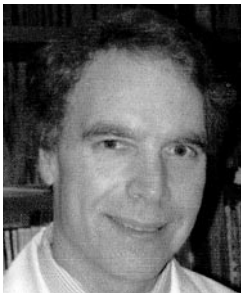
Editorial

Significance of Increased Circulating Proteasome in Autoimmune Disease



THE UBIQUITIN-PROTEIN LIGASE SYSTEM

The ubiquitin-protein ligase system was first described 20 years ago[1]. The ubiquitin system has 3 components consisting of only a few different U1 ligases, a few different homologous U2 ligases, and a larger number of U3 ligases that comprise at least 3 families and are relatively specific for certain substrates. Ubiquitin was named after a 76 amino acid structure that is highly conserved in all eukaryotes. Ubiquitization does not always lead to proteasome degradation of proteins. In addition, there are other regulators of ubiquitin proteasome pathway including SUMO (small ubiquitin modifier) that inhibits the ubiquitinization of proteins.

20S AND 26S PROTEASOME COMPOSITION

The proteolytic activity of the proteasome was described after the ubiquitin ligase system[2], and the proteasome was named in 1988[3]. The 20S core structure is conserved from archaebacteria to eukaryotes and consists of 14 copies each of 2 different but related subunits, an α-type and a ß-type group (Figure 1). The subunits are arranged into four 7-membered rings, with the α-type subunits forming the 2 outer rings, and the ß-type subunits the 2 inner rings. Collectively, they form a barrel-shaped complex, 15 nm in length and 11 nm in diameter, which encloses 3 internal cavities, approximately 5 nm in diameter, bounded by 4 narrow constrictions. Polypeptides to be degraded must pass through a system of internal cavities and constrictions until they reach the active sites in the central cavity. The "26S" proteasome appears as an elongated structure (~45 nm long) consisting of a central 20S complex capped at either one or both ends by the 19S complexes. These 19S caps serve to recognize ubiquitylated proteins and to convert them into a form competent for degradation by the 20S core complex (Figure 1).

INTERFERON-γ INDUCTION OF PROTEIN ACTIVATOR 28 TO FORM THE "IMMUNOPROTEASOME"

The proteasome has an important role in generating immunocompetent peptides to be displayed by the MHC class I complex[4]. The evolution of the proteasome predates the evolution of the immune system, and the availability of peptides between 7 and 9 residues long have been proposed to influence the evolution of the MHC class I system[5]. In turn, the proteasome has responded to the need of the immune system for specific peptides by developing variants of some of its ß-type subunits, which upon induction by interferon-γ (IFN-γ) can replace their constitutive counterparts in the 20S complex, thus allowing further modulation of specificity[6,7].

The 11S or protein activator 28 (PA28) regulator of the 20S proteasome activity is strongly induced by the cytokine IFN-γ[8-11] (Figure 2). This regulator has no hydrolytic activity of its own, but when combined with 20S proteasomes it accelerates the degradation of antigen-processing proteins by the proteasome. IFN-γ also induces synthesis of 3 ß-type subunits of the 20S proteasome, latent membrane protein 2 (LMP2), LMP7, and multicatalytic endopeptidase complex-1 (MECL-1), which replace their constitutive counterparts in the "immunoproteasome." PA28 can also change the single cleavage mode of 20S proteasomes to a coordinated double cleavage mechanism, thereby optimizing the generation of dominant T cell epitopes[12]. PA28 is composed of 2 types of subunits of approximately 30 kDa, PAα and PAß, which are about 50% identical in sequence. 20S proteasomes of tissues from LMP7 knockout mice show reduced MHC class I restricted antigen presentation due to an impairment in peptide generation[13]. Interestingly, the two PA28 subunits have sequence similarity (about 35% identity) to a third protein, the nuclear Ki antigen found in

See Circulating proteasomes are markers of cell damage and immunologic activity in autoimmune diseases, *page 2045-52*.

