Lighting the fires within: the cell biology of autoinflammatory diseases

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Abstract | Autoinflammatory diseases are characterized by seemingly unprovoked pathological activation of the innate immune system in the absence of autoantibodies or autoreactive T cells. Discovery of the causative mutations underlying several monogenic autoinflammatory diseases has identified key regulators of innate immune responses. Recent studies have highlighted the role of misfolding, oligomerization and abnormal trafficking of pathogenic mutant proteins in triggering autoinflammation, and suggest that more common rheumatic diseases may have an autoinflammatory component. This coincides with recent discoveries of new links between endoplasmic reticulum stress and inflammatory signalling pathways, which support the emerging view that autoinflammatory diseases may be due to pathological dysregulation of stress-sensing pathways that normally function in host defence.

Rheumatic and autoimmune diseases have traditionally been categorized according to the target of the abnormal immune response. Type 1 diabetes, for example, is an organ-specific autoimmune disease involving a specific immune attack on pancreatic islet β-cells, whereas systemic lupus erythematosus is the prototypical systemic autoimmune disease, in which autoimmune responses to multiple antigens can be measured and multiple organs are targeted. Recent discoveries have added a second dimension to consider: the degree to which the adaptive versus innate immune system is involved in the disease. Diseases involving abnormal innate immune responses without the involvement of autoantibodies or autoreactive T cells are termed autoinflammatory, whereas diseases that depend on autoreactive B or T cells are classified as autoimmune[1] (FIG. 1).

Autoinflammatory diseases have emerged as a distinct group of diseases that are characterized by pathological inflammation that often stems from the abnormal activation of innate immune cells by endogenous or exogenous stimuli. In the familial autoinflammatory syndromes, mutations in key genes that regulate innate immune cell function have been identified[2]. The uric acid crystals that trigger gout, as well as other particulate materials, have been found to be potent activators of innate immune signalling pathways[3]. These findings have begun to provide a molecular basis for both sporadic and familial autoinflammatory diseases. It is also becoming clear that many diseases are a mix of autoinflammatory and autoimmune components (FIG. 1). In this Review, we explore recent advances in the genetic and cell biological basis of inflammation in Mendelian autoinflammatory diseases (in which inflammation is triggered by mutations) as well as in more common rheumatic diseases, such as spondyloarthropathies and inflammatory muscle diseases. An emerging theme in this field has been the concept that the misfolding and aggregation of protein complexes can trigger autoinflammation with or without triggering the unfolded-protein response (UPR).

Diseases associated with protein misfolding

Both autoimmune and autoinflammatory diseases can be episodic, but autoinflammatory diseases are unique in that symptom flares can have a characteristic frequency and length, which prompted them to be termed periodic fever syndromes. Flares in patients with familial Mediterranean fever (FMF) or gout are particularly acute, generally lasting one week or less. Subclinical signs of autoinflammatory diseases — such as elevated levels of acute-phase proteins or pro-inflammatory cytokines — can often be detected in the absence of symptoms. Although autoreactive memory B and T cells are thought to maintain autoimmunity over the lifetime of an individual, what sustains the inflammation in autoinflammatory diseases? In environmentally triggered autoinflammatory diseases, such as gout, the persistence of the inciting uric acid crystals is responsible for driving recurrent attacks. Therapies that lower the levels of uric acid are highly effective in preventing recurrences of

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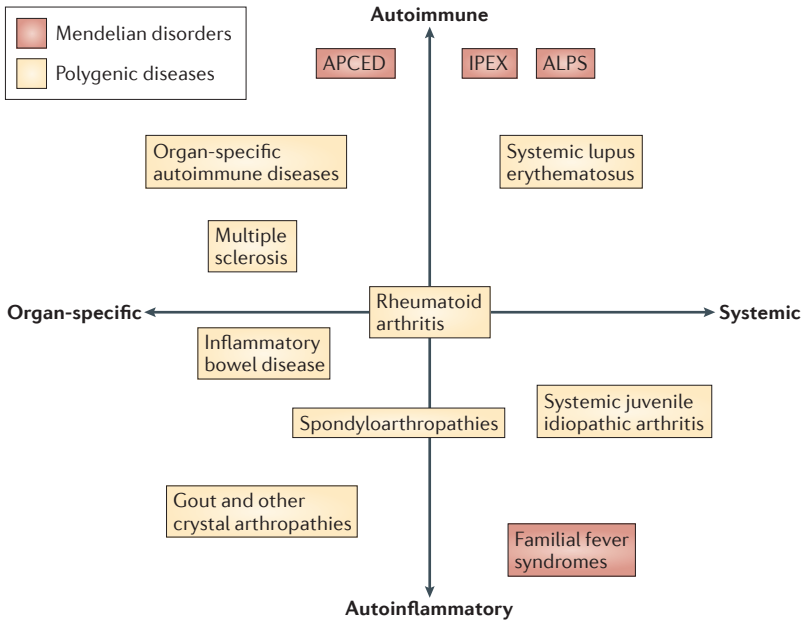

Figure 1 | **The spectrum of autoimmune and autoinflammatory diseases.** Polygenic diseases are boxed in yellow and Mendelian disorders are boxed in red. The horizontal axis depicts the range from organ-specific disease to systemic disease. The vertical axis depicts the degree of involvement of the two strands of the immune system in the immunopathology: autoinflammatory diseases, which involve the innate immune system, are at the bottom; and autoimmune diseases, which involve the adaptive immune system, are at the top. ALPS, autoimmune lymphoproliferative syndrome; APCED, autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy; IPEX, immunodysregulation polyendocrinopathy enteropathy X-linked syndrome.

gout and progression to chronic disease. In the genetic autoinflammatory diseases, the inciting stimulus comes from within, in the form of pathogenic mutations that enhance inflammatory signalling pathways. It is presumed that the disease-associated mutations lower the threshold for triggering an inflammatory response or, in extreme cases, lead to continuous inflammation.

