The Ubiquitin-Proteasome System and Its Role in Inflammatory and Autoimmune Diseases

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Protein degradation through the ubiquitin-proteasome system is the major pathway of non-lysosomal proteolysis of intracellular proteins. It plays important roles in a variety of fundamental cellular processes such as regulation of cell cycle progression, division, development and differentiation, apoptosis, cell trafficking, and modulation of the immune and inflammatory responses. The central element of this system is the covalent linkage of ubiquitin to targeted proteins, which are then recognized by the 26S proteasome, an adenosine triphosphate-dependent, multi-catalytic protease. Damaged, oxidized, or misfolded proteins as well as regulatory proteins that control many critical cellular functions are among the targets of this degradation process. Aberration of this system leads to the dysregulation of cellular homeostasis and the development of multiple diseases. In this review, we described the basic biochemistry and molecular biology of the ubiquitin-proteasome system, and its complex role in the development of inflammatory and autoimmune diseases. In addition, therapies and potential therapeutic targets related to the ubiquitin-proteasome system are discussed as well. Cellular & Molecular Immunology. 2006;3(4):255-261.

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Introduction

The 2004 Nobel Prize in Chemistry was awarded to a group of scientists for the discovery of the function of ubiquitin (1-4). Ubiquitin is part of the ubiquitin-proteasome system (UPS) that is responsible for the degradation of more than 80% of normal and abnormal intracellular proteins. At the heart of this system is the 26S proteasome, a dynamic multisubunit proteolytic complex with a molecular weight of 700 kDa that, in eukaryotes, functions as the key enzyme for non-lysosomal protein degradation. Proteasomal degradation removes denatured, misfolded, damaged or improperly translated proteins from cells and as well as regulating the level of proteins such as cyclins and transcription factors. The products resulting from this enzymatic degradation have different sequences, lengths and biological functions (5). It has been shown that this is a highly regulated, tightly controlled, yet complex system that is central to normal cellular homeostasis including cell cycle regulation, DNA repair, sodium channel function, regulation of immune and inflammatory responses and cellular response to stress (6-8). Derangements of the UPS can lead to many disorders, including malignancies, neurodegenerative diseases and possible systemic autoimmunity (9). Better understanding of the UPS process and identification of the components involved in the degradation of key regulatory proteins has lead to the development of mechanism-based therapeutics in various diseases (10-12). A growing body of evidence suggests that targeting the proteasomal pathway is an attractive approach to treat inflammatory and autoimmune diseases (13-15).

Ubiquitin and ubiquitination

Ubiquitin is a 76-residue protein that is highly evolutionarily conserved in all eukaryotes (16, 17). Selective attachment of ubiquitin to proteins is the initial signal for targeted protein degradation. The linkage of ubiquitin to the target protein is through a branched isopeptide bond between the ubiquitin carboxyl-terminal glycine and an internal lysine on the substrate. Additional ubiquitin moieties are sequentially added to each other to form a polyubiquitin chain that functions as the recognition signal for a downstream proteasome in the UPS. Modification of target proteins by ubiquitin or an ubiquitin-like protein remodels the surface of
target proteins, affecting their stability, activity, interactions
with other proteins, and subcellular localization (18, 19).

The ubiquitination of protein is carried out in three
sequential steps involving three enzymes designated E1, E2
and E3 (Figure 1). E1 and E2 enzymes prepare the ubiquitin
chain that is then attached to proteins by the E3 enzyme.
Ubiquination begins with the adenosine triphosphate (ATP)-
dependent activation of ubiquitin by the ubiquitin-activating
enzyme E1. Ubiquitin is attached to an internal E1 Cys
residue via an intermediate thiol ester generating E1-S~ubiquitin.
Ubiquitin is then transferred to one of several
forms of E2, or ubiquitin conjugating enzyme. In step three,
the addition of ubiquitin to the protein substrate is catalyzed
by one of many E3s - a diverse group of proteins with distinct
motifs (20).

The high specificity and selectivity of the UPS system
lies in the diversity of different ubiquitin-protein ligase E3s
that can recognize a specific substrate (7). One of the most
important recognition patterns is a “destabilizing” N-terminal
amino acid such as arginine and lysine. These unique
N-terminal residues can determine the half-life of an
intracellular protein and has been defined as the N-end rule.
In addition to its substrates, the activity of the ubiquitin
system itself can be modulated by numerous factors such as
thyroid hormones, glucocorticoid steroids, cytokines, and
proteins expressed in malignant cells such as proteolysis-
inducing factor (PIF). Interestingly, several factors, including
interferon γ (IFN-γ) not only regulate the modification of
substrate, such as the IkB family of proteins, but also modify
the components of the enzymatic machinery of the ubiquitin
system, such as the E3 ubiquitin ligase Itch, and the
proteasome complex (21). Similarly, the protein kinase C
and tyrosine kinase pathways are involved in both the
modification of UPS substrates and phosphorylation of E1
and E2, thereby ensuring their activities are carried out
efficiently.

