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Immune and non-immune functions of the immunoproteasome

Arkhjamil Angeles[1], Gabriel Fung[1], Honglin Luo[1]

[1]UBC James Hogg iCAPTURE Centre, Institute for Heart and Lung Health, St. Paul's Hospital, Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada

TABLE OF CONTENTS

1. ABSTRACT

The biological importance of the ubiquitin-proteasome system in the control of myriad cellular processes has been well-recognized; however, the pathophysiological significance of the immunoproteasome, the inducible form of the proteasome, has not been well-comprehended until lately. The primary function of the immunoproteasome was originally believed to improve MHC-I antigen presentation. It now becomes evident that the immunoproteasome possesses broader biological functions. It regulates proinflammatory cytokine production, and T cell differentiation and proliferation. Alongside immune functions, the immunoproteasome has been demonstrated to relieve oxidative stress by the efficient turnover of oxidatively-damaged proteins and by allaying the formation of harmful protein aggregates. Furthermore, it has been implicated to regulate tumor cell growth and control muscle mass. Finally, the immunoproteasome has recently drawn considerable attention as a potential novel therapeutic target for cancer and autoimmune disease. This review will give an overview of the structure and function of the immunoproteasome, highlight its functional diversity in both immune and non-immune responses, and discuss the relationship between the dysregulation of the immunoproteasome and the development of several human diseases. (Words: 174)

2. INTRODUCTION

The ubiquitin-proteasome system (UPS) is a major non-lysosomal pathway for intracellular protein degradation in eukaryotic cells. It helps to maintain homeostatic levels of proteins involved in a myriad of cellular processes, including signal transduction, transcriptional regulation, cell differentiation and apoptosis.(1, 2) The proteolytic activity of the proteasome is attributed to three β-subunits, β1, β2 and β5. However, upon regulatory induction by inflammatory cytokines such as interferon-γ (IFN-γ), the constitutively expressed catalytic β-subunits are replaced by inducible β-counterparts known as immunosubunits (Figure 1).(3, 4) These immunosubunits, β1i (also known as low molecular mass peptide 2 (LMP2); proteasome subunit beta 9 (PSMB9)), β2i (also known as multicatalytic endopeptidase complex-like 1 (MECL-1); PSMB10) and β5i (also known as LMP7; PSMB8), are preferentially incorporated during proteasome assembly to form the immunoproteasome.(3, 4)

The immunoproteasome was named due to the initial identification of its functions in antigenic peptide generation for major histocompatibility complex (MHC) class I presentation during inflammation. The β1i and β5i are encoded by genes within the MHC class II region.(3) The inducible β-counterparts have been shown to exhibit differential cleavage preferences and efficiencies to help

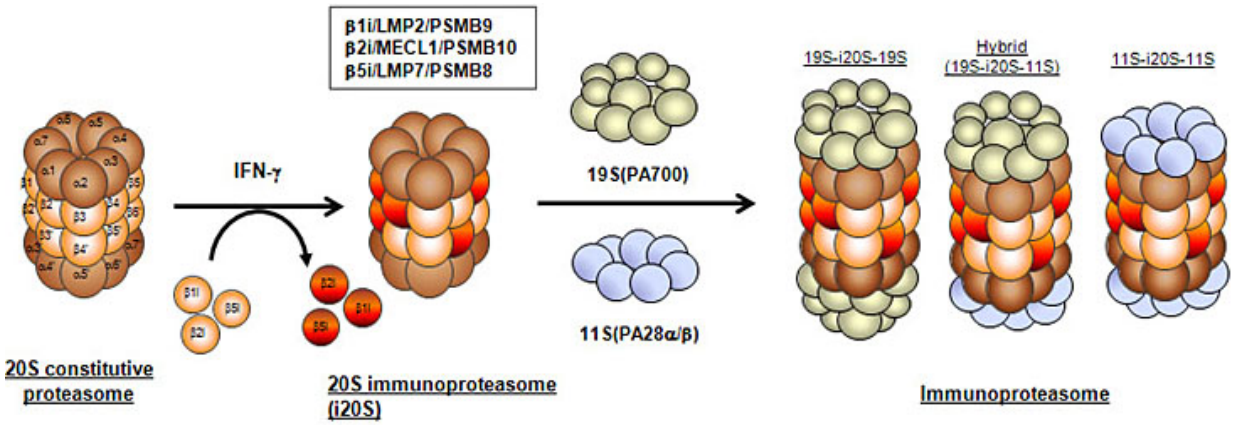

Figure 1. Structure of the immunoproteasome. The 20S proteasome is a cylindrical structure composed of two outer α- and two inner β-rings. Upon stimulation with interferon-γ (IFN-γ), the constitutively expressed catalytic β-subunits of the standard proteasome are replaced by inducible β-counterparts: β1i (also known as low molecular mass peptide 2 (LMP2); proteasome subunit beta 9 (PSMB9); β2i (also known as multicatalytic endopeptidase complex-like 1 (MECL-1); PSMB10); and β5 (also known as LMP7; PSMB8). They form the 20S immunoproteasome (i20S). The mature i20S then binds to either PA700 (19S proteasome), or PA28α/β (11S proteasome), or a combination of both proteasome activators at its two ends to form three different types of immunoproteasome.