Several genetic autoinflammatory diseases are inherited in an autosomal dominant manner (TABLE 1), which suggests that gain-of-function mutations in innate immune signalling proteins may be common triggers for disease. Many of the mutations that are linked to dominant autoinflammatory diseases cause the proteins involved to misfold or form abnormal oligomers. Cellular responses to misfolded proteins can vary dramatically depending on the extent and recognition of protein misfolding[4] (FIG. 2). In the endoplasmic reticulum (ER), misfolded or partially folded polypeptides can be recognized and eliminated through the ER-associated degradation (ERAD) pathway. The resulting loss of protein function can lead to disease, for example in haemophilia caused by factor VIII mutations[5] and in cystic fibrosis resulting from mutations in the cystic fibrosis transmembrane conductance regulator[6]. Misfolded proteins can also accumulate both inside and outside cells. This can lead to a gain of protein function through mechanisms that are related to the physiological role of the protein in signal transduction, or via the activation of other pathways, such as the UPR, which is triggered by ER stress.

Unrestrained ER stress responses can lead to cell death or inflammation. In tissues that divide slowly, such as neurons, glial cells and pancreatic β-cells, the primary outcome of ER stress appears to be cell death, leading to neurodegenerative disease or diabetes. The C96Y mutation found in the insulin 2 gene (*Ins2*) in the Akita mouse alters an essential disulphide bond that normally links the two insulin polypeptides. As a result of the mutation, insulin is misfolded and retained in the ER of pancreatic β-cells, causing ER stress[7], which leads to loss of β-cells and diabetes[8]. In one type of the retinal degenerative syndrome retinitis pigmentosa, mutations in the carbonic anhydrase 4 gene (*CA4*; also known as *CAIV*) cause the encoded protein to aggregate in the ER, triggering the UPR and increased cell death. Dorzolamide — a carbonic anhydrase inhibitor that is thought to function as a cellular chaperone[9] — can rescue cells that have *CA4* mutations from cell death. In other diseases, the mutant protein may acquire functions that are related to its normal role. An example of this is superoxide dismutase 1 (SOD1) in amyotrophic lateral sclerosis. *SOD1* mutations provoke disease in most cases of familial amyotrophic lateral sclerosis. Under normal physiological conditions, SOD1 functions as a scavenger for reactive oxygen species (ROS) by converting superoxide into hydrogen peroxide. However, the mutant form of SOD1 has been shown to bind to the small GTPase RAC1 and to thereby enhance the generation of ROS by NADPH oxidase 2 (NOX2)[10]. This, in turn, leads to increased rates of cell death.

In addition to triggering cell death, protein misfolding in innate immune cells can activate inflammatory pathways that function in host defence. Although the 'classic' UPR is dedicated to restoring homeostasis, in part by inducing a transient blockade of protein synthesis followed by an upregulation of ER chaperone expression, recent studies have identified signalling pathways that are triggered by ER stress that drive inflammatory cellular response programmes. The ER stress sensor inositol-requiring enzyme 1 (IRE1) mediates the splicing of the mRNA encoding X-box-binding protein 1 (XBP1), which activates one arm of the transcriptional response to ER stress. In addition, IRE1 activates JUN N-terminal kinase (JNK) and p38 mitogen-activated protein kinases (MAPKs), which activate signalling cascades that culminate in inflammatory response gene expression[11]. In hepatocytes, the transcription factor cAMP-responsive-element-binding protein H (CREBH; also known as CREB3L3) can be activated by ER stress and leads to transcriptional activation of genes encoding acute-phase proteins, such as serum amyloid P and C-reactive protein[12]. Innate immune stimuli such as lipopolysaccharide (LPS) can trigger low-level *XBP1* splicing, which sustains the transcription of pro-inflammatory genes without activating other arms of the UPR that regulate chaperone expression[13]. This response is independent of JNK and p38 MAPKs but requires the adaptor protein TNFR-associated factor 6 (TRAF6). Taken together, these results indicate that the signalling pathways that were originally discovered in response to ER stress may also be part of the innate immune response and, as such, may be involved in the pathogenesis of autoinflammatory diseases.

Gout
An autoinflammatory disease triggered by crystalline uric acid and characterized by episodic flares of arthritis that can progress to destructive joint damage.

Spondyloarthropathies
A group of immune-mediated inflammatory disorders that affect the vertebral column. Spondyloarthropathies also frequently affect the entheses (the areas of tendon and ligament insertion into bone).

ER stress
Stress caused by the perturbation of endoplasmic reticulum (ER) functions that are necessary for cellular homeostasis. The unfolded protein response is the collective outcome of ER stress and must be tightly regulated, as otherwise it is directly linked to the pathogenesis of several diseases.

