

Dysfunction in protein clearance by the proteasome: impact on autoinflammatory diseases

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Abstract During innate immune responses, proteostasis is greatly impacted by synthesis of pathogen proteins as well as by inflammatory tissue damage through radicals or other damaging molecules released by phagocytes. An adequate adaptation of cellular clearance pathways to the increased burden of damaged proteins is thus of fundamental importance for cells and tissues to prevent protein aggregation, inclusion body formation, and ultimately cell death. We here review the current understanding of the pivotal role of the ubiquitin proteasome system (UPS) in this proteostasis network. The proteolytic capacity of the UPS can be adjusted by differential gene expression, the incorporation and maturation kinetics of alternative active sites, and the attachment of different regulators. Dysregulation of this fine-tuning is likely to induce cell death but seen more often to promote inflammation as well. The link between proteostasis impairment and inflammation may play a crucial role in autoinflammation as well as in age-related diseases and currently uncharacterized diseases. Recent studies on proteasome-associated autoinflammatory syndromes (PRAAS) discovered that IFN signaling drives the inflammation caused by reduction of degradation capacity. Elucidation of these syndromes will reveal further insights in the understanding of inadequate immune responses. Knowledge related to the diversity of this degradation system will raise the awareness of potential pitfalls in the molecular

diagnostics of autoinflammatory syndromes and may help to identify novel drug targets.

Keywords Proteostasis · Proteasome · Autoinflammation · PRAAS

Introduction

Autoinflammation is primarily the result of alterations in the innate immune system and shows a broad range of clinical symptoms. Great advances in genotyping efficiency, sequencing technology, and bioinformatical evaluation of patients with autoinflammatory syndromes have revealed single-gene mutations for example in critical components relating to danger sensing and cytokine regulation of the innate immune response, for example, the interleukin (IL)-1, IL-10, NF- κ B, and interferon (IFN) signaling cascades [1, 2]. Interestingly, mutations or variants in components of the protein clearance pathways such as the cytokine-induced immunoproteasome subunit PSMB8 (LMP7/ β 5i) or components of the autophagy pathway were also identified, clearly linking proteinopathies with innate immune responses [3–5].

Cellular proteostasis is a delicate balance between protein synthesis and protein quality control with protein degradation. Perturbations in the cell physiology through environmental stimuli require a fast and coordinated adjustment of translational systems, chaperone supply, and various proteolytic systems. It is increasingly realized that during innate immune responses proteostasis is severely challenged by the production of radicals and/or the massive synthesis of pathogen proteins. The adequate adaptation of their cellular clearance pathways to the increased requirement for protein degradation is thus of fundamental importance for cells and tissues, which are otherwise faced with the threat of an accumulation of

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faulty, damaged or misfolded proteins, followed by protein aggregation, inclusion body formation, and ultimately cell death. The cells choke on their protein trash as shown for neurodegenerative disorders as classical example for proteinopathies. An impairment of proteostasis systems reflected by intra- and extracellular protein deposits accompanied by oxidative stress, neuroinflammation, and progressive degeneration of specific brain regions all represent hallmarks of these diseases [6–8]. A further example of disturbance in proteostasis in disease is the aging-related sporadic inclusion body myositis, where abnormal intracellular protein accumulation and aggregation is symptomatic combined with proteasome dysregulation and endoplasmic reticulum (ER) stress. Patients suffer from inflammatory myopathy characterized by progressive weakness and wasting of muscle [4]. Restoration of proteostasis network function therefore leads to aggregate detoxification and clearance via chaperone systems and proteolysis by the UPS, autophagy, and/or phagocytosis as an important extracellular pathway. Lysosomal degradation via autophagy has been shown to be crucial for clearance of prion aggregates in skeletal muscle [9] in particular because prions tend to inhibit the ubiquitin proteasome system (UPS) via stabilization of the closed barrel formation [10].

In this review, we will focus on the UPS protein clearance pathway, the inhibition of which, either by aging, mutation, or protein interaction, has a dramatic impact on the proteostasis in cells and leads to proteotoxic stress followed by diverse stress responses such as the unfolded protein response (UPR) with chaperone induction, induction of autophagy, apoptosis, and inflammation via the innate immune response. In particular, the proteasome associated autoinflammatory syndromes (PRAAS), in which the UPS is impaired in all cells of the body, clearly reflects how dynamic adaption of the protein degradation capacity is essential for cell surveillance and a regulated immune response.

The ubiquitin proteasome system

Regulated proteolysis of ubiquitylated and/or oxidative damaged proteins via the UPS plays a key role governing the vitality of eukaryotic cells and tissues, whereby the availability of short-lived proteins is determined and the accumulation of misfolded and damaged proteins is prevented to ensure protein homeostasis. The UPS fulfills the essential regulation of numerous important cellular processes, such as gene transcription, DNA repair, apoptosis, cell cycle, cell differentiation, and signaling, as well as the generation of antigenic peptides presented by major histocompatibility (MHC) class I molecules [11, 12].

Most of the proteins to be degraded are modified with lysine 48 (K48)-linked poly-ubiquitin chains that serve as a signal for degradation. This modification is conjugated to

proteasomal substrates by a three-step thioester cascade involving the E1 ubiquitin-activating enzyme, E2 ubiquitin-conjugating enzymes, and E3-ubiquitin ligases [13]. In line with its multitude of functions, the proteasome appears in mammals in a variety of compositions to allow for adaptation to changing proteolytic requirements (Fig. 1). The modular architecture of the proteasome consists of a 20S catalytic core complex associated with one or two regulator complexes. The barrel-shaped 20S proteasome is formed by four stacked rings of seven subunits each with the arrangement of $\alpha 1-\alpha 7$ ($\beta 1-\beta 7$)₂ $\alpha 1-\alpha 7$. With the $\beta 1$, $\beta 2$, and $\beta 5$ subunits, the two inner β -rings harbor six catalytically active sites with caspase-like, tryptic-like, and chymotryptic-like activities, respectively [14]. Several proteasome isoforms consisting of alternative compositions can exist in parallel to the above-described standard proteasome (SP). Constitutively in hematopoietic cells and in other cells in response to infections or cytokines like type I or type II interferons (IFNs), the immunosubunits $\beta 1i/LMP2/PSMB9$, $\beta 2i/Mec11/PSMB10$, and $\beta 5i/LMP7/PSMB8$ are expressed and rapidly incorporated into nascent proteasome complexes to form immunoproteasomes (IP). There is, however, also the possibility that just one or two of the immunosubunits are incorporated and mixed-type proteasomes (MP) are formed. In addition, a further alternative catalytic subunit namely $\beta 5t/PSMB11$ is solely expressed in thymic cortical epithelial cells forming the thymoproteasome (TP), and finally, the spermatoproteasome containing an alternative α -subunit is expressed in the testis during spermatogenesis. Alongside these different 20S isoforms, the regulatory complexes 19S, PA28 $\alpha\beta$, PA28 γ , and PA200 bind to one or both sides of the core particle, open the catalytic pore, and form several complexes such as the 26S (20S+19S), 30S (20S+2 \times 19S), 20S-PA28, 20S-PA200, and hybrid proteasomes (HP) (20S+19S+PA28 and 20S+19S+PA200) [15–18]. It was reported that PA28 $\alpha\beta$ preferentially binds to IP [19]. While the catalytic activity is located in the 20S proteasome core complex, the 19S regulatory complex governs the recognition, unfolding, and access of ubiquitin-conjugated substrates into the catalytic cavity. Thus, only 19S containing proteasome isoforms (SP, IP, PA28-HP) are involved in the degradation of ubiquitin-conjugated substrates, and their impact in the development of diseases will be further discussed in more detail.

