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REVIEW

Protein misfolding and dysregulated protein homeostasis in autoinflammatory diseases and beyond

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Abstract Cells have a number of mechanisms to maintain protein homeostasis, including proteasome-mediated degradation of ubiquitinated proteins and autophagy, a regulated process of "self-eating" where the contents of entire organelles can be recycled for other uses. The unfolded protein response prevents protein overload in the secretory pathway. In the past decade, it has become clear that these fundamental cellular processes also help contain inflammation though degrading pro-inflammatory protein complexes such as the NLRP3 inflammasome. Signaling pathways such as the UPR can also be co-opted by toll-like receptor and mitochondrial reactive oxygen species signaling to induce inflammatory responses. Mutations that alter key inflammatory proteins, such as NLRP3 or TNFR1, can overcome normal protein homeostasis mechanisms, resulting in autoinflammatory diseases. Conversely, Mendelian defects in the proteasome cause protein accumulation, which can trigger interferon-dependent autoinflammatory disease. In non-Mendelian inflammatory diseases, polymorphisms in genes affecting the UPR or autophagy pathways can contribute to disease, and in diseases not formerly considered inflammatory such as neurodegenerative conditions and type 2 diabetes, there is increasing evidence that cell intrinsic or environmental alterations in protein homeostasis may contribute to pathogenesis.

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Cells must maintain a delicate balance between the demands for protein synthesis and the need to avoid accumulation of incompletely processed or unfolded proteins that can accumulate under normal conditions and even more so when cells face a variety of stresses. The unfolded protein response (UPR) is an evolutionarily conserved mechanism to maintain cellular homeostasis by preventing the accumulation of misfolded proteins in the endoplasmic reticulum (ER). Disturbed protein folding in the ER is primarily detected by three transmembrane (TM) proteins: activating transcription factor 6 (ATF6), inositol-requiring transmembrane kinase/endonuclease 1 (IRE1), and pancreatic ER kinase (PERK). The combined action of these sensors reduces global protein synthesis while upregulating the production of chaperone proteins that can stabilize misfolded proteins. Apart from ER homeostasis, the UPR can modulate other biological functions, including apoptosis, protein secretion, and as we will discuss further, inflammatory responses [1, 2].

A series of molecular changes are initiated in response to cellular stressors in order to minimize damage caused by unfavorable environmental conditions, such as temperature changes, toxins (e.g., bacterial, chemical), radiation, mechanical damage, nutritional status as well as incompletely folded proteins intracellularly (Fig. 1). The UPR serves the adaptive purpose of protecting the cell by activating a series of mechanisms including the induction of molecular chaperones to assist with correct folding—e.g., heat shock proteins and