Table 1 | **Molecular features and molecular causes of familial autoinflammatory disorders**

Disease*	Gene	Protein	Inheritance pattern	Clinical features	Refs
Familial Mediterranean fever (FMF)	*MEFV*	Pyrin	Autosomal recessive or gene-dosage-dependent autosomal dominant	Periodic fevers (lasting 3–7 days), serositis, arthritis	64
Tumour necrosis factor-associated periodic syndrome (TRAPS)	*TNFRSF1A*	Tumour necrosis factor receptor 1	Autosomal dominant with dependence on the wild-type allele	Periodic fevers (lasting 1–6 weeks), serositis, rash, episcleritis	53
Hyper IgD syndrome	*MVK*	Mevalonate kinase	Autosomal recessive	Periodic fevers (lasting 3–7 days), non-destructive arthritis, lymphadenopathy, vasculitic skin lesions	98
Cryopyrin-associated periodic syndromes (FCAS, MWS and NOMID)	*NLRP3*	NLRP3	Autosomal dominant	Cold-induced autoinflammation, cochlear inflammation, fevers, sterile meningitis, bone lesions	99,100
Blau syndrome‡	*NOD2*	NOD2	Autosomal dominant	Granulomatous dermatitis, uveitis, arthritis	101,102
PAPA syndrome	*PSTPIP1*	PSTPIP1	Autosomal dominant	Pyogenic arthritis, pyoderma granulosum, acne	103
Deficiency of IL-1 receptor antagonist (DIRA)	*IL1RN*	IL-1 receptor antagonist	Autosomal recessive	Fevers, pustular skin rash, osteolytic bone lesions	104
Deficiency of IL-36 receptor antagonist (DITRA)	*IL36RN*	IL-36 receptor antagonist	Autosomal recessive	Generalized pustular psoriasis	105
Familial psoriasis (PSORS2) and CARD14-mediated pustular psoriasis (CAMPS)	*CARD14*	Caspase-recruitment domain-containing protein 14	Autosomal dominant	Familial psoriasis and psoriatic arthritis; *de novo* mutation in early-onset generalized pustular psoriasis	17,18
CANDLE syndrome, Nakajo–Nishimura syndrome and JMP syndrome	*PSMB8*	PSMB8 immunoproteasome subunit	Autosomal recessive	Lipodystrophy and multi-organ inflammation	19–22

CANDLE, chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature; FCAS, familial cold autoinflammatory syndrome; IL, interleukin; JMP, joint contractures, muscle atrophy, microcytic anaemia and panniculitis-induced childhood-onset lipodystrophy; MWS, Muckle–Wells syndrome; NLRP3, NOD-, LRR- and pyrin domain-containing 3; NOD2, nucleotide-binding oligomerization domain protein 2; NOMID, neonatal-onset multisystem inflammatory disease; PAPA, pyogenic sterile arthritis, pyoderma gangrenosum and acne; PSTPIP1, proline-serine-threonine phosphatase-interacting protein 1. *Diseases are listed in order of their discovery. ‡Also known as familial juvenile systemic arthrocutaneouveal granulomatosis.

Inflammasome
A large multiprotein complex usually comprising a NOD-like receptor (NLR), the adaptor protein ASC and pro-caspase 1. The assembly of the inflammasome leads to the activation of caspase 1, which cleaves pro-interleukin-1β (pro-IL-1β) and pro-IL-18 to generate the active pro-inflammatory cytokines.

Immunoproteasome
The standard proteasome is composed of 14 α- and β-subunits, of which three (β1, β2 and β5) are involved in peptide-bond cleavage. Interferon-γ induces the expression of the immunosubunits β1i, β2i and β5i, which can replace the catalytic subunits of the standard proteasome to generate the immunoproteasome, which has distinct cleavage-site preferences.

Misfolded proteins that accumulate extracellularly can also trigger the secretion of interleukin-1β (IL-1β), which may be part of the inflammatory cascade that causes tissue damage in autoinflammatory diseases. Extracellular aggregates of amylin (also known as islet amyloid polypeptide) trigger inflammasome activation and the secretion of IL-1β, which results in the dysfunction of pancreatic β-cells[14]. In Alzheimer's disease, aggregates of extracellular β-amyloid can also lead to lysosomal damage followed by activation of the inflammasome[15]. How commonly extracellular protein aggregates trigger other autoinflammatory conditions remains to be determined.

Familial autoinflammatory diseases

In the last decade, the genetic causes of several familial autoinflammatory syndromes have been discovered (TABLE 1). The genes that are responsible for these syndromes have been found to encode key sensors and transducers of inflammatory signal transduction pathways. Some of the genes involved, such as tumour necrosis factor receptor superfamily member 1A (*TNFRSF1A*), were previously well known, whereas the discovery of mutations in others, such as that encoding NOD-, LRR- and pyrin domain-containing 3 (NLRP3; also known as cryopyrin and NALP3), highlighted their importance in inflammatory signalling pathways[16]. The recent identification of gain-of-function mutations in caspase recruitment domain-containing protein 14 (CARD14; also known as CARMA2) that are associated with autosomal dominant familial or *de novo* severe pustular psoriasis has prompted renewed investigation into which receptors activate nuclear factor-κB (NF-κB) through this adaptor protein, particularly in the skin[17,18]. The discovery of mutations in the PSMB8 immunoproteasome subunit that are associated with an autoinflammatory disease was surprising and suggests new links between protein homeostasis and inflammation[19–22].