The proteolytic capacity of the UPS can be tailored to particular proteolytic requirements by differential gene expression of specific subsets of UPS factors and the assembly of different proteasome isoforms. Depending on the cell type or the tissue context, different proteasome modules can be expressed and coexist in cells. Postmitotic cells like neurons or muscle cells mainly contain SP, whereas immune cells like myeloid or lymphoid cells mainly contain IP or HP. The UPS can be adapted to proteotoxic stress by the Nrf1/Nrf2/ARE-

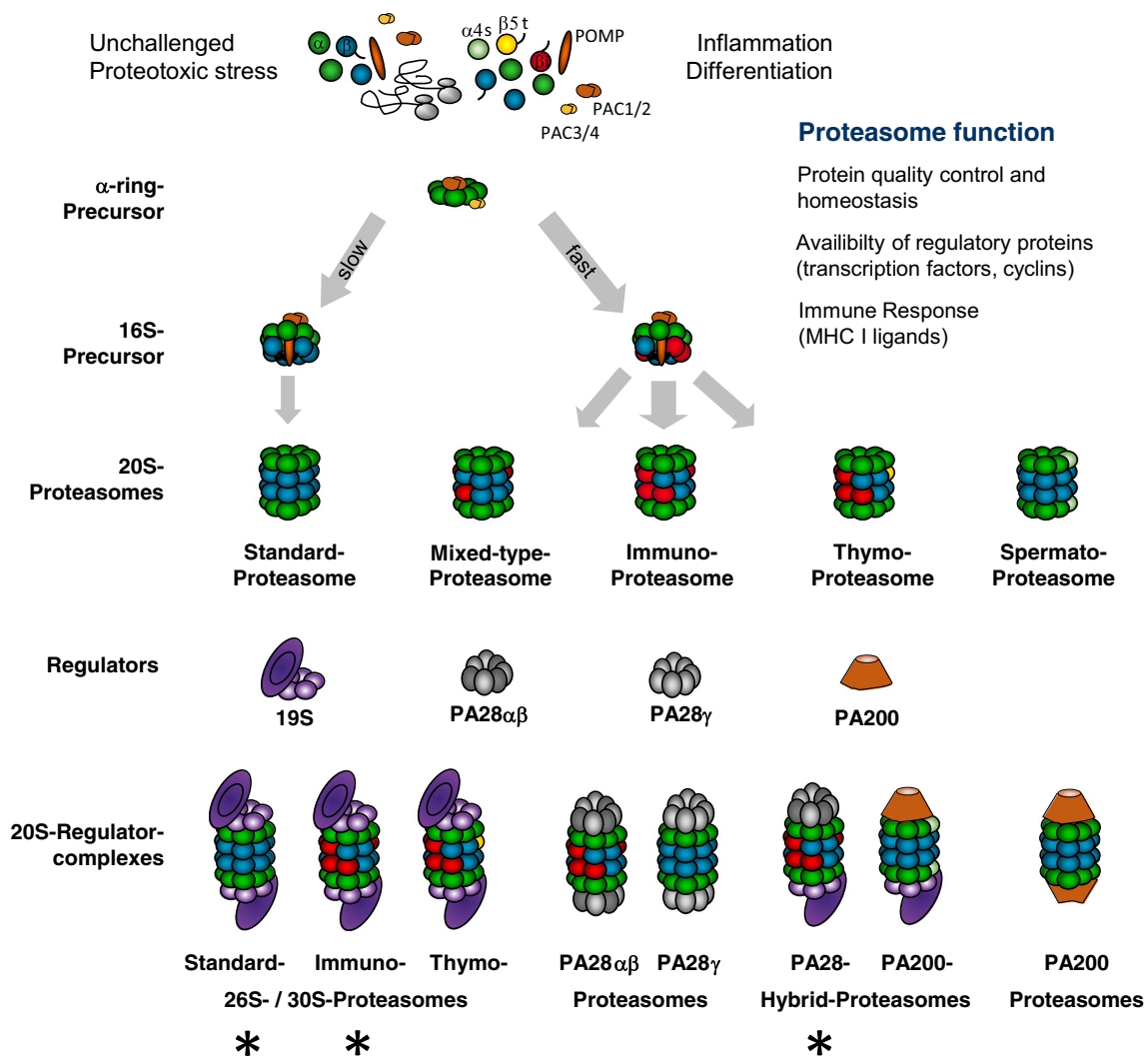


Fig. 1 Proteasome diversity upon cellular requirements. In mammalian cells, a variety of proteasomes is formed according to cell type and challenging status to ensure an appropriate adaption to protein degradation requirements. 20S proteasomes are barrel-shaped and consist of four seven-membered rings in the order of $\alpha 1-\alpha 7$ ($\beta 1-\beta 7$) $_2$ $\alpha 1-\alpha 7$. The stepwise assembly of the 20S proteasome is assisted by the proteasome assembly chaperones (PAC) 1–4 and the proteasome maturation protein (POMP). After 16S precursor dimerization and completed maturation of the β -subunits $\beta 1$, 2, 5, 6, and 7, the latent inactive 20S proteasome is activated through the binding of regulatory particles such as 19S, PA28 $\alpha\beta$, PA28 γ , and PA200 on one or both sides of the proteasome, which open the catalytic pore. According to regulator

combinations, the 26S/30S (20S+19S/20S+2x19S), the 20S-PA28, 20S-PA200, and the hybrid proteasomes with two differing regulators (20S+19S+PA28 or 20S+19S+PA200) can be formed. Besides the standard proteasome with the catalytic subunits $\beta 1$, 2, and 5, alternative catalytic subunits such as $\beta 1i$, $\beta 2i$, $\beta 5i$, and $\beta 5t$ can be incorporated, forming the immuno-proteasome ($\beta 1i$, 2i, 5i), the thymo-proteasome ($\beta 1i$, 2i, 5t), and mixed-type proteasome ($\beta 1$, 2, 5i or $\beta 1$, 2i, 5i). During spermatogenesis, the alternative α -subunit $\alpha 4s$ is expressed, leading to spermatoproteasome formation [16–18, 58]. The asterisk refers to the main isoforms expressed in most cell types and are described in more detail in the text

activated pathway, which induces the SP and certain UPS-related genes [20–22].

