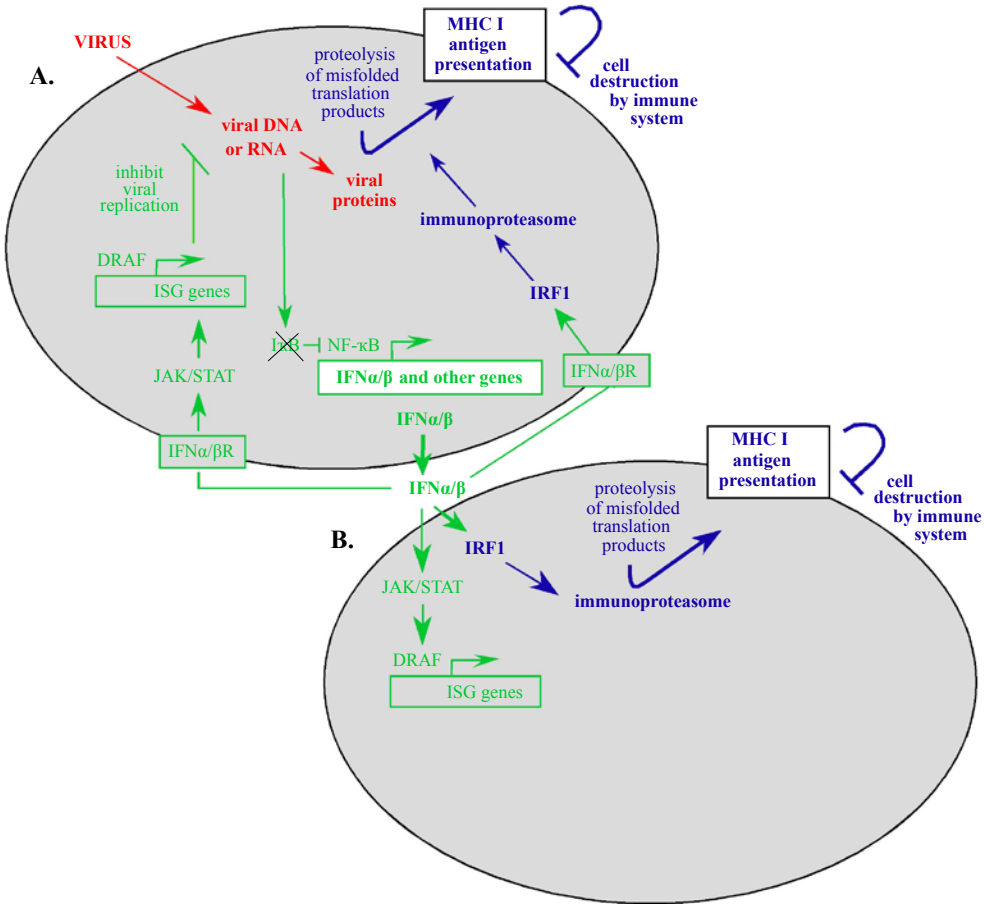

Figure 2: Immunoproteasome as part of the early antiviral response in pancreatic β-cells.

(A). β-cell infected with a virus (red) would secrete type I interferons α and β (IFNα/β) as part of early antiviral response (green). **(B).** Neighboring uninfected β-cells would also undergo early antiviral response (green) as a result of exposure to type I IFNs secreted by the virus-infected β-cell. In each case, induced expression of the immunoproteasome would promote antigen processing for presentation by MHC class I molecules (blue). Antigens derived from β-cell proteins could stimulate the onset of autoimmunity. See text for details.

(12 and 24 hours, respectively); the mechanism of transcriptional activation mediated primarily by the IRF1 and the levels of the inducible mRNAs and proteins. The second interesting finding was the relationship between the expression of regular and inducible subunits. When compared to the expression of regular subunits, which remained unchanged during exposure to IFNs, both IFNβ and IFNγ stimulated the production of inducible mRNAs to a level equal to the regular mRNAs. These features generally agree with the mechanism by which IFNγ activates the immunoproteasome gene expression in other somatic cells. However, they also resurrect the long-standing question about the role of the concurrent expression of regular and immune genes. If complete replacement of regular proteolytic subunits with their immune versions were optimal for the generation of immunogenic peptides, why would the immune subunits be expressed in addition to, not instead of, the regular proteolytic subunits? In the past, this phenomenon was usually interpreted as a slow transition state with no particular significance, possibly because the stimulation of the immune response by IFNγ was thought to be linked to the later stages of anti-viral response, and the late timing could be associated with the eventual replacement of all regular subunits with their immune versions. However, this interpretation does not necessarily apply to the early antiviral response mediated by IFNβ, which would activate the immune response precisely when the immune and regular subunits are expressed at similar levels. Therefore, it appears that in the light of data provided by Freudenburg et al., the possibility needs to be considered that this period of concurrent expression of regular and immune subunits has a unique regulatory significance.