**Proteasome**

**20S proteasome**
The ubiquinated substrates targeted for proteolysis are
recognized by proteasomes – the central element of the UPS.
In the proteasome complex, the core protease (CP) is the
barrel-shaped 20S complex (20S proteasome) (Figure 2). It
consists of four stacked rings each with 7 distinct subunits,
stacked one on top of each other, that are responsible for the
proteolytic activity of the proteasome. There are two identical
outer α rings and two inner β rings. The outer 2 α-rings have
no known function, whereas the β rings contain multiple
catalytic sites. In eukaryotes, two of these sites on the β rings
are chymotrypsin-like (CTL), two of these sites are
tryptsin-like (TL) and two are caspase-like. Essentially all
known synthetic and natural proteasome inhibitors act on the
CTL sites (22).

**26S proteasome**
The 26S proteasome is composed of 2 subcomplexes – one
20S proteasome and two 19S regulatory particles (RP, also
known as PA700) – which cap the ends of the 20S complex
(Figure 2). The 19S RP controls the recognition of the
ubiquinated proteins, the ATP-dependent unfolding and the

opening of the channel in the 20S proteasome that allows entry into the proteolytic chamber.

**Immunoproteasome**

PA28 is an activator of the 20S proteasome and an alternative RP. It is composed of two homologous subunits (α and β) and a separate but related protein termed PA28γ which is also known as the Ki antigen (23). PA28 associates with the 20S proteasome to form a football-like structure, hybrid proteasome (19S RP-20S proteasome-PA28) - a complex referred to as an immunoproteasome due to its enhanced capability to generate major histocompatibility complex (MHC) class I-binding peptides. The expression of PA28 is inducible by IFN-γ, which is different from its constitutively expressed counterparts α and β subunits in 26S proteasome. More recently, it was noted that IFN-γ induces the expression of PA28-α and PA28-β, but not the Ki antigen in human cells, which indicates the PA28 family of proteins may have distinct biological functions (24).

After ubiquinated proteins have been degraded within the CP, the 19S RP removes the polyubiquitin tag from the substrate protein and generates free and reusable ubiquitin. Cleaved peptides generated by proteasome degradation can have different fates. Some are further degraded by cytosolic peptidases. Some can be transported into the endoplasmic reticulum (ER) for binding to MHC class I molecules and cell-surface expression. This latter subset of antigenic peptides may arise from hybrid proteasome that generates peptides trimmed to properly fit the MHC class I binding grooves. In this regard, immunoproteasomes have unique value during immune responses.

**UPS and inflammatory and autoimmune diseases**

The UPS is responsible for the majority of eukaryotic intracellular protein turnover (1, 2). Coordinated UPS function is essential to variety of cellular processes (17, 25). Aberration of the system can lead to the dysregulation of cellular homeostasis and the development of multiple diseases such as malignancy (12, 26-35), neurodegenerative diseases (36), and cardiovascular diseases (37-39). This review, however, will focus on the role of UPS in the immune system and the development of inflammatory and autoimmune diseases.

**UPS and the immune system**

A prime example for the involvement of UPS in the immune system is MHC class I antigen processing by antigen-presenting cells (APC) (40). It has been well established that the majority of peptide antigens presented on MHC I molecules are generated by the UPS (41). As previously mentioned, PA28, a ring-shaped 11S multimeric complex, mediates this line of function within the UPS (42). It has been demonstrated that PA28 binds to the ends of 20S proteasome and dramatically enhances its capability to hydrolyze oligopeptides, which leads to the generation of small oligopeptides suitable for MHC I presentation (43, 44). More specifically, the proteasome is responsible for
generating the precise C termini of many MHC-presented peptides, whereas aminopeptidases in the cytoplasm and endoplasmic reticulum can trim the N terminus of the extended peptides to their proper size (45, 46). Interestingly, IFN-γ is a key pleiotropic regulatory cytokine within this context (47). It controls an inducible proteolytic cascade, which consists of PA28 and other inducible proteasome subunits, and the activity of aminopeptidases, which lead to increased peptide production for MHC I presentation. In addition, IFN-γ also decreases peptide destruction by down-regulating the expression of a metalloprotease, thimet oligopeptidase, that actively destroys many antigenic peptides (48, 49).