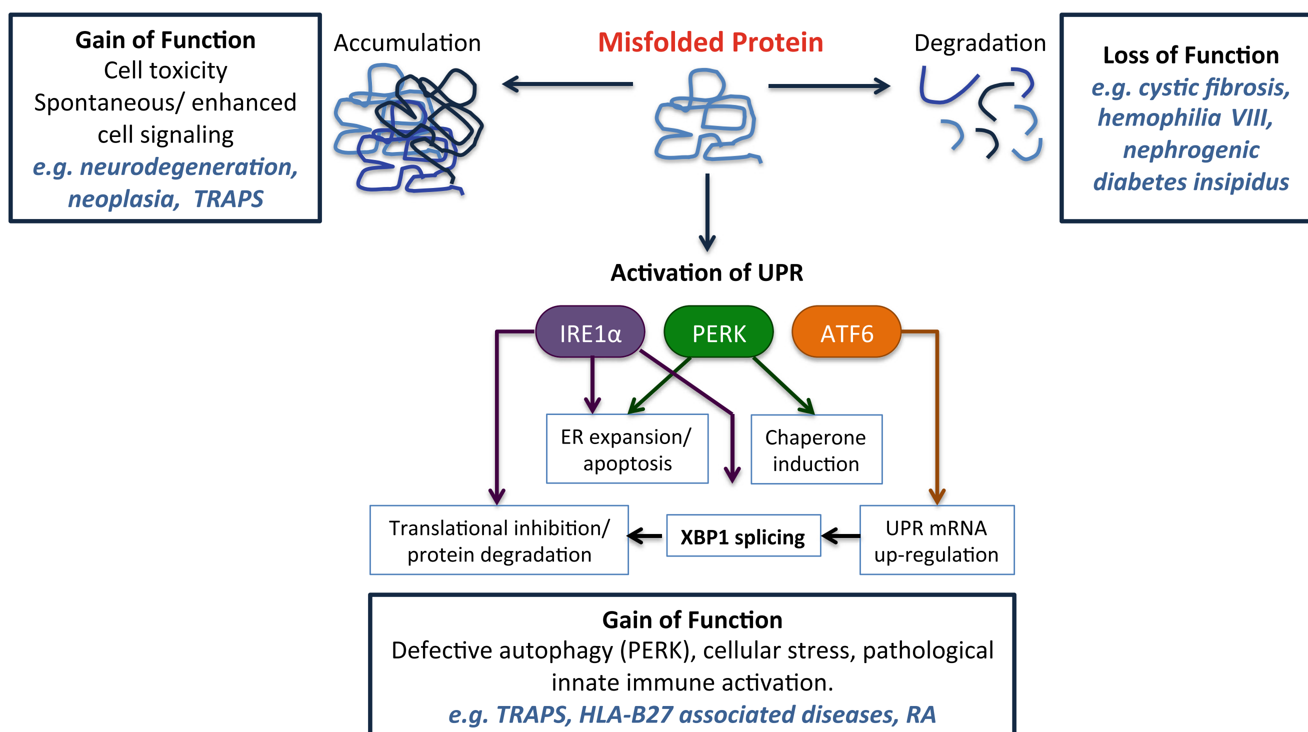

Fig. 1 Effects of protein misfolding that can lead to disease. Misfolded proteins are potentially highly dangerous and may accumulate leading to cell toxicity or inappropriate/excessive cell signaling. Cells therefore attempt to restore protein homeostasis by activating one or more of three major branches of the UPR; IRE1a, PERK, and/ or ATF6; which regulate genes involved in protein production, degradation, and/or refolding. Failure of these mechanisms often results in cell apoptosis; however, chronic UPR activation may promote pathological innate immune activation and defective autophagy. Key: *TRAPS* tumor necrosis factor receptor-associated periodic syndrome, *IRE1α* inositol-requiring enzyme 1-alpha, *UPR* unfolded protein response, *PERK* protein kinase-like endoplasmic reticulum kinase, *ATF6* activating transcription factor 6, *XBP1* X-box binding protein 1, *HLA-B27* human leukocyte antigen B27, *RA* rheumatoid arthritis

foldases. Interestingly, there are a number of connections between the UPR and inflammatory signaling pathways. IRE1 initiates a transcriptional response to ER stress through triggering alternative splicing of the mRNA encoding the XBP-1 bZIP-family transcription factor to remove a 26 nucleotide unconventional intron and allow translation of the functional transcription factor. Independently of XBP-1, IRE1 can also activate the JNK kinase pathway, a well-known mediator of cellular stress that can activate pro-inflammatory gene transcription [3]. Partial activation of the UPR mediated by IRE1 can also be triggered by activation of the innate immune receptors, toll-like receptor (TLR) 2 and TLR4, which results in activation of pro-inflammatory genes. When all reparative UPR mechanisms are overcome, apoptosis can be triggered through PERK and ATF6, which may dampen the inflammatory response to cells dying due to overload of misfolded proteins.

The major degradation mechanisms for misfolded/unfolded proteins outside the secretory pathway include the ubiquitin-proteasome and autophagy-lysosome systems. Addition of K48-linked polyubiquitin tags proteins for rapid elimination by the proteasome. Autophagy involves encapsulation of protein aggregates and organelles within double membrane structures called autophagosomes. Autophagosomes fuse with lysosomes resulting in the degradation of their cargo. A number of abnormalities of the UPR, proteasome, and autophagy-lysosome pathways have been implicated in the pathogenesis of several diseases, including neurodegenerative and cardiovascular disease [4, 5]. In this review, we will discuss the role of misfolded proteins and altered protein homeostasis in autoinflammatory diseases, which has emerged as a theme in the pathogenesis of these diseases over the last few years [6].

Autoinflamamatory diseases linked to disorders in protein misfolding

Autoinflammatory disease is defined as self-directed inflammation distinguishable from autoimmune disease by the absence of high-titer autoantibodies or antigen-specific T cells and involvement of tissue-specific factors [7]. Although originally conceptualized as distinct disease states of aberrant innate and adaptive immunity respectively, it is now widely accepted that the two categories exist along a continuum, with monogenic autoinflammatory and autoimmune diseases at

opposite extremes of the spectrum [8]. Various pathological mechanisms are implicated in the monogenic autoimmune diseases described to date, including inappropriate inflammasome activation, proteasome deficiency, generation of harmful reactive oxygen species (ROS), and, more recently, the UPR and autophagy processes [9]. These latter interconnected cellular environmental responses are currently only described in a select number of autoinflammatory diseases (Table 1), the most notable being tumor necrosis factor (TNF) receptor-associated periodic syndrome (TRAPS).

TRAPS is an autosomal dominant monogenic autoinflammatory disease characterized by periodic fevers, abdominal pain, arthralgia, myalgia, migratory dermatitis and periorbital edema and raised inflammatory markers due to loss of function mutations in the TNF receptor 1 (TNFR1) gene located on Ch12p13.31 [9–11]. The TNFR1 is a homotrimeric receptor complex composed of an extracellular binding domain comprised of four cysteine-rich domains (CRDs), a transmembrane domain, and an intracellular death domain [9]. Currently, 141 TNFR1 mutations are registered on the INFEVERS database for TRAPS, most being missense mutations affecting exons 2–4 which form the CRDs [12]. TRAPS disease-causing mutations are clinically heterogeneous. Mutations in the CRDs are often associated with severe disease, such as the T50M and C88R mutations, whereas other mutations may be less severe; for

Table 1 Diseases associated with defects in protein homeostasis. For each disease, the genes and protein homeostasis pathways which have been implicated in pathogenesis are listed