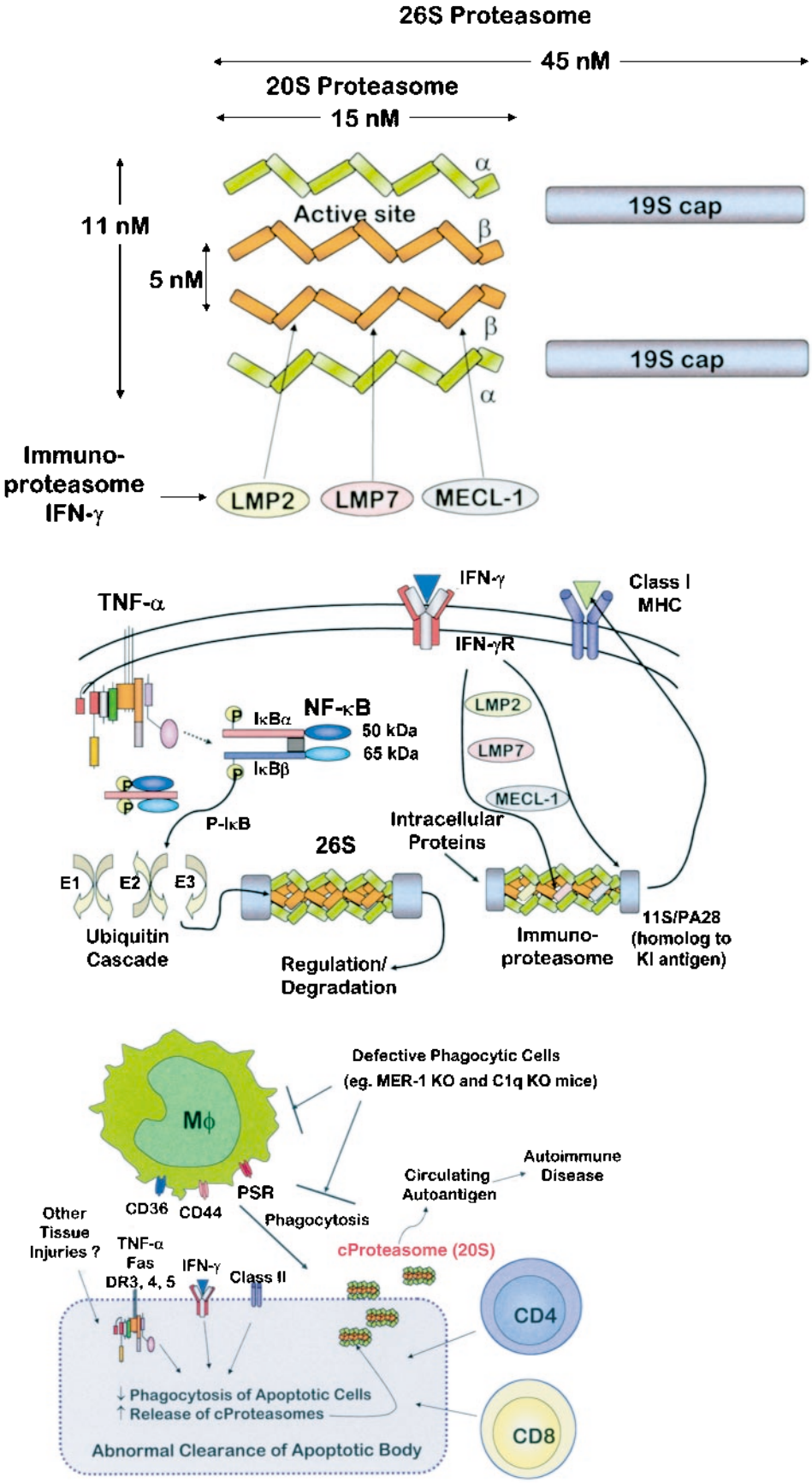


Figure 1. Structure of the 20S and 26S proteasome. The 20S proteasome is the main proteolytic machinery of the proteasome. It consists of 14 inner ß components and 14 outer α components, which are arranged in a barrel-shaped organelle. Under electron microscopy, the 20S proteasome appears as a tubular structure with a length of 15 nM and width of 11 nM. There is a 5 nM core where the peptide to be processed enters. There are 3 cavities and 4 restriction points within the proteasome, and the major proteolytic area is in the central cavity of the proteasome. The proteasome can be modified by addition of the 19S cap, which facilitates entry of ubiquitinated proteins. This 20S proteasome with its 19S caps is referred to as the 26S proteasome. The ß components of the proteasome can be substituted to enhance production of processed antigens. This is accomplished by IFN-γ stimulation and upregulation of protein subunits LMP2, LMP7, and MECL-1, which are incorporated into the ß core of the 20S proteasome.