diversify the antigenic peptide repertoire facilitating an improved adaptive immune response.(5-7) In addition to this function, recent evidence suggests that the immunoproteasome also plays an important role in the regulation of cytokine production,(8-10) and T cell differentiation and survival.(11, 12) Non-inflammatory conditions such as injury to the retina and brain,(13) and oxidatively-stressed cells(14-16) have also been shown to elicit high expression levels of the immunosubunits, suggesting potential roles in other non-immune-related functions. Indeed, the immunoproteasome has been implicated to have anti-oxidant and anti-protein aggregation properties,(15, 16) and to regulate tumor cell growth(17-19) and muscle remodeling.(20, 21) Moreover, recent studies have revealed a possible connection between immunoproteasome dysregulation and human diseases, including cancer,(17-19, 22, 23) and autoimmune,(8, 10) neurodegenerative,(24-27) and cardiovascular diseases.(20, 21, 28) The functions of the immunoproteasome appear largely diverse, but much still remains to be elucidated. Here we highlight the major features of the immunoproteasome, including its structure and assembly, biological properties, expression and regulation, and its immune and non-immune functions. We also discuss recent findings of the involvement of immunoproteasome in various disease settings and the therapeutic potential of targeting the immunoproteasome.

3. STRUCTURE, ASSEMBLY, AND BIOCHEMICAL PROPERTIES

The 20S proteasome is a cylindrical structure composed of α- and β-subunits arranged into four stacked, heteroheptameric rings (Figure 1).(29, 30) The two inner β-rings confer the catalytic properties of the proteasome, specifically within the active sites of the β1, β2 and β5 subunits which face the inner core.(30) The alternate incorporation of the inducible catalytic β-homologues, β1i, β2i and β5i, leads to the formation of the immunoproteasome (Figure 1). Crystallographic analyses depict protrusions of the N-termini of α-subunits into the center of the two outer α-rings, obstructing the entry of protein substrates into the inner catalytic core.(29) Thus, the proteasome activators, PA28α/β (11S proteasome) and/or PA700 (19S proteasome), must associate with the α-subunits to open the configuration of the outer rings to permit substrate passage into the chamber of the 20S proteasome (Figure 1).

The biogenesis of the immunoproteasome shares similar mechanisms to that of the standard proteasome; however, in cells which express both the inducible and constitutive β-subunits, the immunosubunits are preferentially incorporated.(31) β1i is incorporated into the α-ring earlier than β1 and is required for the subsequent incorporation of β2i.(31, 32) In concert, β2i further accelerates the recruitment of β1i.(31, 32) β5i is independently incorporated but is required for the post-translational processing of the β1i and β2i propeptides.(31, 33)

The maturation of the proteasome is also dependent on extrinsic assembly factors such as proteasome maturation protein (POMP), which is also transcriptionally regulated by IFN-γ. (34-36) The evidence that POMP exhibits higher binding affinity for β5i over β5 may explain the observation that the assembly of immunoproteasome occurs preferentially to the standard proteasome.(34) Upon proteasome maturation, POMP is autolytically degraded by β5/β5i.(35, 36) In concordance with this transient and inducible feature of immunoproteasome biogenesis, the