	Causative/ associated gene	UPR activation	Defective autophagy	Defective proteasome	Oxidative stress/ROS
Monogenic autoinflammatory diseases					
TNF receptor-associated periodic syndrome (TRAPS)	TNFRSF1A	✓	✓		✓
Familial Mediterranean fever (FMF)	MEFV		✓		✓
Sideroblastic anemia with immunodeficiency, fevers, and developmental delay (SIFD)	TRNT1	✓			✓
Mevalonate kinase deficiency (MKD)	MVK		✓		✓
Proteasome-associated autoinflammatory syndromes: JMP, NNS, CANDLE, JASL	PSMB8	✓		✓	
Polygenic inflammatory diseases					
Inflammatory bowel disease (IBD): HLA-B27 associated and Crohn's disease	ATG16L	✓	✓		✓
Crystal arthropathies: gout and calcium pyrophosphate dehydrate (CPPD)			✓		✓
Rheumatoid arthritis (RA)		✓	✓		✓
Diseases with an inflammatory component					
Type 2 diabetes (T2D)	IAPP, APP	✓	✓		✓
Alzheimer's disease (AD)	APOE, APP, ADAM10	✓	✓		✓
Parkinson's disease (PD)	PS1, PS2, LRRK2, SNCA, Parkin, DJ-1, PINK1, HTRA2	✓	✓	✓	✓
Amyotrophic lateral sclerosis (ALS)	SOD1, TDP-43, PDI	✓	✓		✓
Huntington's chorea	HTT		✓		✓
Age-related macular degeneration (AMD)		✓		✓	✓
Malignancy (e.g., multiple myeloma)		✓	✓	✓	✓
Cardiovascular diseases		✓	✓		✓
Chronic pancreatitis		✓			✓

TNFRSF1A TNF receptor super family, member 1A; *MEFV*: Familial Mediterranean fever gene; *TRNT1* tRNA nucleotidyltransferase, CCA-adding 1; *JMP* joint contractures, muscle atrophy, microcytic anemia, and panniculitis-induced childhood-onset lipodystrophy; *NNS* Nakajo-Nishimura syndrome; *CANDLE* chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature; *JASL* Japanese autoinflammatory syndrome with lipodystrophy; *MVK* mevalonate kinase; *PSMB8* proteasome subunit beta type 8; *IAPP* islet amyloid polypeptide; *APP* amyloid precursor protein; *APOE* apolipoprotein E; *ADAM10* A disintegrin and metalloproteinase domain 10; *PS1* preselenin 1; *PS2* preselenin 2; *LRRK2* leucine rich repeat kinase 2; *SNCA* synuclein alpha; *PARKIN* aka Parkinson protein 2 (PARK2); *PINK* PTEN-induced putative kinase 1; *DJ-1* oncogene DJ1, aka Parkinson protein 7 (PARK7); *HTRA2* HTRA serine peptidase 2; *SOD1* superoxidase dismutase 1; *TDP-43* transactive response DNA binding protein 1; *PDI* protein disulfide isomerase; *HTT* huntingtin

example, R92Q and P46L variants may be associated with mild disease or can be clinically asymptomatic and have a 1–5 % prevalence in the population [13]. Although the inflammatory features of disease suggests that TRAPS-linked TNFR1 mutations should be gain-of-function, knock-in mice homozygous for TRAPS mutations do not manifest a TRAPS disease phenotype but are resistant to lipopolysaccharide (LPS)-induced septic shock [14]. In keeping with these reports, the majority of TRAPS-causing mutations observed to date are heterozygous, suggesting that expression of both the functional and mutant receptor is required for TRAPS pathogenesis [9].

There are a number of mechanisms by which the mutated TNFR1 may lead to inflammatory disease [9, 13]. The TNFR1 is present physiologically in both the soluble (sTNFR1) and membrane (mTNFR1) bound form, both of which are decreased in TRAPS patients [15–20]. These observations suggest either defective receptor shedding or defective trafficking of mutant receptors to the cell surface. Most probably, the latter mechanism plays a significant role in TRAPS pathogenesis, given that accumulation of mutant TNFR1 can be found in the ER of mutant TNFR1 transfected cell lines associated with increased ER stress [20]. It has been postulated that TNF release stimulated by UPR activation may then signal through the wild-type TNFR1, generating an autocrine positive feedback loop enhancing TNF production [21, 22]. It is also conceivable that mutant intracellular TNFR1 may still stimulate TNF production by activation of their intracellular death domains independent of receptor-ligand binding, particularly as this domain is rarely mutated in TRAPS. However, clear evidence for such a mechanism is lacking [18].

Upregulation of UPR response genes has been reported in TRAPS patients. A study of 16 TRAPS patients with different mutations, 22 healthy controls (HC) and HEK293 wild-type and mutant transfectants, detected increased splicing of X-box binding protein 1 (sXBP1), a key UPR transcription factor, alongside increased protein kinase (PK)-like endoplasmic reticulum kinase (PERK) phosphorylation in TRAPS patient monocytes and in HEK293 mutants compared with HC and wild-type HEK293 cells [23]. Intriguingly, six other UPR genes tested were not differentially upregulated, and activation of UPR genes downstream of sXBP1 was not observed between TRAPS and peripheral blood mononuclear cells (PMBCs) from human controls. Instead, the authors proposed a mechanism of non-canonical XBP1 splicing induced by LPS ligand acting on its receptor, TLR4, and TRAPS monocytes are hyper-responsive to LPS. These results are consistent with previous studies which identify a role for sXBP1 in TLR responses [24]. Interestingly, XBP1 binding sites were also identified in the TNF and IL-6 gene promoters [24]; thus, XBP1 in TRAPS could also contribute to pro-inflammatory cytokine production independent of canonical UPR pathways.