IPs and HPs in turn are permanently expressed in immune cells and can be induced not only by cytokine or developmental signaling, in particular by IFNs, but also by heat stress or during the course of neurodegenerative diseases in almost all other cell types. The UPS is responsible for the generation of the vast majority of intracellular pathogen-derived and self-peptides presented by MHC class I molecules at the cell surface to cytotoxic T lymphocytes. This function of the adaptive

immune system is generally aided by cytokine-mediated induction of IP, which enhances MHC class I antigen presentation by improved antigen processing [11]. An additional IP function has been uncovered in maintaining protein homeostasis under cytokine-induced oxidative stress [21]. IFNs induce the intracellular production of radicals thus increasing the content of oxidant-damaged proteins and increased sensitivity to cytokine-induced cell death. Such oxidant-damaged proteins are immediately ubiquitinated and require the high proteolytic capacity of IP for their efficient removal. As

nascent polypeptides are particularly sensitive to oxidation, IFN-signaling enhances the rate of defective nascent proteins and concomitantly MHC class I antigen presentation, placing IP function at the interface of innate and adaptive immune response. IP-expressing cells such as phagocytes including dendritic cells or microglia are thus better equipped to handle cytokine-induced oxidative protein aggregation. The particular function of HP is still under investigation. However, different studies indicate the involvement of PA28 and HP in the removal of damaged proteins under oxidative stress [20, 23].

Interestingly, LMP7 (PSMB8) knockout mice do not obviously show any lipodystrophy or autoinflammatory defect and are physically undistinguishable from wild-type mice [24], but they are more susceptible to some infections, and pathogen clearance is prolonged or inadequate. In particular, an example for the CNS is that IP deficiency was found to result in significantly increased clinical scores by studying autoimmune encephalomyelitis (EAE) in a mouse model [21]. IP deficiency was further associated with severe heart muscle injury with large inflammatory lesions and severe myocardial tissue damage in a mouse model for Coxsackie virus B3-induced myocarditis [25]. This protective role is also evidenced by studies showing that IP-deficient mice severely suffer from impaired stress responses, survival, and/or clearance rates upon infection or inflammation [26–29] or develop severe LPS-induced hepatitis [21]. Moreover, IP dysfunction is connected with (auto)inflammation and autoimmunity, often accompanied by altered cytokine patterns, such as diabetes or Sjögren syndrome [30–33].

Taken together, these data evidence that UPS-controlled proteostasis is important for immune responses to ensure both a balanced response and protection for non-infected cells.

Proteasome-associated autoinflammatory syndromes

More recently, several previously described autoinflammatory syndromes, such as Nakajo-Nishimura syndrome (NNS) [34], joint contractures, muscle atrophy, microcytic anemia and panniculitis-induced lipodystrophy (JMP) syndrome [35], Japanese autoinflammatory syndrome with lipodystrophy (JASL) [36], and chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE) [5], were found to have a mutated PSMB8/ $\beta 5i$ in common and are now classified as a spectrum of diseases named proteasome-associated autoinflammatory syndrome (PRAAS).

The clinical presentation of PRAAS patients is diverse in severity and shows a broad range of symptoms, including a pernio-like rash and repetitive spiking fever with progressive lipodystrophy starting in early infancy. Other reported symptoms include nodular erythema with infiltration and

induration, erythema on the eyelids, long clubbed fingers and toes with joint contractures, progressive partial lipomuscular atrophy and emaciation mainly of the upper body, hepatosplenomegaly and basal ganglia calcification, chronic anemia, and delayed physical development [5, 34–39]. A detailed summary of the clinical presentation was reviewed by McDermott et al. [40].

To date, an autosomal recessive inheritance of four missense mutations and one non-sense mutation in *PSMB8* (p.T75M; p.G201V; p.A92T; p.M117V; p.C135X) have been implicated to cause PRAAS [5, 34–37, 41]. The reported mutations have different impacts on the proteasome by modulation of gene expression, subunit folding and maturation, assembly of the core complex, and/or structural alterations of the proteolytic pocket [5, 34, 36], but all result in reduced proteasome activity [35, 36] which is probably insufficient to cope with a higher load of damaged proteins. In collaboration with the group of R. Goldbach-Mansky [5], we started to investigate an increasing number of patients with autoinflammatory syndromes similar to CANDLE but without the described mutations in *PSMB8*. From the knowledge about the diversity of the proteasome system, we hypothesize the presence of additional mutation sites in further proteasome components, which may affect the assembly, the activity, and even the regulation of proteasome complexes or other UPS-related components. There is indeed evidence from our collaboration that there are mutations in proteasome components other than *PSMB8*.

These findings strongly suggest that the disturbance of the overall proteasome activity and capacity in response to environmental stress is crucial, not just a disturbance of the inducible catalytically subunit $\beta 5i$. We therefore strongly advise for diagnostic sequencing to broaden the spectrum of candidate genes up to all 20S subunits, assembly helpers, and regulatory particle subunits (Table 1).

Laboratory findings and pathogenesis

A strongly reduced chymotryptic-like activity was observed in Epstein-Barr virus (EBV)-transformed B cells from PRAAS patients compared to healthy controls [35, 36], and an accumulation of poly-ubiquitylated proteins can be detected in skin sections, EBV-transformed B cells, and fibroblasts from patients [34, 36]. In the skeletal muscle cells from a deceased suspected CANDLE patient, intramitochondrial paracrystalline inclusions and cytoplasmic and myeloid bodies were observed [42], indicating accumulation of damaged/aggregated proteins. Aggregates and inclusions increase cellular sensitivity to apoptosis [21], and this could be a cause of muscle loss later in life.