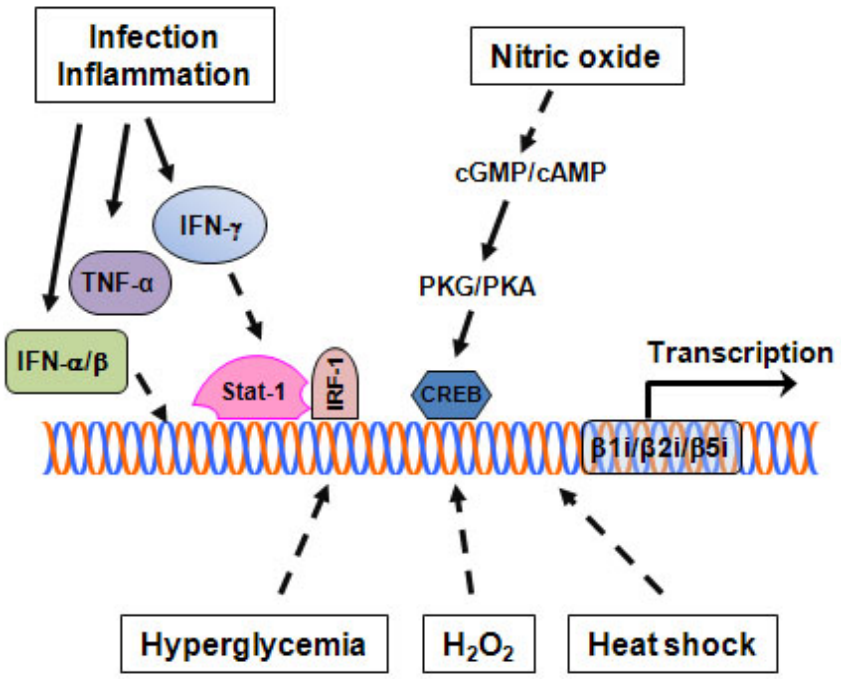

Figure 2. Regulation of the immunoproteasome. Proinflammatory cytokines, interferon (IFN)-γ, IFN-α/β, and tumor necrosis factor (TNF)-α, have been demonstrated to induce immunoproteasome expression. IFN-γ upregulates immunosubunits by activating the signal transducer and activator of transcription (Stat)-1/IFN-γ regulatory factor 1 (IRF-1). Several cytokine-independent regulators of the immunoproteasome have also been identified, which include nitric oxide (NO), which increases immunoproteasome expression through the cGMP/cAMP signaling pathway, heat shock, H_2O_2, and hyperglycemia.

immunoproteasome has also been shown to have a much shorter half-life than the standard proteasome.(34) These findings support a tightly controlled mechanism for immunoproteasome regulation, which allows for a rapid return to basal levels once the immunoproteasome is no longer required.

Like constitutively expressed catalytic β-subunits, the β1i, β2i and β5i subunits display caspase-like, trypsin-like and chymotrypsin-like proteolytic activities and exhibit preferential substrate cleavage after acidic, basic and hydrophobic amino acid residues, respectively.(37) However, immunoproteasomes have distinct proteolytic activities that generate a different spectrum of peptides from standard proteasomes to favor MHC class I antigen presentation. Compared with the standard proteasome, the immunoproteasome is characterized by enhanced chymotrypsin- and trypsin-like activities and reduced caspase-like activity.(4, 6, 38) These enzymatic properties lead to the generation of antigenic peptides with high affinity C-terminals to the MHC class I cleft. The production of such MHC class I ligands is well-known to improve antigen presentation and subsequent cytotoxic T-lymphocyte (CTL) response. However, recent studies have countered the notions that the immunoproteasome is optimized at generating all antigenic peptides. It has been demonstrated that tumor-derived epitopes such as RU1, Melan-A and gp100, all of which contain the branched chain amino acid valine at the C-terminus, are produced efficiently by the standard proteasome but not the immunoproteasome, resulting in a loss of recognition by CTLs.(39)

4. EXPRESSION AND REGULATION

In contrast to the standard proteasome, which is constitutively expressed in the majority of mammalian cells, the expression of the immunoproteasome is generally low under non-stimulating conditions, but can be induced when cells are exposed to various factors (Figure 2).

Among the many factors involved in immunoproteasome regulation, the proinflammatory cytokine, IFN-γ, appears to be the most prominent and well-characterized. IFN-γ induces the expression of the β1i, β2i and β5i subunits along with an array of other genes including MHC class I and II molecules, transporter associated with antigen presentation (TAP), and PA28α/β.(40, 41) The regulatory mechanism involved in the expression of immunosubunits relates to IFN-γ-mediated activation of the transcription factors, signal transducer and activator of transcription (Stat)-1 and IFN-γ regulatory factor 1 (IRF-1).(42, 43). IFN-γ also facilitates the transcriptional upregulation of PA28α/β. It has been reported that PA28α/β preferentially associates with the immunoproteasome upon IFN-γ treatment, coupled with a

concomitant decrease in PA700 association.(44) This preferential association has been attributed to the dephosphorylation of the 20S proteasome caused by IFN-γ.(44)

In addition to type II interferon (IFN-γ)-mediated regulation, type I interferons, IFN-α and IFN-β, have also been linked to increased immunoproteasome expression. *In vitro* experiments in hepatocytes and cardiomyocytes have demonstrated that treatment with IFN-α or IFN-β induces the expression of immunosubunits and promotes their incorporation into the proteasome complex.(45, 46) Animal studies have shown that early induction of type I IFN responses prior to induction of type II IFN responses during hepatitis C virus infection initiates the biogenesis of the immunoproteasome in the liver, which is associated with early antiviral immune responses.(46) In a mouse model of viral myocarditis, it has also been revealed that the secretion of type I interferons early during coxsackievirus infection induces early myocardial immunoproteasome formation and concomitant upregulation of antigenic presentation machinery.(45, 47)

Tumor necrosis factor-α (TNF-α) has also been suggested to regulate immunosubunit expression. Lipopolysaccharide-induced inflammation in the hippocampus of young rats augments TNF-α mRNA expression in concert with immunoproteasome gene upregulation.(48) Concurrently, IFN-γ mRNA expression is minimally affected.(48) Interestingly, Foss *et al* demonstrated that TNF-α alone fails to elicit a significant effect on the expression of immunosubunits, and as such, it may have functions that are either synergistic with IFN-γ, or may be cell- or species-specific.(49)

Cytokine-independent regulation of immunoproteasome has also been described. Nitric oxide (NO) was shown to increase immunosubunit expression through the cGMP/cAMP signaling pathways.(50) NO elevates intracellular levels of cGMP/cAMP which activate protein kinase G (PKG) and protein kinase A (PKA), and in turn phosphorylate cAMP responsive element binding protein (CREB), a nuclear transcription factor reported to facilitate the transcription of immunosubunits.(50) Although the mechanisms are not yet defined, treatment of cells with heat shock(51) or H_2O_2(52, 53) has also been reported to induce the immunosubunit expression and enhance immunoproteasome-dependent epitope generation. Hyperglycemia is another condition recently identified to regulate immunosubunit expression.(21) Isolated murine hearts exposed to high glucose have significantly increased β5i, but decreased β1i protein levels.(21).