Oxidative stress has also emerged as another trigger of enhanced inflammatory responses in TRAPS. Increased IL-6 production in response to LPS stimulation in cells with TRAPS-associated TNFR1 mutations could be reversed with anti-oxidant treatment [23]. Increased reactive oxygen species (ROS) was observed in TRAPS patient cells and cells transfected with TRAPS-associated TNFR1 at baseline and after stimulation with LPS [25]. Specific inhibition of mitochondrial ROS (mtROS), but not nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOX), reduced pro-inflammatory cytokine production. Increased MAPK activity, possibly through inactivation of MAPK phosphatases through oxidation of their catalytic cysteine residues, contributed to enhanced transcription of inflammatory genes [25]. Enhanced activation of NF-κB has also been observed in TRAPS patient cells [26]. Upon activation, NF-κB translates to the nucleus, where it upregulates target gene expression, including genes involved in the production of the pro-inflammatory cytokines, interleukin (IL)-1β, IL-6, and TNF. The importance of IL-1β in TRAPS pathogenesis is reflected in the strong clinical response of TRAPS patients to IL-1β antagonists [27]. The increased IL-6 production in response to LPS in TRAPS cells [23, 25] also raises the possibility that IL-6 antagonists may also have therapeutic benefit in TRAPS.

Defective autophagy has also been reported in TRAPS monocytes. In one study, autophagy, but not proteasome inhibition, increased intracellular mutant and wild-type TNFR1 intracellular aggregation [28]. Moreover, rescue of TNFR1 membrane localization using geldanamycin restored normal ultra-structural appearance in TRAPS monocytes and membrane localization of TNFR1 [28]. This rescue was reversed by the autophagy inhibitor 3-methyladenine (3-MA), suggesting that autophagy defects could be responsible for the failure to clear intracellular mutant TNFR1 aggregates in TRAPS monocytes [28]. Autophagy is also important for the degradation of p62, a protein involved in various processes including ubiquitination, and intracellular aggregate formation [29, 30]. Increased levels of p62 protein, but not mRNA, are reported in certain TRAPS mutations, suggesting defective clearance of p62 by autophagy rather than increased expression of p62 genes [28]. p62 is involved in both caspase 8 activation and apoptosis and also in receptor-interacting protein (RIP) activation, resulting in RIP-dependent I kappa B kinase (IKK), I kappa beta (IκB), and ultimately NF-κB activation [29]. However, p62 also increases ubiquitination and Nod-like receptor protein 3 (NLRP3) inflammasome degradation, which would be expected to have anti-inflammatory effects and promote the clearance of intracellular TNFR1 aggregates. One hypothesis to explain this paradox is that p62-mediated activation of RIP mitigates these beneficial effects [29]; however, this remains to be validated experimentally and, altogether, any potential role for autophagy in TRAPS, either independent or via its effects on p62, remains to be clearly

defined. Nonetheless, current observations point towards a complex model for TRAPS pathogenesis whereby autophagy, the UPR, and ROS-mediated inflammatory pathways operate synergistically to enhance pro-inflammatory cytokine production.

Protein misfolding may also play a role in other autoinflammatory diseases which do not directly involve nod-like receptors (NLRs) (Table 1). Mevalonate kinase deficiency (MKD) is a monogenic disease associated with a defect in isoprenoid synthesis with both autoinflammatory and autoimmune features. MKD patients exhibit periodic fevers and hyper IgD, and PBMCs hypersecrete IL-1β. The ER stress in MKD has been linked to defective mitophagy, and neutralization of mtROS in MKD reduces inflammasome activity. Interestingly, MKD cells were resistant to reduction in IL-1β production by the autophagy activator rapamycin, suggesting that the isoprenoid MKD defect may activate the inflammasome through mechanisms not regulated by autophagy [31]. Genetic defects in the proteasome itself have been recently found to cause autoinflammatory disease. Loss of function mutations in *PSMB8*, which encodes the β5i immunoproteasome subunit, have been associated with a clinical syndrome characterized by systemic inflammation, neutophilic lipodystrophy, and, in older patients, cardiac and hematologic abnormalities [32–34]. Although the immunoproteasome plays a role in processing peptides for antigen presentation to T cells, most of the abnormalities found in this syndrome are in innate immunity. Interestingly, rather than IL-1β, type I interferon-induced gene expression and target proteins are highly induced in this syndrome, perhaps because accumulation of unfolded protein fragments normally degraded by the proteasome induce an interferon response [35, 36].

A role for autophagy in regulating IL-1β secretion and IL-1β-related autoinflammatory diseases

The secretion of pro-inflammatory cytokine, IL-1β requires transcriptional activation of the IL-1β gene and components of the NLRP3 inflammasome: a complex of the NLRP3 protein, also known as cryopyrin; caspase-1; apoptosis-associated speck-like protein containing a CARD (ASC) and CARDINAL, a CARD containing protein. Caspase-1 cleaves IL-1β into its active form. Innate immune stimuli acting through extracellular TLRs and the intracellular NLRs are major activators of transcription of NLRP3 inflammasome components. Diverse particulate stimuli including crystalline forms of uric acid, cholesterol, and ATP acting through membrane receptors and dATP acting directly on the NLRP3 protein are critical for formation of an active inflammasome and allowing IL-1β release from the cell, an event which is also linked to cell death [37, 38]. Activated inflammasomes are visible as microscopic "specks" within the cytosol, and structural studies have shown that they can polymerize into large oligomers with potentially thousands of subunits [39]. Gain-of-function mutations in the *NLRP3* gene cause de novo and inherited autoinflammatory diseases collectively known as cryopyrin-associated periodic syndromes (CAPS) [40]. Pathogenic mutations, predominantly found in exon 3, affect the nucleotide binding domain (NBD) within NLRP3 leading to spontaneous oligomerization and a reduced requirement for the second stimulus, ATP, for IL-1β secretion after activation by innate immune stimuli [41]. NLRP3 mutations lead to a spectrum of diseases ranging from the relatively mild familial cold autoinflammatory syndrome (FCAS), through Muckle-Wells syndrome (MWS), which includes cochlear inflammation leading to hearing loss, to the neonatal-onset multisystem inflammatory disease (NOMID), in which multi-organ inflammation including sterile meningitis can lead to neurological impairment and can be fatal without treatment [42–46]. It has recently become apparent that autophagy plays a physiological role in disposing of the components of the activated NLRP3 inflammasome through targeting of ubiquitinated components to the inflammasome and recruitment of the autophagy adapter p62 [47–49]. These studies reveal a physiological function for autophagy in controlling inflammation. Although CAPS patients can be successfully treated with IL-1 receptor (IL-1R) antagonists [50, 51], whether activation of autophagy would be another avenue for treatment remains an open question.