Interestingly, adipocyte differentiation seemed to be reduced after *PSMB8* siRNA treatment of preadipocytes,

Table 1 Main components of the ubiquitin proteasome system

Subunit nomenclature	Alternative nomenclature	Gene (HGNC)	Reference transcript NCBI	Function	
20S proteasome core particle					
α-Subunits					
α 1	iota	PSMA6	NM_002791	Guided assembly of 20S together with assembly helpers, gating of catalytic pore, interaction with regulator particles	
α 2	C3	PSMA2	NM_002787		
α 3	C9	PSMA4	NM_001102667		
α 4	C6	PSMA7	NM_002792		
α 5	zeta	PSMA5	NM_002790		
α 6	C2	PSMA1	NM_002786		
α 7	C8	PSMA3	NM_0027883		
α 4s	PSMA7L	PSMA8	NM_144662		In spermatids and sperm, may support interaction with PA200 [16–18]
β-Subunits					
β 1	Y	PSMB6	NM_002798	Active site subunit caspase-like activity	
β 2	Z	PSMB7	NM_002799	Active site subunit tryptic-like activity, c-terminal clamp to β 3	
β 3	C10	PSMB3	NM_002795	Structural function	
β 4	C7	PSMB2	NM_002794	Structural function	
β 5	X, MB-1	PSMB5	NM_002797	Active site subunit chymotryptic-like activity	
β 6	C5	PSMB1	NM_002793	Structural function	
β 7	N3	PSMB4	NM_002796	Structural function, c-terminal clamp to trans β -ring	
β 1i	LMP2	PSMB9	NM_0028004	Caspase-like activity	
β 2i	MECL-1	PSMB10	NM_0028013	Tryptic-like activity	
β 5i	LMP7	PSMB8	NM_1489193	Chymotryptic-like activity	
β 5t		PSMB11	NM_001099780	Only in thymus, chymotryptic-like activity [11, 16–18]	
Assembly helpers					
PAC1		PSMG1	NM_003720	PAC1-PAC2-dimer binds the outer surface of α -ring and prevents α -ring dimerization and off-pathways	
PAC2		PSMG2	NM_020232		
PAC3		PSMG3	NM_001134340		PAC3-PAC4-dimer binds on the inner surface of α -ring around α 5 and prevents faulty arrangement of α -subunits
PAC4		PSMG4	NM_001128591		
POMP	Ump1	POMP	NM_015932	Assist stepwise β -subunits incorporation and dimerization at ER [16–18, 59–61]	
19S regulator					
ATPase subunits					
RPT1	S7	PSMC2	NM_002803	Binding to α -ring surface and opening of catalytic pore, ATP-dependent unfolding of substrates, and translocation into the catalytic pore	
RPT2	S4	PSMC1	NM_002802		
RPT3	S6b	PSMC4	NM_006503		
RPT4	S10b	PSMC6	NM_002806		
RPT5	S6a	PSMC3	NM_002804		
RPT6	S8	PSMC5	NM_002805		

Table 1 (continued)

Subunit nomenclature	Alternative nomenclature	Gene (HGNC)	Reference transcript NCBI	Function	
Non-ATPase subunits					
RPN1	S2	PSMD2	NM_002808	Large subunits which serve as scaffolds onto other substrates and subunits can bind	
RPN2	S10b	PSMD1	NM_002807		
RPN3	S3	PSMD3	NM_002809		
RPN5	p55	PSMD12	NM_002816		
RPN6	S9	PSMD11	NM_002815		
RPN7	S10a	PSMD6	NM_014814		
RPN8	S12	PSMD7	NM_002811		
RPN9	S11	PSMD13	NM_002817		
RPN10	S5a	PSMD4	NM_002817		
RPN11	S13	PSMD14	NM_005805		
RPN12	S14	PSMD8	NM_002812		
RPN13		ADRM1	NM_00700		
RPN15	Sem1, Dss1	SHFM1	NM_006304		
Assembly helpers					
RPN14	PAAF	PAAF1	NM_001267803		Solubilize and bind to c-termini of ATPase subunits, stabilization, assist during the temporally sequence of assembly process
S5b		PSMD5	NM_005047		
p27		PSMD9	NM_002813		
p28	Gankyrin	PSMD10	NM_002814		
PA28 regulator					
PA28 α	11S α	PSME1	NM_006263	Cytokine inducible, heteroheptameric ring binds on α -ring surface of 20S complex, may enhance the generation of hydrophilic peptides	
PA28 β	11S β	PSME2	NM_002818		
PA28 γ	11S γ	PSME3	NM_005789	Only in the nucleus, supports degradation of regulatory proteins [64–66]	
Associated proteins					
PA200		PSME4	NM_014614	Proteasome activator of caspase-like activity, promotes degradation of N-acetylated histones, involved in DNA repair	
ECM29		KIAA0368		Quality control of 26S assembly, localization of proteasomes to secretory compartments, proteasome stabilizer [67–69]	

suggesting that a high proteasomal activity is needed for adipocyte differentiation [36].

In skin biopsy, Kitamura et al. observed a much higher interleukin-6 (IL-6) expression (tenfold) compared to a healthy control patient. They further showed an IL-6 hyperactivation of the patient's EBV-B cells (PSMB8 G201V +/+) after ionomycin/PMA stimulation, which could be partially rescued by p38 inhibition or PSMB8 WT retroviral overexpression. In another study using mouse macrophages, inhibition of the proteasome with lactacystin results in an increased p38 and JNK phosphorylation [43], supporting the results from Kitamura et al. Arima et al. detected higher p38 phosphorylation in patients' fibroblasts as well and found no downregulation of NF- κ B, JNK1/2/3, and ERK1/2 [34]. In contrast, Liu et al. showed that cytokine analysis of blood serum revealed only moderately elevated levels of monocyte chemoattractant protein 1 (MCP1), IL-6, and IL-1 receptor antagonist. However, a characteristic persistent IFN signature evidenced by high levels of IP-10 (CXCL10) was observed and verified by microarray analysis of whole blood, revealing 48 IFN-regulated genes. Furthermore, the downstream mediator of IFN signaling, STAT-1, showed a much stronger phosphorylation after IFN γ stimulation of patient monocytes compared to a healthy control patient [5]. In line with Liu et al., reported abnormally high levels of IL-6 [34, 35, 37], IL-8, IP-10, and IFN- γ in serum of affected patients [35, 37]. This together with the accumulation of ubiquitylated proteins strongly suggests that reduced proteasome activity leads to proteotoxic stress, which is linked by an as yet unidentified pathway to the innate immune response via IFN signaling and p38.

Model of pathomechanism

We here suggest a model of the pathomechanism involved in PRAAS (Fig. 2), an overview of observed findings which will need to be further elaborated in a cell type-specific manner. Because PRAAS flares coincide with infections and other stressful events [5], we suggest environmental stress as the trigger, initiating overwhelming inflammation. The skin and cells from the hematopoietic system, such as dendritic cells and macrophages, establish the first line of defense. A bacterial or viral infection leads to activation of pattern recognition receptors (PRR) such as Toll-like receptors (TLRs) via identification of pathogen-associated molecular patterns (PAMP), such as lipopolysaccharides or nucleic acids. Multiple signaling cascades can be induced after pathogen recognition, which result in expression of genes involved in the specific immune response such as cytokines, particularly IFN type I, enzymes for production of reactive oxygen species (ROS), reactive nitric species (RNS), and antimicrobials [44, 45]. This initial "normal" inflammation leads to a higher level of defective

ribosomal products (DRiPs) and oxidant-damaged proteins due to temporal attenuation of global protein synthesis as result of eIF2a phosphorylation and release of ROS such as superoxide anions, hydrogen peroxide, and hydroxylradicals [46, 47]. Due to the cell's inability to adapt the proteasomal capacity to levels required to degrade the raising amount of impaired proteins, aggregates of damaged proteins are formed [21, 47, 48]. Furthermore, ER stress is induced through the activity of ROS, and the unfolded protein response (UPR) is activated. Four different pathways are induced by ER stress; these are the pancreatic ER kinase (PERK) pathway, the inositol-requiring transmembrane kinase/endonuclease 1 (IRE1) pathway, the activating transcription factor 6 (ATF6) pathway, and the inflammasome pathway [49–52]. We can, however, exclude the inflammasome pathway as a main mechanism in PRAAS because patients are nonresponders for IL-1 β antagonistic drugs [5]. The detailed mechanism which leads from accumulation of damaged proteins in the cytosol and ER to the induction of IFN type I expression needs to be further elucidated, but with this induction, a vicious circle of overwhelming inflammation starts.