Several types of non-immune cells, such as the retina,(13) ocular lens, (54) and aortic endothelial cells,(50) have been found to constitutively express the immunoproteasome at basal levels. Although the functional significance remains to be elucidated, basal expression of immunoproteasome has been shown to occur independently of IFN-γ, as IFN-γ-deficient mice have similar β1i and β5i basal levels as wild-type mice.(55) Other speculations include a partial role of unphosphorylated Stat-1 promoter interactions for basal immunoproteasome expression.(55) High levels of endogenous NO have also been considered to be responsible for the basal expression of the immunoproteasome in endothelial cells.(50)

5. IMMUNE FUNCTIONS

The role that the immunoproteasome plays in immunity has been studied extensively, particularly its function in antigen presentation (Figure 3). The catalytic immunosubunits generate short peptides that are transported into the endoplasmic reticulum (ER) via TAP complexes and are subsequently loaded onto MHC class I molecules with high affinity for cell surface presentation to CTLs.(56) The increased proteolytic capacity and the high affinity, MHC I-binding peptides generated by the immunoproteasome are what define its greater efficiency over the constitutive proteasome. Furthermore, many epitopes generated by the immunoproteasome have also been described to be immunodominant, which is known to elicit a stronger CTL response. Accordingly, some immunodominant epitopes have been shown to be severely downregulated upon immunoproteasome inhibition. For example, the CTL response to M45, an immunodominant epitope in cytomegalovirus-infected mice, is notably lost in LMP7/β5i-deficient mice.(57) As well, CTL responses to two dominant epitopes in influenza virus-infected B6 mice, $NP_{366-374}$ and $PA_{224-233}$, are also profoundly reduced in LMP2/β1i-deficient mice.(58)

Despite the recognized role of the immunoproteasome in optimizing the presentation of certain MHC class I antigens, mice lacking immunosubunits have no discernible abnormalities, and do not exhibit significant alterations in general CTL responses to viral epitopes.(59-61) Lymphocytic choriomeningitis virus-infected LMP2/β1i- or LMP7/β5i-deficient mice were also shown to display no noticeable difference in viral load and the number of CTL responses as compared to wild-type mice.(62) These results suggest that antigen processing may not be the major biological function of the immunoproteasome.

Indeed, the immunoproteasome has also been implicated in other aspects of the regulation of immune responses. Several studies have demonstrated a key role of the immunoproteasome in the regulation of cytokine production by immune cells (Figure 3). Bone marrow-derived dendritic cells from LMP2/β1i-deficient mice infected with influenza virus have considerably reduced levels of IFN-α, interleukin (IL)-1β, IL-6 and TNF-α as compared to wild-type counterparts, suggesting an important role for the immunoproteasome in innate immune responses.(9) The reduced ability to produce cytokines in LMP2/β1i-deficient cells has been associated with compromised NF-κB signaling.(9) It was also shown that selective inhibition of β5i by a small molecule inhibitor, PR-957, blocks IL-23 production in monocytes,

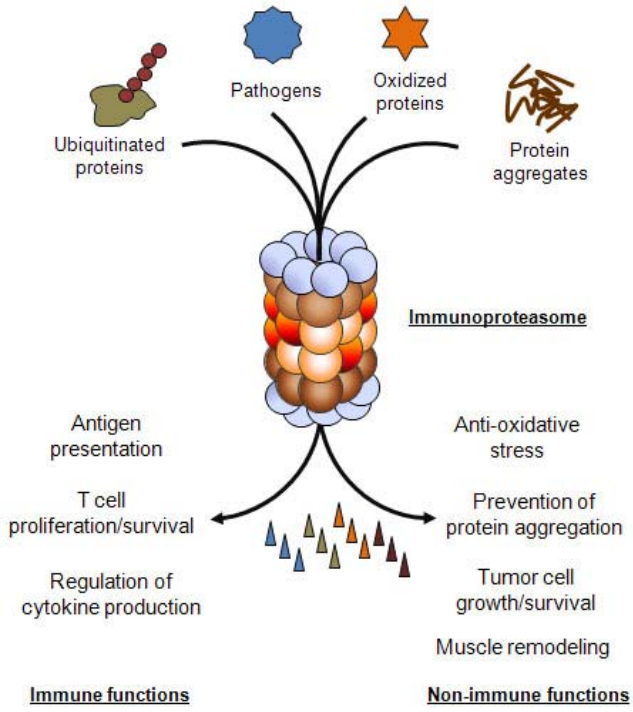

Figure 3. Biological functions of the immunoproteasome. The known substrates of the immunoproteasome include pathogens, ubiquitinated proteins, oxidatively-damaged proteins, and protein aggregates. In addition to the well-defined immune functions of the immunoproteasome in the regulation of MHC class I antigen presentation, the immunoproteasome is involved in cytokine production, and T cell proliferation and survival. Non-immune related function of the immunoproteasome has recently emerging, such as anti-oxidative stress, anti-protein aggregation function, and regulation of muscle mass.