Figure 2. Different functions of the proteasome. Two main functions of the proteasome are to regulate signal transduction and to process antigens for presentation on class I MHC. An example of how signal transduction is regulated by the ubiquitin-proteasome pathway is shown by the proteasome degradation of phosphorylated and ubiquitinated IκB. One regulator of anti-apoptosis signaling by tumor necrosis factor-α (TNF-α) is phosphorylation of IκB. Recognization of the phosphorylated IκB by ubiquitin ligase proteins, resulting in ubiquitinization and degradation of IκB by the 26S proteasome. This also results in nuclear translocation of nuclear factor-κB and transcription of anti-apoptosis genes. The second function of the proteasome is antigen processing. To enable generation of peptides of the correct length to fit into the class I MHC groove, IFN-γ induces expression of LMP2, LMP7, and MECL-1, which can be substituted for other ß subunits to facilitate antigen processing for presentation by class I. Other functions of the immunoproteasome are to facilitate presentation of antigens by class II MHC and T cell receptor.

Figure 3. Abnormal clearance of apoptotic bodies. Apoptosis is induced by signaling through apoptosis molecules of the death domain family, or by factors from cells such as IFN-γ from CD4 T cells, and perforin-granzyme B from CD8+ T cells. Signaling through TNF-α might affect proteasome activity or decrease phagocytosis of apoptotic cells. This leads to release of conserved proteins such as cProteasome that can circulate and lead to increased anti-cProteasome autoantibodies. Abnormal clearance of apoptotic bodies is especially seen in the MER-1 knockout mice, which have abnormal phagocytotic signaling, or in the C1q knockout mice, which exhibit defective clearance. Phagocytosis is enhanced and made possible by receptors on macrophages including CD36, CD44, and the phosphatidyl serine receptor (PSR). Under normal conditions, there is high phagocytosis of cells at early stages of apoptosis. Under abnormal conditions, defective clearance of apoptotic bodies may lead to release of protein components from these cells and development of autoantibodies.

patients with systemic lupus erythematosus (SLE)[14]. The function of this highly conserved protein is still unknown. Its relationship to the subunits of PA28 and the fact that it is upregulated by IFN-γ point to a possible role in immunity.

APOPTOSIS IN PROTEASOME PROCESSING OF PROTEINS

Apoptotic cell clearance is a complex process that inhibits the initiation of inflammation and the immune response[15,16]. Clearance involves several components on the phagocytic cell. This is mediated by at least 3 receptors including phosphatidyl serine (PS) receptor, CD44, CD36 that are expressed in the macrophage, resulting in uptake of the cell expressing PS and undergoing apoptosis[17,18] (Figure 3). This method of elimination of cells leads to intracellular degradation of antigens as well as intramacrosomal degradation of the cell. Complement deficiencies, the strongest susceptibility genes for the development of SLE, have been shown to correlate an impairment in the phagocytosis of apoptotic cells by macrophages *in vivo*[19]. Dying cells are thought to be a major source of the autoantigens of SLE, and impairment of their removal by complement may explain the link between hereditary complement deficiency and the development of SLE. The MER knockout mice, which lack the c-mer membrane tyrosine kinase, exhibit a defect in phagocytosis[20]. These mice exhibit impaired clearance of infused apoptotic cells, leading to development of progressive lupus-like autoimmunity, with antibodies to chromatin, DNA, and IgG[21].

In this issue, Egerer, *et al*[22] show that there are increased levels of circulating, released 20S proteasomes (circulating proteasomes, cProteasome) associated with a spectrum of autoimmune disease. The highest levels of cProteosomes are associated with myositis, autoimmune hepatitis, and SLE. Autoimmune disease is associated with destruction of cell types. For example, in myositis, muscle cell necrosis is quite evident and is associated with increased serum levels of tissue-specific proteins such as CPK; and hepatitis is associated with tissue-specific proteins such as AST and ALT. Increased cProteasome that can induce an autoimmune response might be due to the evolutionary conserved nature of this complex, as observed for the Ku autoantigen[14]. Increased IFN-γ in autoimmune disease, which leads to higher ratios of the "immunoproteasome" (which contains a 20S core), may also play a role in higher levels of cProteasome[23]. Thus the proteasome plays a critical role in antigen processing, protein degradation, and signal transduction and as an autoantigen in autoimmune diseases.