Diagnostics for proteasome-associated autoinflammatory syndromes

Many patients pass through a long period of misdiagnosis associated with variable but not often beneficial treatments because the full features of PRAAS develop over time. First described for NNS but also suitable for CANDLE, JMP and JALS, a tentative set of eight features is used as criteria for clinical diagnosis, whereby at least five of these features need to be fulfilled. The criteria are the following: 1. autosomal recessive inheritance (parental consanguinity and/or familial occurrence), 2. pernio-like purplish rash on hands and feet (appearing in winter since infancy), 3. haunting nodular erythema with infiltration and induration (sometimes circumscribed), 4. repetitive spiking fever (periodic, not necessarily), 5. long clubbed fingers and toes with joint contractures, 6. progressive partial lipomuscular atrophy and emaciation (marked in the upper part of the body), 7. Hepatosplenomegaly, and 8. basal ganglia calcification [53]. It is clear that there is an urgent need for improved diagnosis in the early infancy before full symptoms are apparent, helping to avoid misdiagnosis like dermatosis, lupus profundus, systemic lupus erythematosus, Weber-Christian disease, cryopyrin-associated periodic syndrome, and inclusion body myositis [53].

For differential diagnosis, a genomic confirmation is indicated, but this is normally time consuming. There are two features observed in patient blood samples that could serve

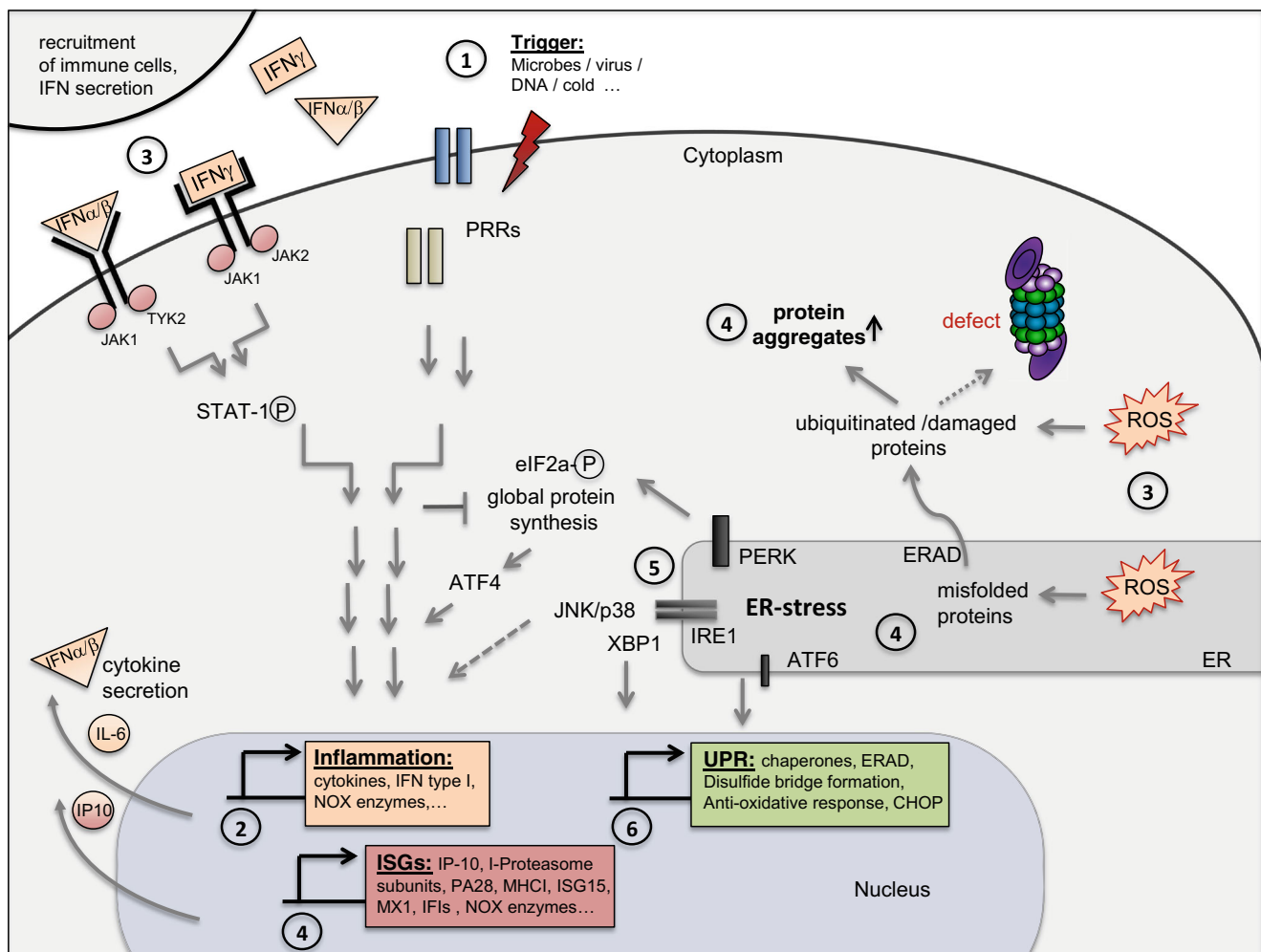


Fig. 2 Overwhelming inflammation caused by proteasome impairment. Sensing of stressors such as virus, bacteria, and low temperatures through pattern recognition receptors (PRRs) leads to the induction of the innate immune response with secretion of cytokines and activation and expression of NOX (NADPH-oxidase) enzymes. Reactive oxygen species (ROS) are produced, which lead to an increased load of oxidative damaged and misfolded foreign- and self-proteins in the cytosol and endoplasmic reticulum (ER). An impairment of the upregulation of the proteasomal degradation capacity results in the