and TNF-α and IL-6 in T cells.(10) A similar trend was observed in animal models of autoimmune diseases such as experimental colitis and rheumatoid arthritis, in which β5i inhibition by PR-957 (8, 10) or deletion in LMP7/β5i-deficient mice (63) lowers inflammatory cytokine secretion, effectively reducing inflammation and disease manifestation. Administration of a higher dose of PR-957 also blocks the β1i and β2i subunits, further lowering TNF-α and IL-6 production, indicating that these two subunits are also involved in cytokine regulation.(8) Impaired NF-κB signaling observed in LMP7$^{-/-}$ mice has been suggested to be responsible for the reduction of cytokine secretion. (63) In addition, NF-κB-independent pathways that require specific enzymatic activity of the immunoproteasome have also been reported to be involved in such action.(10) The underlying mechanisms by which the immunoproteasome activates specific intracellular signaling pathways, such as the NF-κB signaling, more efficiently than the standard proteasome remain unclear. It is postulated that predominant expression of immunoproteasome relative to standard proteasome in immune cells may contribute to the preferential activation of the NF-κB signaling at the site of inflammation. (63)

Additionally, studies have demonstrated the involvement of the immunoproteasome in T cell differentiation, survival, and proliferation (Figure 3). Administration of PR-957 prevents IL-6 and TGF-β induction of CD4$^+$ T cell differentiation into T_H17 cells, implying that the immunoproteasome may be required for the processing of certain differentiation factors.(10) Moreover, β2i deficiency has been shown to increase the CD4$^+$/CD8$^+$ T cell ratio that is speculated to be through intrinsic regulatory mechanisms unrelated to antigen processing.(11, 12) Studies have also suggested a role for the immunoproteasome in T cell survival, as T cells transferred from LMP2/β1i- or MECL-1/β2i-deficient mice into influenza or lymphocytic choriomeningitis virus-infected wild-type mice, respectively, did not improve survival.(58, 59) Proliferation has also shown to be altered in immunosubunit-deficient T cells. T cells from mice lacking both MECL-1/β2i and LMP7/β5i were observed to be highly proliferated in response to polyclonal T cell mitogens; however, the deficiency of only one of the immunosubunits does not result in such phenotype.(11) This may be explained by the possible formation of specific, mixed proteasomes containing β1i, β2 and β5 that may have anomalous function, as mature β1i was detected.

6. ANTI-OXIDATIVE STRESS FUNCTION

Beyond its role in the immune system, recent studies have begun to unravel the non-immune functions of the immunoproteasome. It has recently been demonstrated that the immunoproteasome plays a critical role in maintaining protein homeostasis under cytokine-induced

oxidative stress by preventing protein aggregate formation (Figure 3).(15, 16) Oxidative stress is defined as an imbalance between pro-oxidants and anti-oxidants, resulting in increased release of free radicals and subsequent accumulation of damaged proteins. The pool of damaged proteins can accumulate rapidly under oxidative stress condition to an extent that exceeds the proteolytic capacity of the standard proteasome, and subsequently instigates the formation of harmful protein aggregates, leading to apoptosis.(15, 16) Due to the rapid induction properties and the enhanced proteolytic activities compared to the constitutive proteasome, the immunoproteasome has been suggested to play an important role in efficiently removing the nascent, oxidatively-damaged proteins.(14, 15) In the absence of functional immunoproteasomes, oxidized and polyubiquitinated proteins were shown to accumulate in the liver and brain of LMP7/β5i- and LMP2/β1i-deficient mice.(15, 64) In line with this observation, LMP7/β5i-deficient mice develop more severe oxidative damage of the central nervous system (CNS) in a mouse model of experimental autoimmune encephalomyelitis.(15) The findings that polyubiquitinated oxidative proteins are accumulated in LMP7/β5i-deficient mice suggest a function of the immunoproteasome in controlling the turnover of polyubiquitinated proteins beyond the previous reports that the immunoproteasome mainly degrades oxidative proteins in a ubiquitin-independent manner. (14) Ubiquitin-dependent oxidative protein degradation is likely executed through the action of the 19S-i20S-19S, or hybrid immunoproteasomes (Figure 1), whereas ubiquitin-independent turnover is mediated by 11S-i20S-11S immunoproteasomes (Figure 1). Together, these findings demonstrate that immunoproteasome-mediated proteolytic machineries are crucial to cellular relief from oxidative stress and the maintenance of protein homeostasis.