Cryopyrin-associated periodic syndromes and the inflammasome. Cryopyrin-associated periodic syndromes (CAPS) are a group of autosomal dominant autoinflammatory diseases. Examples include familial cold autoinflammatory syndrome (FCAS), Muckle–Wells syndrome (MWS) and neonatal-onset multisystem inflammatory disease (NOMID; also known as chronic infantile neurological, cutaneous and articular syndrome (CINCA)). All of these syndromes have been linked to mutations in the *NLRP3* gene[23]. FCAS is characterized by cold-induced urticaria and mild symptoms of systemic inflammation. In addition to showing these symptoms, patients with MWS develop progressive sensorineural deafness (owing to cochlear inflammation), arthralgias and

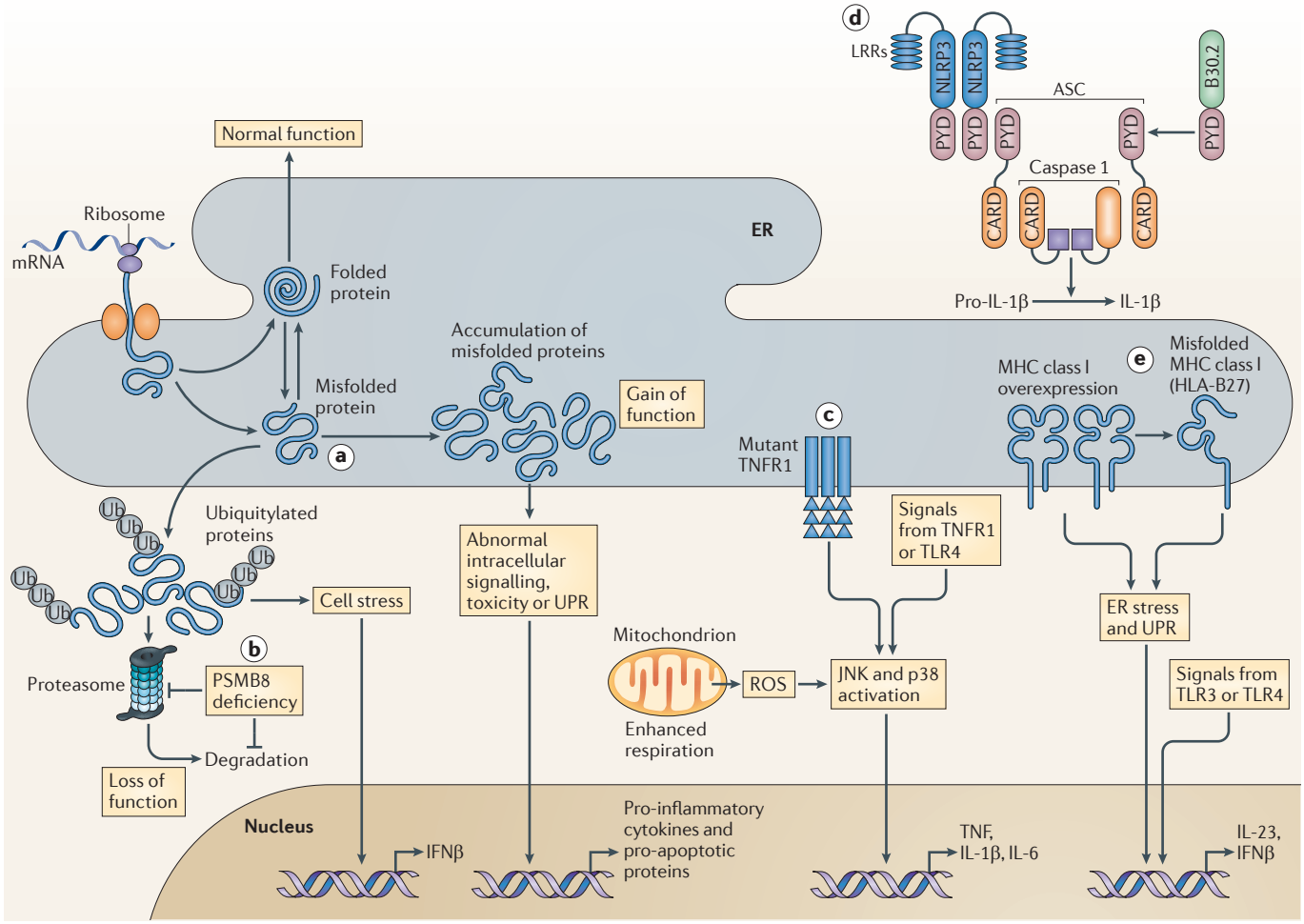

Figure 2 | **Consequences of protein misfolding and intracellular signalling complexes that activate autoinflammatory disease.** **a** | The effects of the misfolding of secretory proteins in the endoplasmic reticulum (ER) are depicted at the bottom of the figure. The degradation of misfolded proteins can cause a loss of function, whereas the accumulation of misfolded proteins can trigger abnormal intracellular signalling or, at higher levels, the induction of the unfolded-protein response (UPR), which can also lead to the induction of inflammation and programmed cell death. Different foci of abnormal cellular signalling that trigger autoinflammatory diseases are depicted in the cell. **b** | In PSMB8 deficiency, reduced degradation of misfolded proteins and peptides by the immunoproteasome leads to the accumulation of ubiquitylated proteins and cellular stress. This can lead to the production of interferon-β (IFNβ), which in turn upregulates the synthesis of immunoproteasome subunits, perpetuating the abnormalities. **c** | In tumour necrosis factor-associated periodic syndrome (TRAPS), mutations in the extracellular region of tumour necrosis factor receptor 1 (TNFR1) lead to accumulation of the mutant receptor in the ER, which triggers an abnormal inflammatory response that is amplified by TNF or lipopolysaccharide (LPS) signalling through cell-surface receptors. **d** | In the cryopyrin-associated perodic syndromes (CAPS), mutations in NOD-, LRR- and pyrin domain-containing 3 (NLRP3) enhance the activation of the NLRP3 inflammasome and the processing of pro-interleukin-1β (pro-IL-1β) into its active form. In familial Mediterranean fever, mutant pyrin is thought to associate with the inflammasome adaptor protein ASC and increase IL-1β processing. **e** | In the spondyloarthropathies, HLA-B27 is expressed at a high level (which is enhanced in inflammation), fails to fold properly and is retained in the ER, triggering a partial ER stress response that leads to type I IFN and IL-23 production. CARD, caspase recruitment domain; JNK, JUN N-terminal kinase; LRR, leucine-rich repeat; PYD, pyrin domain; ROS, reactive oxygen species; TLR, Toll-like receptor.

recurrent fevers. In patients with NOMID, inflammatory symptoms are nearly continuous and typically begin in infancy. Patients with NOMID also suffer from chronic meningitis that can affect central nervous system development, and from hypertrophic bone and cartilaginous lesions in the epiphyses of the long bones that can lead to severe deformities and disability. Mutations in *NLRP3* occur *de novo* in NOMID, whereas they are often inherited in the other less severe syndromes.

The identification of *NLRP3* mutations in CAPS coincided with the realization that the NLRP3 protein is a key component of an inflammasome — a multiprotein complex that can activate caspase 1. Inflammasomes are required to process the precursor forms of the pro-inflammatory cytokines IL-1β and IL-18 into products that can be secreted from the cell[24]. There are at least four distinct inflammasome complexes, which sense distinct classes of stimuli