Recently, activating mutations in the gene coding for another NLR, *NLRC4*, have been associated with an autoinflammatory disease that differs from CAPS [52, 53]. Patients with these *NLRC4* mutations exhibited early-onset spontaneous fevers, gastrointestinal (GI) inflammation, urticaria, splenomegaly, and malaise. These symptoms are consistent with macrophage activation syndrome (MAS), a severe systemic inflammatory disease involving decreased erythrocytes, leukocytes and platelets, abnormal natural killer (NK) cell function, elevated levels of triglycerides and ferritin and recurrent fevers. Overexpression of the NLRC4 mutants resulted in constitutive activation of the inflammasome and caspase-1 leading to elevated levels of IL-1β and IL-18. In contrast to CAPS but like MAS, monocytes and macrophages with the NLRC4 mutations showed hypersecretion of IL-18 [52]. Romberg et al. showed that NLRC4 mutant macrophages also exhibited increased apoptosis [53]. Treatment with IL-1 blocking agents resulted in partial amelioration of symptoms [52]. Given the role of autophagy in regulating IL-1β secretion, it could be a potential additional therapeutic target in treating the autoinflammation in patients with disease due to *NLRC4* mutations.

Protein misfolding and proteotoxic stress in non-Mendelian inflammatory diseases

Increasing evidence suggests that in polygenic inflammatory diseases, autoinflammation may be triggered by misfolded proteins, defects in the UPR, or protein degradation pathways. Ankylosing spondylitis (AS) is a prototypic spondyloarthropathy involving immune dysregulation, chronic inflammation, and a strong genetic predisposition. Many genes confer susceptibility to AS, but the HLA-B27 class I allele has by far the strongest association, with over 90 % of AS patients carrying this allele. Rather than functioning in its classical role to present specific peptides to $CD8^+$ T cells, evidence has been accumulating to implicate misfolding of HLA-B27 heavy chain as a pathogenic factor in AS. HLA-B27 molecules tend to misfold [54, 55], and after induction of class I expression with cytokines such as interferons, HLA-B27 can induce ER stress and activate the UPR leading to production of IL-6, TNF, IL-23, IFN-β, and possibly IL-1α [56, 57]. In AS patients, ileal biopsies revealed abundant misfolded/unfolded MHC class 1 heavy chains co-localizing with the E3 ubiquitin ligase, synoviolin/HRD1 [58]. Studies using rodent models of AS showed that activation of UPR in macrophages led to increased levels of IL-23 and upregulation of Th-17 in $CD4^+$ T cells within inflamed tissue [59]. Other studies have implicated autophagy defects in the hypersecretion of IL-23 in the gut but not in synovium or PBMCs from $HLA\text{-}B27^+$ patients with AS [58, 60]. Systemic overexpression of IL-23 in mice leads to IL-17 production by innate-like T cells present in the enthesis, the bone-tendon interface where much of the inflammation begins in AS, leading to a striking phenocopy of many of the clinical features of AS [61]. Taken together, these data implicate HLA-B27 misfolding with the induction of the UPR as a pathogenic factor in AS upstream of inflammatory cytokine production.

Polymorphisms in the autophagy regulatory gene, *Atg16L1*, have been associated with Crohn's disease, a subtype of inflammatory bowel disease [62]. ATG16L1 deficient macrophages produce excessive amounts of cytokines after stimulation with LPS [63]. Partial deficiency of Atg16L1 can lead to reduced production of antimicrobial peptides by Paneth cells in the intestine, impairing antimicrobial immunity which may predispose to intestinal inflammation [64]. The ER stress signal transducing protein XBP1 is critical in regulating the survival of Paneth cells. In the DSS-induced colitis model, conditional deletion of *Xbp1* in the intestinal epithelium resulted in Paneth cell disappearance and increased susceptibility to colitis due to impaired production of antimicrobial peptides [65]. These genetic and functional data build a strong case that in the intestine, autophagy and the UPR are important for the survival and function of Paneth cells, and when these processes are defective, inflammatory bowel disease can ensue due to defects in control of commensal intestinal flora.

Gout is a crystal arthropathy characterized by the deposition of monosodium urate (MSU) crystals in joints and tissues, leading to inflammation and significant morbidity. Uric acid released by dying cells interacts with extracellular sodium to form MSU, which acts as a danger signal. The phagocytosis of cell debris combined with the MSU signal induce maturation and activation of dendritic cells [66], possibly also caused by MSU interactions with CD14, an adaptor molecule for TLR2 and TLR4 [67]. MSU and calcium pyrophosphate dehydrate (CPPD) crystals can induce activation of the NLRP3 inflammasome resulting in production of IL-1β [68–70]. In addition to activating the NLRP3 inflammasome, MSU crystals can also activate autophagosome formation and impair proteasome function resulting in p62 accumulation. Inhibition of autophagy through siRNA against ATG16L1 was shown to increase caspase-1 activation and IL-1β production [71]. Peripheral neutrophils from healthy patients treated with MSU crystals or synovial fluid from patients with active gout lead to neutrophil extracellular trap (NET) formation. MSU-induced NET formation was dependent on IL-1β and phagolysosomal fusion [72, 73]. Combined with the previously discussed findings that autophagy helps to terminate activation of the NLRP3 inflammasome, these results suggest that autophagy may help to control inflammation in environmentally triggered IL-1β-related autoinflammatory diseases, such as gout.