The efficiency of the immunoproteasome and the proteasome regulator PA28α/β in the removal of oxidized proteins has also been carefully characterized *in vitro* in comparison to the 20S and the 26S proteasome.(14) It was shown that the 26S proteasome exhibits no preferential degradation of oxidized proteins, whereas the 20S proteasome, the immunoproteasome, and the PA28α/β are all capable of degrading oxidatively-damaged proteins.(14) Further investigation using purified immunoproteasome and 20S proteasome demonstrated that the immunoproteasome can selectively degrade oxidized hemoglobin at a much higher rate than the standard 20S proteasome.(14) Together, available data suggests that turnover of oxidized proteins is enhanced, either by the proteolytic activities of the immunosubunits or by the subsidiary role of PA28α/β, which preferentially associates with the immunoproteasome, or possibly a combination of both. Further investigation is warranted to elucidate the factor which contributes to the specialized function of immunoproteasome in turnover of oxidized proteins.

7. IMMUNOPROTEASOME AND DISEASES

7.1. Cancer

As the relationship between cancer and the UPS continues to emerge, the proteasome has become increasingly targeted as a novel candidate for anti-cancer therapy.(65) Several proteasome inhibitors have been developed in an attempt to mitigate oncogenesis, particularly in patients with multiple myeloma. Bortezomib has been approved by the FDA for clinical usage for relapsed and refractory multiple myeloma.(66) It non-specifically inhibits what is considered the most significant, rate-limiting proteolytic activity of both the constitutive proteasome and the immunoproteasome, the chymotrypsin-like activity (β5 and β5i).(66) Such treatment has been proposed to effectively inhibit NFκB activation, block myeloma cell adhesion to the bone marrow stroma, and reduce angiogenesis.(67) It has also been thought to cause the accumulation of unfolded/misfolded proteins, resulting in ER stress and the induction of apoptosis.(68, 69) Despite its efficacy, treatment with bortezomib can result in cytotoxicity, such as neuropathy,(70) and the development of drug resistance.(18) Although several alternative general proteasome inhibitors such as Carfilzomib(71) and NPI-0052(72, 73) have recently been developed to overcome such drug resistance, the treatment of multiple myeloma remains challenging due to off-target side effects and the high risk of relapse.

Immunoproteasome has been reported to be highly expressed in hematological cancers,(74) suggesting that selective inhibition of the immunosubunits may provide a novel therapeutic option. The expected advantage of using immunoproteasome inhibitors is that they will not target standard proteasomes, which are constitutively expressed in all eukaryotic cells, thereby reducing the toxicities of broad proteasome inhibitors. Immunoproteasome-specific inhibitors such as IPSI-001 (a selective β1i inhibitor) and PR-924 (a selective β5i inhibitor) are being developed to selectively target infected cells.(18, 19) It has been shown that IPSI-001 significantly inhibits cell proliferation and promotes apoptosis in myeloma patient samples and other hematologic malignancies.(18) Preclinical studies have also demonstrated that selective inhibition of β5i by *in vivo* administration of PR-924 reduces tumor size in both human plasmacytoma xenografts and SCID-hu mouse models.(19) In addition to hematological cancers, recent studies have revealed a potential application of immunoproteasome inhibitors in the treatment of solid tumors. PC3 prostate cancer cells treated with a β1i-specific inhibitor showed a marked decrease in cancer cell growth.(17) Interestingly, it was observed that cancer cells expressing high levels of β1i are more susceptible to the inhibitor than cells with lower levels of β1i. However, it has been reported that, in some cases, proteasome-specific inhibitors that discriminately target either the chymotrypsin-like activity of the β5 or β5i subunit fail to produce a strong antitumor effect against multiple myeloma cells.(71) A broad-spectrum inhibitor targeting both subunits was required to reduce the viability of tumor cells of hematopoietic origin. Nevertheless, the immunoproteasome has proven to be an important factor in cancer development and merits further study (Figure 3).

On the other hand, several studies have shown that some tumor cells can employ different techniques to manipulate the immunoproteasome function to escape immune surveillance. For instance, downregulation of the

immunoproteasome has been implicated to be an important immune evasion strategy for some tumor types.(75-77) Human cancer lines have also been identified to preferentially express a nonfunctional, immunosubunit variant known as LMP7E1.(78) LMP7E1 and its functional form LMP7E2 are encoded in the same LMP7/β5i gene but are transcribed into two separate mRNAs that have different propeptides due to different initial exons.(78) This incongruity renders LMP7E1 unable to associate with POMP, an important factor critical to the maturation of the immunoproteasome. Importantly, treatment of LMP7E1-expressing cancer cells with IFN-γ fails to induce immunoproteasome formation. The lack of functional immunoproteasomes benefits cancer cells by inhibiting antigen presentation in tumor cells. Thus, efforts to restore the immune regulation and clearance of cancer cells through targeting the immunoproteasome remain an attractive prospect for certain tumors.