using different sensor and adaptor proteins, but they all converge on IL-1β activation (FIG. 3). In addition, NLRP6 has recently been identified as a component of an inflammasome that activates IL-18 and negatively regulates colonic inflammation through alterations of the intestinal microbiota[25–27]. The NLRP3 inflammasome is the most intensively studied and has been linked to autoinflammatory disease[28]. NLRP3 is a large protein containing a carboxy-terminal leucine-rich repeat (LRR) domain, which probably acts as a sensor, a central nucleotide-binding domain that binds ATP or dATP, and an amino-terminal pyrin domain. The activation of NLRP3 inflammasomes is thought to be triggered by conformational changes in the NLRP3 LRR domain, which result in the release of NLRP3 from an autoinhibited basal state. Activated NLRP3 oligomerizes and interacts with the adaptor protein ASC and pro-caspase 1, resulting in the enzymatic activation of caspase 1. Studies in NLRP3-deficient mice have shown that NLRP3 is required for IL-1β processing in response to a wide variety of stimuli, including bacterial and viral RNA, Gram-positive bacteria such as *Staphylococcus aureus* and *Listeria monocytogenes,* uric acid and calcium pyrophosphate crystals, and aggregated proteins such as amyloid-β. The molecular diversity of these NLRP3-activating ligands suggests that at least some of them may not bind to the LRRs of NLRP3 directly, but may instead trigger a common intracellular mediator that activates NLRP3. An initial priming step is also required to induce the expression of inflammasome components and substrates such as pro-IL-1β and NLRP3. This signal can be provided by Toll-like receptor (TLR) ligands, such as LPS.

Gain-of-function missense mutations in *NLRP3* have been identified in the majority of CAPS patients, although one patient with atypical CAPS symptoms was reported to be heterozygous for an *NLRP3* truncation mutation[29]. Somatic missense *NLRP3* mutations have also been detected in some patients who have biochemical and clinical features of CAPS but do not have germline mutations in *NLRP3* (REFS 23,30).

Mechanisms of normal inflammasome activation and disruption in familial fever syndromes. Several mechanisms have been proposed to explain how diverse stimuli gain access to the cytoplasmic NLRP3 inflammasome and trigger its activation. The first model ascribes inflammasome activation to the formation of a pannexin 1 hemichannel, through which pathogen-associated molecular patterns (PAMPs; such as muramyl dipeptides) and endogenous damage-associated molecular patterns (DAMPs) may enter the cell[31,32]. Although studies have shown a crucial role for pannexin 1 in the phagocytosis of bacterial muramyl dipeptide[33], it is unlikely that all NLRP3-activating stimuli enter the cell through the pannexin 1 pore, particularly in the case of crystals or particulates. For these stimuli, 'frustrated phagocytosis' — a process that can lead to the rupture of phagolysosomes — has been suggested to trigger inflammasome activation. In this second model, lysosomal rupture (perhaps triggered by the mechanical disruption of lysosomes by crystalline material) may allow the diffusion of phagocytosed particles into the cytoplasm, where they may directly interact with the NLRP3 inflammasome. Alternatively, lysosomal enzymes, such as cathepsins, may trigger inflammasome activation in the cytoplasm through proteolytic cleavage[34,35]. However, cathepsin B-deficient mice do not consistently show diminished levels of caspase 1 cleavage and IL-1β secretion, suggesting that the leakage of other lysosomal components may also be required[36].