formation of damaged protein aggregates. This effect is enhanced by further autocrine and paracrine IFN α/β and IFN γ responses. The accumulation of damaged proteins induces ER stress with the following unrelated protein response (UPR) acting via the pancreatic ER kinase (PERK) pathway, the inositol-requiring transmembrane kinase/endonuclease 1 (IRE1) pathway, and the activating transcription factor 6 (ATF6) pathway. Furthermore, through a still unknown mechanism, proteasome impairment triggers IFN type I expression and leads to a vicious circle of IFN signaling and response

for differential diagnostics, a strong IFN signature [5] and impaired proteolytic activity [35, 36]. A strong IFN signature is characteristic for PRAAS patients [5], but this is not a unique feature, as STING-associated vasculopathy with onset in infancy, Aicardi-Goutières-syndrome, severe lupus, ISG15 deficiency, and some undifferentiated interferonopathies show similar induction of IFN-responsive genes [54–57]. The establishment of an additional diagnostic-approved proteasome-specific assay is therefore essential to verify a significantly reduced proteolytic activity in at least one of the three protease activities in PBMCs. From our own data with preclinical mouse models and cell culture, we assume that syndromes with increased interferon signature but without

proteasome defects may display induced proteolytic activity due to the induction of IP [21]. If both features, induced IFN signature and significantly reduced proteasome activity, are present, a final confirmation of PRAAS could be achieved by identification of a disease-related mutation in one or two proteasome components such as those listed in Table 1. To date, only mutations in the 20S proteasome component PSMB8/ $\beta 5i$ have been identified, but it is worth expanding sequencing to include regulatory particle components, as the full catalytic capacity of the 20S core particle is only possible by interaction with regulators. Mutations can affect not only coding sequence regions but also regulatory sequences, splice sites, or the translational control of proteasome subunits.

Treatment

Some of the symptoms such as skin lesions, fever, and acute-phase reactants can be alleviated by application of high doses of systemic steroids. But these treatments fail to halt the progression of lipodystrophy and even worsens the central obesity and can cause severe side effects including growth retardation and glaucoma. Administration of various antirheumatic and immunosuppressive drugs, like IL-1 receptor antagonists, IL-6 receptor antagonists, and TNF- α inhibitors, either have no effect or showed only a temporal clinical improvement. Many other immunosuppressant drugs have been used such as methotrexate, hydroxychloroquine, azathioprine, cyclosporine, tacrolimus, colchicine, and dapsone, but they proved ineffective in most patients [5, 40, 53].

Because laboratory investigations identified IFN signaling as the most deregulated pathway in PRAAS patients, inhibition of this pathway seems to be the most promising target for therapeutic treatment. Preliminary investigations with the biological drug group of JAK inhibitors are promising. The JAK inhibitor tofacitinib (mainly inhibiting JAK3) has been shown to decrease phosphorylation of STAT-1, a downstream signaling molecule of IFN α/β and IFN γ receptors which is constitutively upregulated in CANDLE patients [5]. In an ongoing compassionate run by the National Institute of Health (NIH) administration of the JAK1/2 inhibitor baricitinib, which is in phase three clinical development for rheumatoid arthritis and phase two development for psoriasis and diabetic nephropathy, produced clinical improvement for CANDLE patients (NCT01724580) [40] (oral communication from R. Goldbach-Mansky).

Conclusions

The accumulation of protein aggregates (misfolded or damaged proteins) is common to proteinopathies and proteasome dysfunction disorders (PRAAS), and it seems likely that in both cases, this imbalance in proteostasis triggers an inflammatory outcome and cell death. Recent publications showed that some proteinopathies [3–5] are accompanied with the dysregulation of the proteasome or autophagy, and this may also be true for other proteinopathies. More investigations are needed to clarify the exact signaling pathway; however, recent studies on autoinflammation caused by mutations of proteasomal components indicate the involvement of IFN type I signaling. A detailed knowledge about the molecular pathway linking proteotoxic stress to type I IFN production will likely disclose novel targets for therapeutics in several inflammatory diseases driven by intracellular stress coupled with IFN production. Besides the development of new drugs, the development of diagnostic tools to differentiate

inflammation types and to monitor activity of different clearance pathways would be invaluable.

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Conflict of interest The authors declare that they have no conflict of interest.

References

- Liu Y, Jesus AA, Marrero B et al (2014) Activated STING in a vascular and pulmonary syndrome. *N Engl J Med* 371:507–18. doi:10.1056/NEJMoa1312625
- Sanchez GAM, de Jesus AA, Goldbach-Mansky R (2013) Monogenic autoinflammatory diseases: disorders of amplified danger sensing and cytokine dysregulation. *Rheum Dis Clin North Am* 39:701–34. doi:10.1016/j.rdc.2013.08.001
- Yamanaka K, Sasagawa Y, Ogura T (2012) Recent advances in p97/VCP/Cdc48 cellular functions. *Biochim Biophys Acta* 1823:130–7. doi:10.1016/j.bbamcr.2011.07.001
- Askanas V, Engel WK (2006) Inclusion-body myositis: a myodegenerative conformational disorder associated with Abeta, protein misfolding, and proteasome inhibition. *Neurology* 66: S39–48. doi:10.1212/01.wnl.0000192128.13875.1e
- Liu Y, Ramot Y, Torrello A et al (2012) Mutations in proteasome subunit β type 8 cause chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature with evidence of genetic and phenotypic heterogeneity. *Arthritis Rheum* 64:895–907. doi:10.1002/art.33368
- LaFerla FM (2010) Pathways linking Abeta and tau pathologies. *Biochem Soc Trans* 38:993–5. doi:10.1042/BST0380993
- Sulistio YA, Heese K (2015) The ubiquitin-proteasome system and molecular chaperone deregulation in Alzheimer's disease. *Mol Neurobiol*. doi:10.1007/s12035-014-9063-4
- LaFerla FM, Green KN, Oddo S (2007) Intracellular amyloid-beta in Alzheimer's disease. *Nat Rev Neurosci* 8:499–509. doi:10.1038/nm2168
- Joshi-Barr S, Bett C, Chiang W-C et al (2014) De novo prion aggregates trigger autophagy in skeletal muscle. *J Virol* 88:2071–82. doi:10.1128/JVI.02279-13
- Deriziotis P, André R, Smith DM et al (2011) Misfolded PrP impairs the UPS by interaction with the 20S proteasome and inhibition of substrate entry. *EMBO J* 30:3065–77. doi:10.1038/emboj.2011.224
- Ebstein F, Kloetzel P-M, Krüger E, Seifert U (2012) Emerging roles of immunoproteasomes beyond MHC class I antigen processing. *Cell Mol Life Sci* 69:2543–58. doi:10.1007/s00018-012-0938-0
- Krüger E, Kloetzel P-M (2012) Immunoproteasomes at the interface of innate and adaptive immune responses: two faces of one enzyme. *Curr Opin Immunol* 24:77–83. doi:10.1016/j.coi.2012.01.005
- Ciechanover A (2013) Intracellular protein degradation: from a vague idea through the lysosome and the ubiquitin-proteasome system and onto human diseases and drug targeting. *Bioorg Med Chem* 21:3400–10. doi:10.1016/j.bmc.2013.01.056
- Groll M, Ditzel L, Löwe J et al (1997) Structure of 20S proteasome from yeast at 2.4 Å resolution. *Nature* 386:463–71. doi:10.1038/386463a0