7.2. Autoimmune diseases

Given the demonstrated function of the immunoproteasome in processing self-antigens, recent evidence suggests that the immunoproteasome may be involved in the development of autoimmune disease and serve as a potential target for the treatment of this disease. Increased expression of the immunosubunits has been observed in several autoimmune diseases such as rheumatoid arthritis(10, 79) and inflammatory bowel disease. (8, 63, 80, 81) Using two different mouse models of rheumatoid arthritis (collagen-induced and anti-collagen antibody-induced arthritis), it has been shown that administration of a selective inhibitor of β5i (PR-957) results in reduced inflammatory infiltration, cytokine and autoantibody production, and attenuated disease symptoms.(10) In a case of dextran sulfate sodium-induced experimental colitis, β5i inhibition by PR-957 (8) or deletion in LMP7/β5i-deficient mice (63) lowers inflammatory cytokine secretion, effectively reducing inflammation and disease manifestation. Despite these exciting findings, the contribution of the immunoproteasome to the pathogenesis of autoimmune diseases remains largely unknown and warrants further investigations.

7.3. Neurodegenerative diseases

Unlike the standard proteasome, which is highly expressed in the brain, the expression of immunosubunits is low in young rodent and human brains.(24, 26, 82) Expression of β1i and β5i is significantly upregulated in mice upon CTL-induced brain injury.(13) Furthermore, increased expression of immunosubunits in the hippocampus of aged rats as compared to their younger counterparts was observed,(48) consistent with earlier reports that the contents of the immunoproteasome were markedly increased in aged muscle.(83, 84) It is speculated that chronic inflammation and cellular stress may contribute to such upregulation during aging and that the increased immunoproteasome levels may be involved in protecting the CNS from stress and injury by repairing damage more efficiently than the standard proteasome.(13)

Increased expression of immunoproteasome has also been described in neurodegenerative diseases. Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by CAG repeats coding for poly-glutamine sequences of the huntingtin protein.(85) The expanded glutamine repeats induce the formation of neurotoxic huntingtin aggregates which can directly impair the function of the UPS and are thought to cause abnormal neuronal physiology and viability, possibly leading to altered turnover of regulatory proteins and neuronal cell death.(86) In contrast, β1i and β5i subunits were observed to be upregulated in these same aggregate-containing regions of the brain in HD94 mice, a mouse model of HD, and in patients with HD.(24, 25) The mechanisms of increased immunoproteasome expression in HD remain unclear. It was speculated that the immunoproteasome may be upregulated to compensate for the loss of the UPS function to help clear protein aggregates.(24) Studies have further demonstrated that the progressive signs of neurodegeneration correlate with increasing levels of β1i and β5i in neurons.(24) A similar elevated expression of immunosubunits (β1i and β5i) was also observed in the brain of patients with Alzheimer's disease(26) and in the CNS of a mouse model of familial amyotrophic lateral sclerosis (ALS);(27) however, the pathophysiology relevance of the upregulation of the immunoproteasome in neurological diseases remains to be elucidated.

7.4. Cardiovascular diseases

Individual immunosubunits, such as LMP2/β1i, have been demonstrated to play a vital role in cardioprotection.(20) Ischemic-preconditioning induces cardioprotection by activating multiple signaling pathways including protein kinase C (PKC), mitogen-activated protein kinase (MAPK), and phosphatidylinositol-3-kinase (PI3K).(87) It has been shown that the protective effects of ischemic-preconditioning on cardiac function and infarct size during ischemia-reperfusion injury are lost in LMP2/β1i-deficient mice.(20) Furthermore, it was observed that ischemic-preconditioning-induced degradation of phosphatase and tensin homolog (PTEN), and subsequent activation of Akt is disrupted in LMP2/β1i-deficient mice, suggesting a potential link between impaired PTEN degradation and the loss of cardioprotective signaling mechanism in these mice.(20) In addition, LMP2/β1i has also been shown to be important in normal cardiac development and in the regulation of muscle mass under disease conditions (Figure 3).(20, 21) LMP2/β1i-deficient mice were observed to exhibit reduced cardiac mass and decreased heart rate.(20) At resting conditions, LMP2/β1i and LMP7/β5i are constitutively expressed in the heart. However, the protein level of