A third model proposes a role for ROS, which are generated as a result of various types of cellular stress, in inflammasome activation. A direct effect of ROS on inflammasome activation was suggested by studies on thioredoxin-interacting protein (TXNIP), a protein that was previously found to be involved in glucose homeostasis[37]. Under resting conditions, TXNIP associates with the oxidoreductase thioredoxin at two redox-sensitive cysteine residues. With increasing levels of ROS, TXNIP was found to dissociate from thioredoxin and associate with NLRP3 through the LRR and nucleotide-binding domains of NLRP3. However, TXNIP-deficient macrophages exhibited reduced, but not absent, IL-1β secretion in response to NLRP3 agonists[38], which suggests that other mechanisms of IL-1β processing also exist. Chemically induced ER stress has recently been shown to activate the NLRP3 inflammasome via ROS production, but is independent of classical UPR activation[39].

The source of the ROS that activate the inflammasome has been of considerable interest. Initially, the NOX family of NADPH oxidases was implicated in the production of inflammasome-activating ROS, as these enzymes are known to have a role in innate immunity through the generation of the bactericidal respiratory burst. Indeed, NLRP3 inflammasome activation was reduced by pharmacological inhibitors of NADPH oxidases, such as diphenyleneiodonium (DPI) or apocynin, and by small interfering RNA-mediated knockdown of the expression of p22phox (also known as CYBA), an essential component of NOX1 to NOX4 complexes[36,40,41]. Moreover, antioxidants that are thought to act on NADPH oxidases reduced IL-1β secretion by cells from patients with CAPS and from healthy donors[42]. However, DPI and apocynin can impair other flavin-containing enzyme complexes that generate ROS, such as those involved in the electron transport pathway in mitochondria, which calls into question their use as specific inhibitors of NADPH oxidases[43]. Furthermore, macrophages from patients with chronic granulomatous disease who are deficient in subunits of the NOX2 NADPH oxidase produce normal levels of IL-1β in response to LPS, and NOX2-deficient mice exhibit increased pro-inflammatory responses in response to various sterile pro-inflammatory stimuli[44–46]. For these reasons, it seems likely that ROS generated through other pathways besides NADPH oxidases may enable NLRP3 inflammasome activation.

NADPH oxidases
Plasma membrane- and phagosomal membrane-bound enzyme complexes that transfer electrons from NADPH to molecular oxygen, promoting the generation of the reactive oxygen species superoxide.

Mitochondrial damage and consequent ROS production have been recently implicated in the activation of the NLRP3 inflammasome. Blocking mitophagy (the removal of damaged mitochondria by autophagy) resulted in increased mitochondrial ROS production and NLRP3 inflammasome activation, whereas the inhibition of ROS reversed the increase in IL-1β production[47,48]. Oxidative damage to mitochondrial DNA may provide a link between ROS generation and inflammasome activation, as oxidized mitochondrial DNA can directly trigger NLRP3-dependent IL-1β processing[49]. However, kinetic studies have shown that mitochondrion-targeted antioxidants also block the transcription of pro-inflammatory genes, including *NLRP3,* which occurs before inflammasome activation, suggesting a role for mitochondrial ROS in the priming phase of inflammasome activation[45,50].

How do the *NLRP3* mutations that occur in patients with CAPS enhance inflammasome activation? Most of the 110 known disease-associated mutations in *NLRP3* are concentrated in exon 3, which encodes the nucleotide-binding domain, suggesting that this domain has functional importance in the disease process[51]. The NLRP3 nucleotide-binding domain binds ATP and functions as an ATPase[52], and intact nucleotide binding is required for the oligomerization of NLRP3 and for IL-1β processing[52]. Importantly, mutant NLRP3 proteins from patients with CAPS still bind to ATP. Moreover, modelling has suggested that CAPS-associated NLRP3 mutant proteins can oligomerize more readily, potentially as a result of having an open form that favours nucleotide binding. This oligomerization promotes inflammasome activation in response to reduced or absent exposure to activating stimuli. Cells that have CAPS-associated *NLRP3* mutations release IL-1β spontaneously and are hyperresponsive to pro-inflammatory stimuli such as TLR ligands. Notably, cells that have *NLRP3* mutations lose the requirement for extracellular ATP to activate IL-1β processing. These effects are seen in cells from patients with CAPS who are heterozygous for *NLRP3* mutations, suggesting that the mutations produce dominant gain-of-function alleles.