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REFERENCES

1. Hershko A, Heller H, Elias S, Ciechanover A. Components of ubiquitin-protein ligase system. Resolution, affinity purification, and role in protein breakdown. J Biol Chem 1983;258:8206-14.
2. Dahlmann B, Kuehn L, Ishiura S, et al. The multicatalytic proteinase: a high-Mr endopeptidase. Biochem J 1988;255:750–1.
3. Arrigo AP, Tanaka K, Goldberg AL, Welch WJ. Identity of the 19S "prosome" particle with the large multifunctional protease complex of mammalian cells (the proteasome). Nature 1988;331:192–4.
4. Ben-Neriah Y. Regulatory functions of ubiquitination in the immune system. Nat Immunol 2002;3:20-6.
5. Niedermann G, Grimm R, Geier E, et al. Potential immunocompetence of proteolytic fragments produced by proteasomes before evolution of the vertebrate immune system. J Exp Med 1997;186:209–20.
6. Goldberg AL, Gaczynska M, Grant E, Michalek M, Rock KL. Functions of the proteasome in antigen presentation. Cold Spring Harb Symp Quant Biol 1995;60:479–90.
7. Heemels MT, Ploegh H. Generation, translocation, and presentation of MHC class I–restricted peptides. Annu Rev Biochem 1995;64:463–91.
8. Ma CP, Slaughter CA, DeMartino GN. Identification, purification, and characterization of a protein activator (PA28) of the 20S proteasome (macropain). J Biol Chem 1992;267:10515–23.
9. Dubiel W, Pratt G, Ferrell K, Rechsteiner M. Purification of an 11S regulator of the multicatalytic proteases. J Biol Chem 1992;267:22369–77.
10. Realini C, Dubiel W, Pratt G, Ferell K, Rechsteiner M. Molecular cloning and expression of a gamma-IFN-inducible activator of the multicatalytic protease. J Biol Chem 1994;269:20727–32.
11. Ustrell V, Realini C, Pratt G, Rechsteiner M. Human lymphoblast and erythrocyte multicatalytic proteases: differential peptidase activities and responses to the 11S regulator. FEBS Lett 1995;376:155–8.
12. Dick TP, Ruppert T, Groettrup M. Coordinated dual cleavages induced by the proteasome regulator PA28 lead to dominant MHC ligands. Cell 1996;86:253–62.
13. Stohwasser R, Kuckelkorn U, Kraft R, Kostka S, Kloetzel PM. 20S proteasome from LMP7 knock out mice reveals altered proteolytic activities and cleavage site preferences. FEBS Lett 1996; 383:109-13.
14. Francoeur AM, Peebles CL, Gompper PT, Tan EM. Identification of Ki (Ku, p70/p80) autoantigens and analysis of anti-Ki autoantibody reactivity. J Immunol 1986;136:1648-53.
15. Casiano CA, Tan EM. Recent developments in the understanding of antinuclear autoantibodies. Int Arch Allergy Immunol 1996;111:308-13.
16. Rosen A, Casciola-Rosen L. Autoantigens as substrates for apoptotic proteases: implications for the pathogenesis of systemic autoimmune disease. Cell Death Differ 1999;6:6-12.
17. Savill J, Fadok V, Henson P, Haslett C. Phagocyte recognition of cells undergoing apoptosis. Immunol Today 1993;14:131-6.
18. Rosen A, Casciola-Rosen L. Clearing the way to mechanisms of autoimmunity. Nat Med 2001;7:664-5.
19. Taylor PR, Carugati A, Fadok VA, et al. A hierarchical role for classical pathway complement proteins in the clearance of apoptotic cells in vivo. J Exp Med 2000;192:359-66.
20. Scott RS, McMahon EJ, Pop SM, et al. Phagocytosis and clearance of apoptotic cells is mediated by MER. Nature 2001;411:207-11.
21. Cohen PL, Caricchio R, Abraham V, et al. Delayed apoptotic cell clearance and lupus-like autoimmunity in mice lacking the c-mer membrane tyrosine kinase. Exp Med 2002;196:135-40.
22. Egerer K, Kuckelkorn U, Rudolf PE, et al. Circulating proteasomes are markers of cell damage and immunologic activity in autoimmune disease. J Rheumatol 2002;29:2045-52.
23. Masutani K, Akahoshi M, Tsuruya K, et al. Predominance of Th1 immune response in diffuse proliferative lupus nephritis. Arthritis Rheum 2001;44:2097-106.