Rheumatoid arthritis (RA) is a polygenic autoimmune disease with a significant inflammatory component. There have been a number of interesting connections identified in RA between aberrant UPR and increased inflammatory responses. As in TRAPS, synovial macrophages, synovial fibroblasts, and PBMCs from RA patients were found to have increased *Xbp1* splicing but not increased expression of classical UPR response genes, leading to increased production of pro-inflammatory cytokines, IL-6 and TNF [74, 75]. Endogenous TLR ligands which have been found in the joint, such as SNAPIN, may act to induce this aberrant *Xbp1* splicing and sustain inflammatory responses [76].

Proteotoxic stress in the pathogenesis of diseases not formerly considered inflammatory

The proteotoxic effects of amyloid-like deposits are a hallmark of many diseases, including type II diabetes (T2D) and neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, and age-related retinal degeneration. Interesting links have been made between insulin insensitivity, obesity, ER stress, and chronic inflammation in the pathogenesis of T2D [77–80]. T2D is a multifactorial disease characterized by insensitivity of target organs such as skeletal muscle, liver and adipose tissue, to the effects of insulin. Obesity develops because insulin resistance leads to increased lipolysis and abnormal fat deposits, decreased glucose uptake in

skeletal muscle, and enhanced gluconeogenesis in the liver. Adipose tissue, which consists of preadipocytes, adipocytes, and vascular cells, has important endocrine functions including the secretion of adipokines (TNF, IL-6, leptin, adiponectin, and more) and monocyte chemoattractant protein 1 (MCP-1) [80, 81]. During inflammation, infiltrating macrophages are found in adipose tissue. Studies show that markers of ER stress and UPR are elevated in tissues from diabetic and/or obese humans and rodents [78, 82–84]. The enormous adipocyte size, accumulation of lipids, and increased cellularity of adipose tissue in obesity are thought to contribute to local tissue hypoxia [85]. Hypoxia induces ER stress and PERK-dependent eIF2α phosphorylation leading to protein synthesis inhibition [86]. In hypertrophic adipocytes, ER stress upregulates UPR proteins, including CHOP and GRP78, the inflammatory and apoptotic pathways [78, 87]. Analysis of *Xbp1*$^{+/-}$ mice on a high-fat diet revealed persistent hyperinsulinemia, hyperglycemia, elevated C-peptide, and suppression of insulin signaling in adipocytes [78]. Thus, ER stress may reinforce insulin resistance and may lead to pro-inflammatory cytokine secretion by adipocytes. TNF and IL-6 have been shown to activate ER stress in adipocytes, and subclinical inflammation has been observed in T2D and insulin resistance states [88]. Obese mice lacking either TNF or its receptors, TNFR1 and TNFR2, showed decreased insulin resistance and low blood levels of free fatty acids [88]. In a study of patients with obesity-induced insulin resistance, adipose tissue expression of TNF and IL-6 mRNA was significantly elevated, and in vitro stimulation of human adipocytes with TNF caused increased PPARβ/δ mRNA production but decreased its target genes and DNA binding activity in a NF-κB dependent manner [89]. In skeletal muscle cells, PPARβ/δ agonism inhibited palmitate-induced ER stress and significantly decreased levels of pro-inflammatory cytokines, TNF and IL-6, in an AMPK-dependent manner [90]. The inflammatory environment in T2D could be abrogated by increased activity of PPARβ/δ target genes such as *SIRT1* [91, 92].

Emerging evidence suggests that IL-1β may also play an important role in T2D pathogenesis. Pancreatic β cells secrete insulin and islet amyloid polypeptide (IAPP), a major component of extracellular amyloid β aggregates which are frequently found in the pancreas of diabetic patients. In primed dendritic cells, IAPP oligomers can activate the NLRP3 inflammasome leading to production of IL-1β [93]. IL-1β secretion was significantly decreased with exposure to inhibitors of caspase-1, glucose metabolism, and lysosomal acidification. Glyburide, a sulfonylurea used in T2D treatment, also inhibited IL-1β production [93]. Soluble IAPP oligomers, and not the higher order fibrils, appear to be critical for IL-1β production. One rodent model of islet amyloid deposition is the transgenic mouse expressing human IAPP (IAPP-TG) on a high-fat diet for 1 year [94]. Pancreatic islets from IAPP-TG mice showed regions of decreased insulin, abundant amyloid β deposits, and increased intracellular IL-1β. The amyloid β protein co-localized with IL-1β within IAPP-TG islets. However, there was no significant difference in the proportion of macrophages in islets from IAPP-TG mice versus wild-type controls. A functional analysis of phagocytes isolated from pancreatic islets in IAPP-TG mice could provide definitive proof of which cells (beta cells, macrophages, or dendritic cells) produce IL-1β. Studies using IL-1 antagonists have been shown to ameliorate insulin resistance and obesity [95, 96], and this collectively suggests an autoinflammatory component in T2D.