15. Vigneron N, Van den Eynde BJ (2014) Proteasome subtypes and regulators in the processing of antigenic peptides presented by class I molecules of the major histocompatibility complex. *Biomolecules* 4:994–1025. doi:10.3390/biom4040994
16. Gu ZC, Enenkel C (2014) Proteasome assembly. *Cell Mol Life Sci* 71:4729–45. doi:10.1007/s00018-014-1699-8
17. Sahara K, Kogleck L, Yashiroda H, Murata S (2014) The mechanism for molecular assembly of the proteasome. *Adv Biol Regul* 54:51–8. doi:10.1016/j.jbior.2013.09.010
18. Kniepert A, Groettrup M (2014) The unique functions of tissue-specific proteasomes. *Trends Biochem Sci* 39:17–24. doi:10.1016/j.tibs.2013.10.004
19. Fabre B, Lambour T, Garrigues L et al (2015) Deciphering preferential interactions within supramolecular protein complexes: the proteasome case. *Mol Syst Biol* 11:771. doi: 10.15252/msb.20145497
20. Kriegenburg F, Poulsen EG, Koch A et al (2011) Redox control of the ubiquitin-proteasome system: from molecular mechanisms to functional significance. *Antioxid Redox Signal* 15:2265–99. doi: 10.1089/ars.2010.3590
21. Seifert U, Bialy LP, Ebstein F et al (2010) Immunoproteasomes preserve protein homeostasis upon interferon-induced oxidative stress. *Cell* 142:613–24. doi:10.1016/j.cell.2010.07.036
22. Ebstein F, Voigt A, Lange N et al (2013) Immunoproteasomes are important for proteostasis in immune responses. *Cell* 152:935–7. doi:10.1016/j.cell.2013.02.018
23. Pickering AM, Koop AL, Teoh CY et al (2010) The immunoproteasome, the 20S proteasome and the PA28 $\alpha\beta$ proteasome regulator are oxidative-stress-adaptive proteolytic complexes. *Biochem J* 432:585–94. doi:10.1042/BJ20100878
24. Fehling H, Swat W, Laplace C et al (1994) MHC class I expression in mice lacking the proteasome subunit LMP-7. *Science* 265(80):1234–1237. doi:10.1126/science.8066463
25. Opitz E, Koch A, Klingel K et al (2011) Impairment of immunoproteasome function by β 5i/LMP7 subunit deficiency results in severe enterovirus myocarditis. *PLoS Pathog* 7:e1002233. doi:10.1371/journal.ppat.1002233
26. Strehl B, Joeris T, Rieger M et al (2006) Immunoproteasomes are essential for clearance of *Listeria monocytogenes* in nonlymphoid tissues but not for induction of bacteria-specific CD8⁺ T cells. *J Immunol* 177:6238–6244. doi:10.4049/jimmunol.177.9.6238
27. Ishii K, Hisaeda H, Duan X et al (2006) The involvement of immunoproteasomes in induction of MHC class I-restricted immunity targeting *Toxoplasma SAG1*. *Microbes Infect* 8:1045–53. doi: 10.1016/j.micinf.2005.10.023
28. Hussong SA, Kappahn RJ, Phillips SL et al (2010) Immunoproteasome deficiency alters retinal proteasome's response to stress. *J Neurochem* 113:1481–90. doi:10.1111/j.1471-4159.2010.06688.x
29. Chou B, Hisaeda H, Shen J et al (2008) Critical contribution of immunoproteasomes in the induction of protective immunity against *Trypanosoma cruzi* in mice vaccinated with a plasmid encoding a CTL epitope fused to green fluorescence protein. *Microbes Infect* 10:241–50. doi:10.1016/j.micinf.2007.11.010
30. Zaiss DMW, Bekker CPJ, Gröne A et al (2011) Proteasome immunosubunits protect against the development of CD8 T cell-mediated autoimmune diseases. *J Immunol* 187:2302–9. doi:10.4049/jimmunol.1101003
31. Eleftheriadis T, Pissas G, Antoniadi G et al (2013) CD8⁺ T-cell auto-reactivity is dependent on the expression of the immunoproteasome subunit LMP7 in exposed to lipopolysaccharide antigen presenting cells and epithelial target cells. *Autoimmunity* 46:439–45. doi:10.3109/08916934.2013.801460
32. Krause S, Kuckelkorn U, Dörner T et al (2006) Immunoproteasome subunit LMP2 expression is deregulated in Sjogren's syndrome but not in other autoimmune disorders. *Ann Rheum Dis* 65:1021–7. doi:10.1136/ard.2005.045930
33. Hayashi T, Faustman D (2000) Defective function of the proteasome in autoimmunity: involvement of impaired NF-kappaB activation. *Diabetes Technol Ther* 2:415–28
34. Arima K, Kinoshita A, Mishima H et al (2011) Proteasome assembly defect due to a proteasome subunit beta type 8 (PSMB8) mutation causes the autoinflammatory disorder, Nakajo-Nishimura syndrome. *Proc Natl Acad Sci U S A* 108:14914–9. doi:10.1073/pnas.1106015108
35. Agarwal AK, Xing C, DeMartino GN et al (2010) PSMB8 encoding the β 5i proteasome subunit is mutated in joint contractures, muscle atrophy, microcytic anemia, and panniculitis-induced lipodystrophy syndrome. *Am J Hum Genet* 87:866–72. doi:10.1016/j.ajhg.2010.10.031
36. Kitamura A, Maekawa Y, Uehara H et al (2011) A mutation in the immunoproteasome subunit PSMB8 causes autoinflammation and lipodystrophy in humans. *J Clin Invest* 121:4150–60. doi:10.1172/JCI58414DS1
37. McDermott A, Jesus AA, Liu Y et al (2013) A case of proteasome-associated auto-inflammatory syndrome with compound heterozygous mutations. *J Am Acad Dermatol* 69:e29–e32
38. Mégarbané A, Sanders A, Chouery E et al (2002) An unknown autoinflammatory syndrome associated with short stature and dysmorphic features in a young boy. *J Rheumatol* 29:1084–7
39. Torreló A, Patel S, Colmenero I et al (2010) Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE) syndrome. *J Am Acad Dermatol* 62:489–95. doi:10.1016/j.jaad.2009.04.046
40. McDermott A, Jacks J, Kessler M et al (2014) Proteasome-associated autoinflammatory syndromes: advances in pathogenesis, clinical presentations, diagnosis, and management. *Int J Dermatol*. doi:10.1111/ijd.12695
41. Kluk J, Rustin M, Brogan PA et al (2013) CANDLE syndrome: a report of a novel mutation and review of the literature. *Br J Dermatol*. doi:10.1111/bjd.12600
42. Oyanagi K, Sasaki K, Ohama E et al (1987) An autopsy case of a syndrome with muscular atrophy, decreased subcutaneous fat, skin eruption and hyper gamma-globulinemia: peculiar vascular changes and muscle fiber degeneration. *Acta Neuropathol* 73:313–9
43. Qureshi N, Perera P-Y, Shen J et al (2003) The proteasome as a lipopolysaccharide-binding protein in macrophages: differential effects of proteasome inhibition on lipopolysaccharide-induced signaling events. *J Immunol* 171:1515–1525. doi:10.4049/jimmunol.171.3.1515
44. Kawai T, Akira S (2010) The role of pattern-recognition receptors in innate immunity: update on toll-like receptors. *Nat Immunol* 11:373–84. doi:10.1038/ni.1863
45. Katsuyama M (2010) NOX/NADPH oxidase, the superoxide-generating enzyme: its transcriptional regulation and physiological roles. *J Pharmacol Sci* 114:134–46
46. Yewdell JW (2011) DRiPs solidify: progress in understanding endogenous MHC class I antigen processing. *Trends Immunol* 32:548–58. doi:10.1016/j.it.2011.08.001
47. Wamatsch A, Bergann T, Krüger E (2013) Oxidation matters: the ubiquitin proteasome system connects innate immune mechanisms with MHC class I antigen presentation. *Mol Immunol* 55:106–9. doi:10.1016/j.molimm.2012.10.007
48. Szeto J, Kaniuk NA, Canadien V et al (2014) ALIS are stress-induced protein storage compartments for substrates of the proteasome and autophagy. *Autophagy* 2:189–199. doi:10.4161/auto.2731
49. Senft D, Ronai ZA (2015) UPR, autophagy, and mitochondria crosstalk underlies the ER stress response. *Trends Biochem Sci*. doi:10.1016/j.tibs.2015.01.002