TNFR-associated periodic syndrome. TNFR-associated periodic syndrome (TRAPS) is an autosomal dominant autoinflammatory disease associated with mutations in *TNFRSF1A,* which encodes TNFR1 (REF. 53). Patients with TRAPS experience recurrent prolonged episodes of fever lasting for up to 6 weeks, rash, abdominal pain and internal inflammatory manifestations, such as serositis, fasciitis and episcleritis[54]. Systemic amyloidosis occurs in approximately 10% of patients with TRAPS. Seventy-nine distinct mutations in *TNFRSF1A* have been found to be associated with TRAPS[51] in the heterozygous state[55]. Strikingly, almost all TRAPS-associated mutations in *TNFRSF1A* are missense mutations that result in amino acid substitutions or small deletions and insertions in the TNFR1 extracellular domain, which is responsible for receptor pre-association and TNF binding. About half of these mutations occur at highly conserved cysteines and other conserved residues that are important for maintaining the secondary structure of the TNFR1 extracellular domain. Another class of mutations associated with TRAPS also occur in 1–3% of asymptomatic individuals, suggesting that they may be more akin to functional polymorphisms. Such mutations include those that lead to R92Q and P46L amino acid substitutions.

The autoinflammatory phenotype of TRAPS was originally thought to result from reduced proteolytic cleavage of the soluble extracellular domain of TNFR1, but only one mutation (V173D) has been shown to be in or near the metalloproteinase cleavage site in TNFR1 (REF. 56). The extent to which cleavage of cell-surface TNFR1 is reduced has been variable in heterozygous cells that express both wild-type and mutant forms of TNFR1 (REF. 54). In addition, the treatment of patients with TRAPS with TNF-blocking agents, such as etanercept (a TNFR2–Fc fusion protein), improves symptoms but does not fully suppress disease or prevent amyloidosis, indicating that there may be alternative mechanisms by which mutant TNFR1 causes inflammation in TRAPS[57] (E. A. Hull & D.L.K., unpublished observations).

TRAPS-associated TNFR1 mutant proteins have several abnormalities that probably stem from aberrant folding of the extracellular domain of the protein. Mutant TNFR1 receptors that fail to bind to TNF do not physically interact with wild-type receptors and have reduced or absent expression on the cell surface. The majority of mutant TNFR1 molecules are retained intracellularly, primarily in the ER, probably because of abnormal protein folding through non-physiological disulphide bonding, and these mutants accumulate intracellularly to levels more than tenfold higher than those of wild-type TNFR1 (REFS 58–61).

These findings raise the question of whether mutant TNFR1 proteins might trigger ER stress and the UPR. Cells harbouring heterozygous TRAPS-associated *TNFRSF1A* mutations do not have altered baseline levels of the ER chaperone protein BIP (also known as GRP78) or the ER stress-responsive protein CHOP (also known as DDIT3) or aberrant increases in these proteins following induction of the UPR[58,61]. However, JNK and p38 MAPKs are spontaneously activated in cells containing TRAPS-associated *TNFRSF1A* mutations, and further increases in their activities occur following treatment with LPS[61]. In addition, some elevation in the levels of XBP1 can be seen[62]. These findings suggest that mutant TNFR1 alters the balance of MAPK activation, either through spontaneous TNF-independent signalling owing to intracellular protein accumulation or through the induction of a low level of ER stress. These alterations in kinase activation have inflammatory consequences, as cells from patients with TRAPS and from mice with heterozygous *Tnfrsf1a* mutations homologous to those linked to TRAPS produce excess pro-inflammatory cytokines in response to LPS in a JNK- and p38 MAPK-dependent manner[61]. Interestingly, full expression of the inflammatory phenotype that occurs in TRAPS seems to require wild-type as well as mutant TNFR1 proteins,

Mitophagy
Selective removal of mitochondria under conditions of nutrient starvation or mitochondrial stress.

Autophagy
An evolutionarily conserved process in which acidic double-membrane vacuoles sequester intracellular contents (such as damaged organelles and macromolecules) and target them for degradation through fusion to secondary lysosomes.