Proteotoxic stress and inflammation have been found to play an increasingly essential role in the initiation and/or progression of neurodegenerative diseases. Parkinson's disease (PD) is characterized by chronic inflammation, neurotoxicity, progressive loss of dopaminergic neurons culminating in a movement disorder, and progressive dementia. PD pathogenesis involves genetic abnormalities, protein misfolding, defective mitophagy, and neuroinflammation. Loci multiplication and mutations in the α-synuclein gene, *SNCA*, predispose to autosomal dominant PD [97–99]. However, most patients have sporadic forms of PD in which aging and inefficient proteasome degradation results in accumulation of α-synuclein and inflammation [100–104]. Misfolded α-synuclein accumulates into cytoplasmic protein aggregates called Lewy bodies in presynaptic neurons [105, 106]. Additional components of Lewy bodies include ubiquitin, the E3 ubiquitin ligase, Parkin, and the α-synuclein interacting protein, synphilin-1. Synphilin-1 can be ubiquitinated by Parkin leading to its degradation [107].

In a rat model of PD, overexpression of human α-synuclein in the substantia nigra leads to ER stress and upregulation of the UPR response. The study showed a trend towards increased splicing of XBP1, with significantly increased levels of ATF4, pATF6, and CHOP, indicating activation of the PERK and ATF6 pathways and their culmination in apoptosis [108]. Overexpressed human α-synuclein was found to associate with GRP78/BiP leading to its effective removal and prevention of neuronal apoptosis [108]. In addition to the proteasome and UPR, autophagy has been implicated in the degradation of α-synuclein and PD pathogenesis [109, 110]. Aging neurons exhibit increasingly impaired chaperone-mediated autophagy (CMA). Interestingly, CMA has been shown to degrade α-synuclein [103]. However, the mutant, oligomeric or dopamine-treated α-synuclein prevents its CMA-mediated degradation by blocking uptake into lysosomes [111, 112]. Winslow et al. showed that α-synuclein overexpression inhibited autophagosome formation in autophagy [113]. Defective mitochondria accumulate in aging neurons leading to neuronal toxicity and loss. Mitophagy is another form of autophagy that selectively degrades these defective mitochondria. Parkin reduces ER stress-mediated

mitochondrial damage by preventing excessive fragmentation and induction of autophagy [114]. Caspase-1 cleavage and Parkin activation could generate a positive feedback loop whereby increased ER stress leads to increased caspase-1 mediated cleavage of Parkin. Thus, the protective effects of Parkin are suppressed [115]. Neuroinflammation observed in PD is attributed to activated microglia, which are abundant in the postmortem brains of PD patients [116, 117]. CSF and brain tissue from PD patients have elevated TNF and IFN-γ levels [118, 119]. Rodent models of chronic LPS infusion lead to brain inflammation with subsequent delay and selective degeneration of dopaminergic neurons [120]. Peripheral LPS administration resulted in TNF production, which crosses the blood-brain barrier via TNF receptors, leading to neuroinflammation [121, 122]. The microglia in PD express pro-inflammatory cytokines, such as IL-1β, TNF, nitric oxide (NO), and ROS [120, 123–125]. These studies suggest that the establishment and maintenance of an inflammatory microenvironment and failure of protein degradation pathways may together speed the destruction of dopaminergic neurons in PD.

Alzheimer's disease (AD) is characterized by the progressive accumulation of extracellular amyloid β plaques, intracellular neurofibrillary tau, neuroinflammation, extensive neuronal cell death, and dementia [126]. Increased expression of GRP78 was found in brain tissue of AD patients at the early pathological stage of AD compared to controls without dementia [127]. In addition, neurons from AD patients have increased phosphorylated PERK, IRE1α, and eILF2α [128, 129]. The activated UPR was enhanced in neurons with a diffuse pattern of phosphorylated tau [128], suggesting that the UPR activation requires tau but precedes formation of neurofibrillary tau tangles. Microglia and infiltrating mononuclear phagocytes are recruited to amyloid β plaques, become activated and phagocytose amyloid β leading to activation of the NLRP3 inflammasome and production of pro-inflammatory cytokines, specifically IL-1β [130–134]. AD patients and rodent models showed increased IL-1β expression in microglia isolated from amyloid β plaques and increased CSF levels of IL-1β [135]. Studies show that like PD, autophagy may prevent the accumulation of amyloid β plaques in AD. Brain tissue from AD patients shows decreased levels of Beclin-1 and mice deficient in Beclin-1 exhibited amyloid β accumulation and neurodegeneration [136]. Lentiviral expression of Beclin-1 in these mice significantly decreased the amyloid β-meditated pathology [136]. Thus, inefficient UPR combined with dysfunctional autophagy lead to failed clearance of misfolded protein aggregates, subsequent activation of microglia, and significant neuroinflammation in AD.

Age-related macular degeneration (AMD) is characterized by progressive destruction of retinal photoreceptors in the macula and retinal pigment epithelium (RPE) resulting in blindness [137, 138]. AMD is associated with multiple risk factors including age, race, genetic susceptibility, smoking, obesity, and high blood pressure [137, 139–141]. Proposed mechanisms of AMD pathogenesis include chronic oxidative stress, ER stress, light damage, increased polyunsaturated fatty acids, abnormal phagocytosis, complement activation, and inflammation [142–152]. In human RPE, induction of oxidative stress by H_2O_2 or photooxidation led to proteasome inhibition and accumulation of polyubiquitinated proteins and aggregates [152]. Under conditions of proteasome inhibition, the CMA pathway plays an important role in the removal of accumulated proteins [153]. Interestingly, inhibition of both the proteasome and CMA resulted in increased cell death. Thus, retinal cells require protective measures to ensure cell survival. In a murine model of light-induced retinal degeneration, *Xbp1* deficiency caused a decrease in antioxidant genes, superoxide dismutase (SOD) 1, SOD2, and glutathione synthase. In addition, increased oxidative stress and susceptibility to oxidative damage were observed. This study showed that XBP1 has an antioxidant function that may facilitate cell survival and prevent retinal degeneration in AMD [154].