Stochastic Combinations of Immune and Regular Subunits may Promote the Generation of Unique Self-Antigens, but Only Under Conditions of Low ATP Levels

Freudenburg et al. showed that, as would be expected from the concurrent expression of immune and regular proteolytic subunits, the immunoproteasome particles assembled in pancreatic β-cells exposed to IFNβ had stochastic combinations of regular and immune active sites [32]. This type of replacement differs from the complete replacement of proteolytic subunits typical of the classic immunoproteasome (Figure 1B, subunits in black) and would be likely to increase the number of variations in protein cleavage, thereby also increasing the probability with which unique immunogenic peptides are produced. However, while the immunoproteasome 20S cores co-existed with the regular 19S and the inducible 11S activators, the 11S stimulated

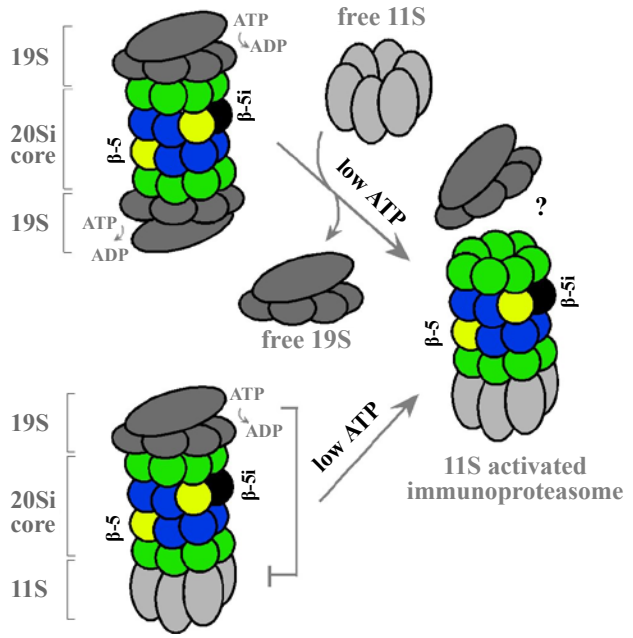

Figure 3: Reduction in ATP levels stimulates immunoproteasome activation by the 11S.

In β-cells exposed to IFNβ, the 20S proteolytic cores contain a stochastic arrangement of regular and immune proteolytic subunits, and coexist with the 19S and 11S activators. However, the 11S activator stimulates the proteolytic rates only under conditions of ATP depletion. Two mechanisms could explain this observation, as discussed in the text.

proteolysis only under conditions of ATP depletion. A mechanism that restricts the function of the 11S activator in cells with high ATP levels could have a major regulatory significance, as it could suppress the generation of immunogenic peptides even if cells accumulated the immunoproteasome and the 11S, and could rapidly activate antigen processing but only when ATP levels drop. While it is yet unknown whether this mechanism represents a more general phenomenon, or is limited to pancreatic β-cells, where the insulin secretory function is tightly linked to changes in ATP concentrations, this finding has powerful implications.

First and foremost, this finding suggests that any change in the cell that would lead to reduction in ATP levels could act as a rapid stimulus of antigen generation in cells expressing the immunoproteasome and the 11S activator as a result of IFNβ or IFNγ treatment. The dependence on two factors (the 11S expression and ATP depletion) could limit the probability of stimulating the production of unusual self-antigens in otherwise healthy, uninfected, β-cells that express the immunoproteasome as part of the early antiviral response (Figure 2B). While little is known about how viral infections affect ATP levels in pancreatic β-cells, a 5-fold reduction in total ATP content of uninfected pancreatic β-cells was observed in response to inflammatory cytokines, such as IL-1 and combinations of IL-1 and IFNs, and this change depended on expression of the inducible nitric oxide synthase (iNOS) and production of nitric oxide [35,36]. Since iNOS expression, nitric oxide production, and the generation of inflammatory cytokines are stimulated by synthetic dsRNA that is used to mimic virus infections [37], it is tempting to speculate that a reduction in ATP levels may link the effects of iNOS expression and nitric oxide production to immunoproteasome activation by the 11S, and to production of unusual self-antigens.

What could be the mechanism by which reduction in ATP levels stimulates activation of the immunoproteasome by the 11S? In general, the 20S proteolytic cores, regular or immune, are thought to exist in excess over the constitutive 19S activator, implicating that free 20S cores are always available in cells to assemble with the inducible 11S activator. However, if the 19S activators were expressed at levels sufficiently high to saturate the 20S proteolytic cores, they could restrict premature access of the 11S activator and make it dependent on dissociation of at least one 19S (Figure 3, top). Another possibility is that the function of the two activators within 11S-20S-19S particle is coordinated by an allosteric mechanism dependent on ATP levels (Figure 3, bottom). Perhaps most interestingly, the link between activation by the 11S and reduction in cellular ATP levels also suggests that the activation could be associated with a major change in the pool of proteasomal substrates, thereby further contributing to the production of unique antigens. Indeed, while the 19S activator recruits substrates in a manner primarily dependent on substrate polyubiquitination, which would be limited at low ATP levels, the 11S could recruit substrates independently of polyubiquitination. An interesting possibility is that the 11S facilitates ubiquitin-independent degradation of proteins damaged by reactive oxygen and nitrogen species that are robustly produced in cells exposed to IFNs, and that this role ensures pancreatic β-cell survival, similarly to the protective role of the immunoproteasome during IFNγ-induced oxidative stress [38]. However, if this fundamentally protective role was linked to the early stimulation of the immune response against β-cells, it could also predispose to autoimmune responses under any conditions that would cause even a modest accumulation of the immunoproteasome and 11S in combination with compromised ATP levels.

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