50. Urano F, Wang X, Bertolotti A et al (2000) Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. *Science* 287:664–6
51. Menu P, Mayor A, Zhou R et al (2012) ER stress activates the NLRP3 inflammasome via an UPR-independent pathway. *Cell Death Dis* 3:e261. doi:10.1038/cddis.2011.132
52. Lerner AG, Upton J-P, Praveen PVK et al (2012) IRE1 α induces thioredoxin-interacting protein to activate the NLRP3 inflammasome and promote programmed cell death under irremediable ER stress. *Cell Metab* 16:250–64. doi:10.1016/j.cmet.2012.07.007
53. Kanazawa N (2012) Nakajo-Nishimura syndrome: an autoinflammatory disorder showing pemphig-like rashes and progressive partial lipodystrophy. *Allergol Int* 61:197–206. doi:10.2332/allergolint.11-RAI-0416
54. Zhang X, Bogunovic D, Payelle-Brogard B et al (2014) Human intracellular ISG15 prevents interferon- α/β over-amplification and auto-inflammation. *Nature* 517:89–93. doi:10.1038/nature13801
55. Baechler EC, Batliwalla FM, Karypis G et al (2003) Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. *Proc Natl Acad Sci U S A* 100:2610–5. doi:10.1073/pnas.0337679100
56. Crow YJ (2013) Aicardi-Goutières syndrome. *Handb Clin Neurol* 113:1629–35. doi:10.1016/B978-0-444-59565-2.00031-9
57. Briggs TA, Rice GI, Daly S et al (2011) Tartrate-resistant acid phosphatase deficiency causes a bone dysplasia with autoimmunity and a type I interferon expression signature. *Nat Genet* 43:127–31. doi:10.1038/ng.748
58. Qian M-X, Pang Y, Liu CH et al (2013) Acetylation-mediated proteasomal degradation of core histones during DNA repair and spermatogenesis. *Cell* 153:1012–24. doi:10.1016/j.cell.2013.04.032
59. Stadtmueller BM, Kish-Trier E, Ferrell K et al (2012) Structure of a proteasome Pba1-Pba2 complex: implications for proteasome assembly, activation, and biological function. *J Biol Chem* 287:37371–82. doi:10.1074/jbc.M112.367003
60. Fricke B, Heink S, Steffen J et al (2007) The proteasome maturation protein POMP facilitates major steps of 20S proteasome formation at the endoplasmic reticulum. *EMBO Rep* 8:1170–5. doi:10.1038/sj.embor.7401091
61. Hirano Y, Hendil KB, Yashiroda H et al (2005) A heterodimeric complex that promotes the assembly of mammalian 20S proteasomes. *Nature* 437:1381–5. doi:10.1038/nature04106
62. Paraskevopoulos K, Kriegenburg F, Tatham MH et al (2014) Dss1 is a 26S proteasome ubiquitin receptor. *Mol Cell* 56:453–61. doi:10.1016/j.molcel.2014.09.008
63. Kim Y-C, DeMartino GN (2011) C termini of proteasomal ATPases play nonequivalent roles in cellular assembly of mammalian 26S proteasome. *J Biol Chem* 286:26652–66. doi:10.1074/jbc.M111.246793
64. Cascio P (2014) PA28 $\alpha\beta$: the enigmatic magic ring of the proteasome? *Biomolecules* 4:566–84. doi:10.3390/biom4020566
65. De Graaf N, van Helden MJG, Textoris-Taube K et al (2011) PA28 and the proteasome immunosubunits play a central and independent role in the production of MHC class I-binding peptides in vivo. *Eur J Immunol* 41:926–35. doi:10.1002/eji.201041040
66. Mao I, Liu J, Li X, Luo H (2008) REG γ , a proteasome activator and beyond? *Cell Mol Life Sci* 65:3971–80. doi:10.1007/s00018-008-8291-z
67. Lehmann A, Niewianda A, Jechow K et al (2010) Ecm29 fulfils quality control functions in proteasome assembly. *Mol Cell* 38:879–88. doi:10.1016/j.molcel.2010.06.016
68. Gorbea C, Goellner GM, Teter K et al (2004) Characterization of mammalian Ecm29, a 26S proteasome-associated protein that localizes to the nucleus and membrane vesicles. *J Biol Chem* 279:54849–61. doi:10.1074/jbc.M410444200
69. Tomko RJ Jr, Hochstrasser M (2013) Molecular architecture and assembly of the eukaryotic proteasome. *Annu Rev Biochem*. doi:10.1146/annurev-biochem-060410-150257