The early stage of macular degeneration in AMD is characterized by local inflammation leading to deposition of drusen, an extracellular debris-like material that accumulates beneath RPE cells and Bruch's membrane [155]. Studies show that drusen contains oxidized proteins, complement and amyloid β. Oxidative stress causes modifications in drusen proteins that may facilitate the formation of drusen. Complement pathway dysregulation is thought to play a critical role in the formation of drusen [142, 144, 148]. An analysis of drusen components showed an abundance of regulatory proteins of the common complement pathway. In addition, RPE cells overlying drusen had increased cytoplasmic levels of complement inhibitors. Similar to T2D and Alzheimer's disease, drusen consists of amyloid beta, a pro-inflammatory molecule that also activates complement [142] and the NLRP3 inflammasome [130, 132]. Drusen components, complement C1q and oxidized lipids, activate the NLRP3 inflammasome resulting in increased production of the pro-inflammatory cytokines, IL-1β and IL-18 [156]. IL-18 is thought to protect against AMD by inhibiting vascular endothelial growth factor (VEGF) and thus preventing the pathological neovascularization in wet AMD. These studies show that drusen biogenesis and maintenance is dependent on an inflammatory milieu with autoinflammatory features.

The interplay of the UPR and various metabolic pathways has raised the possibility that targeted intervention at key points in specific metabolic pathways could be effective in a range of autoinflammatory pathologies. sXBP1 is a direct transcriptional activator of the hexosamine biosynthetic (HBP) pathway, via sXbp1-dependent transcription of genes coding for key, rate-limiting enzymes [157]. This UPR-HBP axis is triggered in a variety of stress conditions, including ischemia-reperfusion (I/R) injury, where acute stimulation of

sXbp1 confers robust cardio-protection in part via induction of HBP. A separate study of I/R injury showed that ischemic accumulation of succinate controls reperfusion injury through mtROS [158]. Therefore, limiting succinate accumulation has marked potential for management of a range of conditions, including autoinflammatory diseases resistant to standard therapies, as well as more widespread conditions, where dysregulation of the UPR underlies pathogenesis. Blockade of *XBP1* splicing by inhibition of IRE1α has shown promise in the treatment of myeloma [159]. Collectively, these studies reveal that the effects of sXbp1 are very context dependent and that the UPR may play a key role to protect cells under stress in addition to the more publicized contribution to causing disease.

Conclusions and implications for future therapies

The diverse diseases arising from protein misfolding, defective clearance, and autoinflammation form a continuum in which cumulative cellular stress results in significant pathology and, in some cases, severe disease and death. In TRAPS and MKD deficiency, accumulation of mutant proteins leads to ER stress, UPR activation, abnormal signaling, and autoinflammation. In CAS and MAS, NLRP3 and NLRC4 mutants undergo improper oligomerization with constitutive activation of the inflammasome that results in IL-1β and IL-18 hypersecretion. In spondyloarthropathies, abnormal UPR activation leads to overproduction of pro-inflammatory cytokines. Interestingly, the UPR and autophagy have dual roles in both promoting and controlling inflammation. Autophagy gene polymorphisms and XBP1 deficiency lead to impaired integrity of Paneth cells, resulting in IBD. Furthermore, the protective functions of XBP1 are demonstrated in I/R injury and multiple myeloma. In T2D and AMD, accumulating extracellular amyloid deposits may be phagocytosed by macrophages/microglia leading to inflammasome activation and increased IL-1β production. Intracellular aggregates of proteins such as α-synuclein accumulate in PD and AD due to marked impairment of protein disposal mechanisms.

The advances in our understanding of the pathogenesis of autoinflammatory diseases and recognition that other diseases have an autoinflammatory component related to altered protein homeostasis have underlined the pressing need for development of novel therapies for these conditions and raised the possibility that these therapies may also treat a wider spectrum of diseases. The goals of such therapies include effective prevention of protein accumulation, enhancement of clearance mechanisms, suppression of ROS, and inflammation. Protein misfolding within the ER leads to activation of the UPR, thus therapies focused on augmenting the UPR could be highly beneficial. However, given the potential for an activated UPR to lead to inflammation and increased disease severity, tight regulation is essential to the success of any pharmacological strategy. In diseases with aberrant ROS production and oxidative stress like AMD and TRAPS, useful therapies may include antioxidants as adjunct therapies [158]. Patients with autoinflammatory diseases who are treated with cytokine blockade show significant attenuation in symptoms and disease progression. IL-1 blockade is effective in treating CAPS, gout and T2D. TNF blockade therapy resulted in limited success in TRAPS patients. Since proteasome degradation and autophagy are anti-inflammatory, possible interventions may involve enhancing these pathways to effectively reduce NLRP3 activation and inflammation. Small molecules that can directly block the NLRP3 inflammasome and related signaling pathways have recently shown promise in pre-clinical studies [160, 161]. Clinical trials of agents designed to modulate proteotoxic stress and the inflammasome, in combination with traditional therapies, will determine the therapeutic impact of these new insights into the connections between protein homeostasis and autoinflammation.

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