UPS plays a significant role in the regulation of both T cell receptor (TCR) and costimulatory CD28 signaling through the action of ubiquitin ligases of the Cbl family (50). CD28 costimulation results in the ubiquitination and degradation of Cbl-β, which eliminates the negative regulators and allows the expression of proinflammatory cytokines and their receptors. However, the most important link between the UPS and inflammation is related to NF-κB. NF-κB is a master regulator of many inflammatory cytokine genes, and its activation is mediated through the UPS. NF-κB is actively inhibited when bound to IκB. NF-κB activation follows the degradation of IκB, which is dependent on ubiquitination of IκB followed by proteasomal degradation. Hence, alterations in the UPS would have profound effects on immune responses including the regulation of an array of inflammatory cytokines. In addition, proteasome has been shown to regulate inflammatory pathway by controlling the function of macrophages (51).

It has also been reported that the deubiquitinating enzyme CYLD positively regulates proximal T cell receptor signaling in thymocytes by selectively binding to and deubiquitinating the active form of the kinase Lck. Due to cell-autonomous defect in T cell development, CYLD-deficient mice had substantially fewer mature CD4+ and CD8+ single-positive thymocytes and peripheral T cells (52).

Autoimmunity to components of UPS

Autoantibodies to the α and β subunits of 20S proteasome, PA28 and other components of the UPS have been detected in systemic lupus erythematosus (SLE), primary Sjögren’s syndrome, myositis and other autoimmune diseases (53, 54). Autoantibodies to Ki antigen were detected in 6-21% of patients with SLE. The antibodies were associated with certain clinical features such as sicca syndrome [xerostomia (dry mouth), xerophthalmia (dry eyes), and lymphocytic infiltration of the exocrine glands], persistent arthritis, pericarditis, positive anti-Sm antibody and skin involvement in lupus (54, 55). The functional relevance of these autoantibodies remains to be determined. However, it has been shown that autoantibodies against 20S proteasome have the capability to block proteasome activation by PA28 in vitro, which indicates that autoantibodies may have a regulatory role in proteasome function, and antibody targeting of the interaction between proteasome subunits represents a novel mechanism of proteasome inhibition (56, 57).

Increased levels of circulating proteasomes has also been observed in autoimmune myositis, SLE, primary Sjögren’s syndrome, rheumatoid arthritis (RA) and other autoimmune diseases (58). In addition, circulating proteasome levels correlate with disease activity in RA and SLE patients. Hence, it has the potential to serve as a biomarker for cellular damage and disease activity in these patients (57).

UPS and inflammatory and autoimmune diseases

The UPS is involved in the development of inflammatory and autoimmune diseases through multiple pathways, including MHC-mediated antigen presentation, cytokine and cell cycle regulation, and apoptosis (59).

1) Inflammatory arthritis. NF-κB regulates multiple critical cytokines involved in the pathogenesis of RA (60, 61). In the peptoglycan/polysaccharide-induced inflammatory arthritis model, a proteasome inhibitor improved the arthritis score by suppressing the activation of NF-κB, reducing the expression of cell adhesion molecules and IL-6. In addition, proteasome inhibition may regulate the development of inflammatory arthritis by controlling angiogenesis (62).

2) Psoriasis. Psoriasis is one of the prototypical T cell-mediated diseases, and its development is related to the activation of NF-κB. Administration of a proteasome inhibitor reduced the size of psoriatic lesions in human skin explants grafted onto mice. The treatment also resulted in reduced superantigen-mediated T-cell activation, attenuated cell adhesion molecule expression, decreased expression of T-cell activation markers that were significantly elevated during the disease process (63).

3) Allergy and asthma. Abnormal activation of type 2 helper T cells (Th2) results in asthmatic and allergic symptoms (64). E3 ubiquitin ligase Itch plays a critical role in maintaining immune tolerance mediated through Th2 cells both in vitro and in vivo. Itch deficient mice failed to block the development of airway inflammation in an allergic model (65). Consistent with these findings, encouraging therapeutic effects were observed in a rodent model of allergen-induced asthma (66).