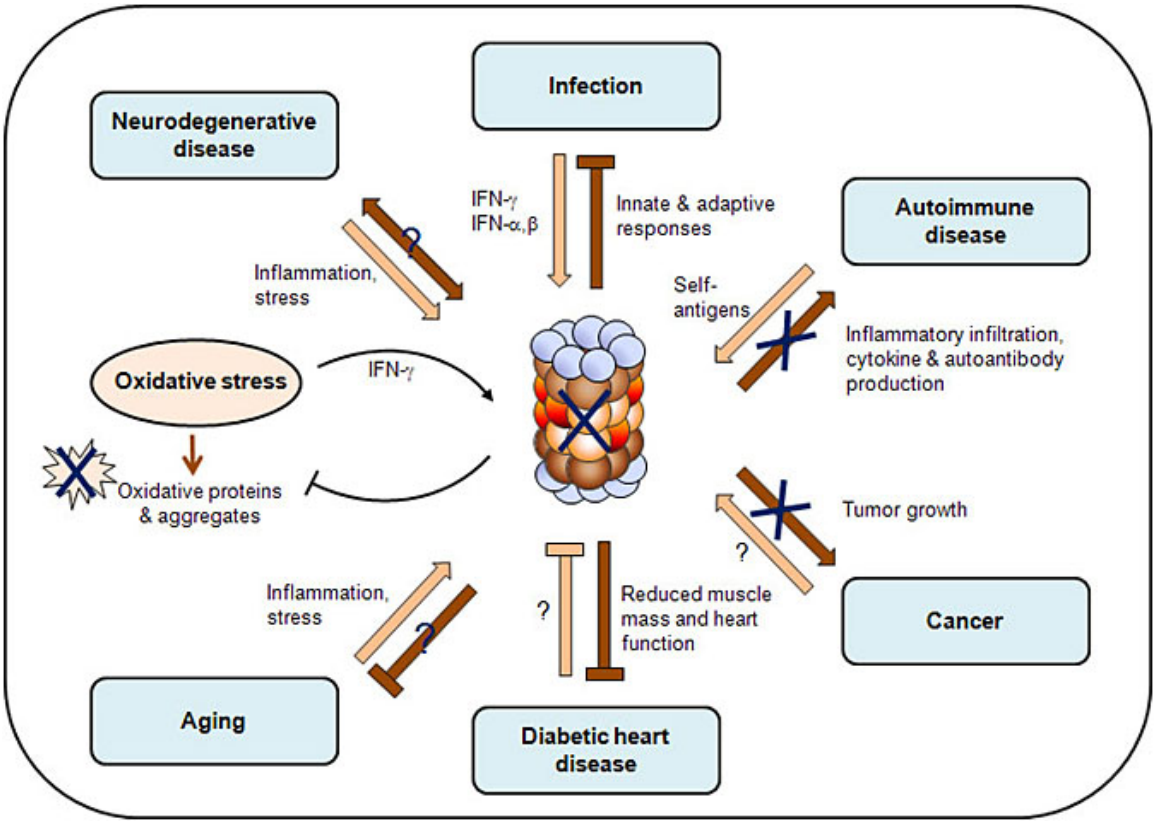

Figure 4. The immunoproteasome and diseases. Dysregulation of the immunoproteasome has been associated with various clinical disorders, including cancer, autoimmune disease, neurodegenerative disease, heart disease, aging, and infection, as well as the conditions of oxidative stress. The immunoproteasome can regulate both the innate and adaptive immune responses, and maintain cellular protein homeostasis, which may be beneficial to the cells under infection, aging or oxidative stress. On the other hand, excessive and prolonged induction of the immunoproteasome may be harmful, contributing to the pathogenesis of autoimmune disease and cancer. Inhibition of the immunoproteasome function leads to decreased tumor size, attenuated symptoms of autoimmune diseases, and relief from oxidative stress.

LMP2/β1i and its corresponding trypsin-like activity are significantly reduced in diabetic mouse hearts and isolated mouse hearts exposed to high glucose.(21) More importantly, LMP2/β1i-deficient mice were shown to display reduced cardiac mass and impaired cardiac function associated with increased accumulation of PTEN, implying a role of the LMP2/β1i in the regulation of muscle mass.(21) Together, these studies support a role of the basal level of LMP2/β1i in cardiac muscle remodeling.

A recent study has also revealed a role for immunoproteasome in the control of vascular apoptosis and plaque rupture.(28) Chronic inflammation is thought to be a key factor contributing to the development of atherosclerosis.(88) In response to IFN-γ, cells isolated from human atherosclerotic lesions were demonstrated to be more sensitive to Fas-induced apoptosis.(28) Further investigation showed that siRNA knockdown of β5i, one of the IFN-γ responsive genes identified by microarray analysis in these samples, blocks IFN-γ sensitivity to Fas treatment and prevents Fas-induced degradation of the proapoptotic protein Mcl-1, resulting in increased cell survival.(28)

The relationship between the immunoproteasome and human diseases is summarized and shown in Figure 4.

8. CONCLUSION

Immunoproteasome-mediated proteolysis has emerged as an important molecular mechanism for regulating wide-ranging functions. It plays a key role not only in modulating both the innate and adaptive immune responses, but also in protecting against oxidative stress and maintaining cellular protein homeostasis. In addition to these protective functions observed under basal, stress or inflammatory conditions, deregulation of the immunoproteasome, for example, excessive and prolonged induction of the immunoproteasome activity can be detrimental to the cells and has been associated with the pathogenesis of several clinical disorders including autoimmune disease and cancer (Figure 4). Elucidating the exact pathological contribution of the dysregulated immunoproteasome to the development of disease and the underlying causal mechanisms will undoubtedly bring valuable insights into the pathogenesis of these diseases and lead to a novel avenue for therapeutic intervention.

9. ACKNOWLEDGEMENTS

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Send correspondence to: Honglin Luo, UBC James Hogg iCAPTURE Centre, Institute for Heart and Lung Health, University of British Columbia St. Paul's Hospital, 1081 Burrard Street, Vancouver, BC, Canada, V6Z 1Y6, Tel: 604-682-2344 ext. 62847, Fax: 604-806-9274, E-mail: honglin.luo@hli.ubc.ca

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