4) Other inflammatory and autoimmune diseases. Sero-negative spondyloarthropathies (SpA) are a group of diseases characterized by, but not limited to, axial joint inflammation. Ankylosing spondylitis (AS) is the prototypical SpA. Most patients with AS carry the MHC class I HLA-B27 gene, and so much research effort has been directed at understanding the role of this gene in the disease pathogenesis. Much interest has been focused on determining the origin and nature of the peptides being presented by HLA-B27 and the cell surface expression of misfolded HLA-B27, two areas in which the UPS is known to play a role. The UPS is involved in the regulation or induction of apoptosis. Apoptosis has been implicated in both experimental models and clinical SLE. In mature, activated lymphocytes, the proteasome inhibitor lactacystin induces DNA fragmentation and apoptosis in a dose-dependent fashion, indicating that proteasome suppresses apoptosis in these cells. Altered clearance of autoantigens is thought to allow for targeting by
the immune system and the development of autoimmunity. The involvement of UPS in regulating the levels of Ku70 and other autoantigens has been reported (67-69).

**Proteasome inhibitors as novel therapeutic agents**

An array of synthetic and natural inhibitors of the proteolytic sites on the 20S proteasome have been developed both as research tools, and more importantly, as therapeutic agents (9, 13, 70-72). Currently there are several inhibitors either approved or in clinical trials for the treatment of multiple cancers and strokes (71, 73). These inhibitors have the capability of entering cells and blocking protein degradation by the ubiquitine-proteasome pathway. They have also proven to be valuable tools for investigating the basic intracellular function of proteasomes and have facilitated the discovery of numerous novel regulatory functions of UPS.

Proteasome inhibitors represent a novel class of anticancer drug (74). Preclinical studies have demonstrated that bortezomib, a dipetidyl boronic acid, that is a selective and potent inhibitor of the 26S proteasome, decreases proliferation, induces apoptosis, enhances the activity of chemotherapy and radiation, and reverses chemoresistance in a variety of hematologic and solid malignancy models both in vitro and in vivo (75). It has been shown that bortezomib inhibits PKR-like endoplasmic reticulum kinase (PERK), and sensitizes pancreatic cancer cells to endoplasmic reticulum stress-mediated apoptosis (33, 76). Bortezomib was the first proteasome inhibitor to enter clinical trials. Recently, bortezomib received Food and Drug Administration (FDA) approval for the use of multiple myeloma and is being evaluated for the treatment of solid tumors (33, 34, 74, 77). The antineoplastic effects of bortezomib have been attributed, at least in part, to inhibition of IκB degradation leading to inactivation of the pivotal pro-survival transcription factor, NF-κB (74).

In addition to anti-tumor effects, proteasome inhibitors also have direct impact on the inflammatory pathway. With the help of a proteasome inhibitor, IκB was the first substrate of UPS identified (78). Through regulation of IκB degradation, the UPS controls the activity of NF-κB, which activates the expression of many genes encoding key inflammatory mediators such as cytokines (TNF and IL-1), leukocyte adhesion molecules (ICAM, VCAM) (79), and enzymes (cyclooxygenase, nitric oxide synthetase) (71). In preclinical animal models, selective proteasome inhibitors effectively suppress inflammatory arthritis and other inflammatory conditions (80-84). In recent years, numerous novel drugs for the treatment of rheumatologic diseases have been successfully developed. The main approach has been to target cytokines, immune cells and their activation pathways (85). Hence proteasome inhibitors could represent a novel class of drugs through regulation of catabolism of pro-inflammatory proteins. On the other hand, proteasome inhibition stabilizes short-lived cyclooxygenase II, a critical enzyme involved in synthesis of inflammatory prostaglandins in the neuronal cell line, so their use could potentially exacerbates inflammation (86).

**Conclusion**

The UPS is a complex system that controls many important aspects of cell function. The covalent linkage of ubiquitin to protein substrates can selectively and specifically alter their fate through proteolytic and non-proteolytic pathways. Loss of normal homeostasis though many mechanisms, including stress or infection, can lead to aberrant cellular function and diseases. Increase in ubiquitinated products has been noted in a variety of pathophysiologic states associated with increased oxidative stress such as neurodegenerative diseases and coronary atherogenesis (39, 87). So far, dysregulation of the UPS has been linked to the pathogenesis of a variety of inherited and acquired diseases such as cancer, diabetes, stroke, graft injection, Alzheimer’s disease, amyotrophic lateral sclerosis, multiple sclerosis, asthma, inflammatory bowel disease, autoimmune thyroiditis, inflammatory arthritis and SLE. The involvement of the UPS in the pathogenesis of inflammatory and autoimmune diseases is mediated through various mechanisms. Multiple lines of evidence have indicated UPS has the potential to be an exciting novel therapeutic target for the treatment of inflammatory and autoimmune diseases.

**References**

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