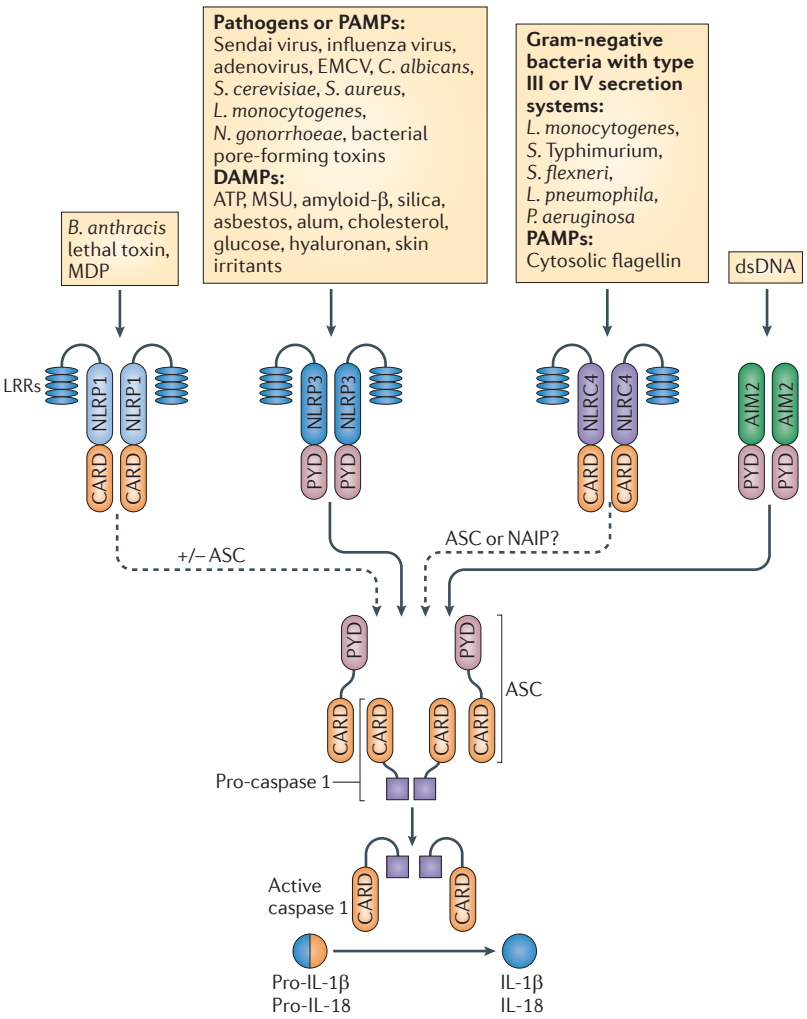

Figure 3 | **The NLRP1, NLRP3, NLRC4 and AIM2 inflammasomes.** The NOD-, LRR- and pyrin domain-containing 1 (NLRP1) inflammasome is activated by *Bacillus anthracis* lethal toxin and muramyl dipeptide (MDP). The NLRP3 inflammasome is activated by exposure to whole pathogens, pathogen-associated molecular patterns (PAMPs), damage-associated molecular patterns (DAMPs) and environmental irritants. The NOD-, LRR- and CARD-containing 4 (NLRC4; also known as IPAF) inflammasome is activated by Gram-negative bacteria with type III or IV secretion systems, and the absent in melanoma 2 (AIM2) inflammasome senses double-stranded DNA (dsDNA). Other complexes (not shown) — such as NLRP6-containing complexes that regulate interleukin-18 (IL-18) production — also exist[24], and pyrin probably regulates an inflammasome complex containing ASC but not NLRP3 that activates IL-1β processing[69]. CARD, caspase recruitment domain; EMCV, encephalomyocarditis virus; LRR, leucine-rich repeat; MSU, monosodium urate; PYD, pyrin domain.

Colchicine
An inhibitor of microtubule polymerization that has anti-inflammatory properties, possibly through blockade of neutrophil chemotaxis. Colchicine is effective in treating acute attacks of gout and protecting against recurrent flares. It is also an effective treatment for familial Mediterranean fever, but not for other genetic autoinflammatory syndromes.

because cells from mice homozygous for TRAPS-associated *Tnfrsf1a* mutations mimic TNFR1-deficient mice in their resistance to LPS-induced septic shock[61]. Thus, the mutant TNFR1 in TRAPS seems to function as an unusual gain-of-function protein, signalling from within the cell to enhance inflammatory responses, but requiring cooperation with the wild-type receptor to produce the clinical manifestations of TRAPS. These results may explain the partial efficacy of TNF blockade in this syndrome[57], as TNF blockade would only affect extracellular TNF binding to wild-type TNFR1, not the intracellular effects of the mutant receptor.

The study of *TNFRSF1A* mutations in TRAPS has also revealed new insights into mitochondrial biology and the origin of pro-inflammatory ROS. The enhanced MAPK activation seen in cells with TRAPS-associated *TNFRSF1A* mutations depends on ROS, probably because MAPK phosphatases are inactivated by ROS. As shown in recent studies of inflammasome activation, the increased inflammation in TRAPS is dependent on mitochondrial respiration rather than on NADPH oxidases. Elevated levels of mitochondrial ROS were seen in cells from patients with TRAPS and in mice with engineered TRAPS-associated *Tnfrsf1a* mutations[43]. Mitoquinone (MitoQ), a mitochondrially targeted coenzyme Q antioxidant, could reduce inflammatory responses in these cells. Unlike cells in which mitophagy is blocked and ROS leak from a damaged respiratory chain, cells from patients with TRAPS have increased oxygen consumption and respiratory capacity. Whether other diseases display this type of increased ROS production owing to enhanced respiration is an important subject for future investigation.

Familial Mediterranean fever and NLRP3-independent inflammasome activation. Mutations in the *MEFV* gene, which encodes the protein pyrin, were found to underlie most cases of the autoinflammatory disease FMF, which is characterized by short episodes of fever and inflammation involving the joints and serosal surfaces[63]. Patients with FMF previously also suffered from high rates of amyloidosis until the introduction of colchicine therapy in the 1970s. Pyrin is an adaptor protein that contains an N-terminal eponymous pyrin domain and a domain at the C-terminal end termed the B30.2 or PRY–SPRY domain. Interestingly, the B30.2 domain is present in primate and human pyrin but not in rodent pyrin[64], and most missense mutations associated with FMF in humans are in this domain. Pyrin can assemble with ASC and pro-caspase 1 in human cells to facilitate the cleavage of pro-caspase 1 (REF. 65). However, pyrin has also been reported to function as a negative regulator of inflammasome function, through interactions of its B30.2 domain with caspase 1 (REFS 66–68). FMF-associated mutations in the B30.2 domain reduce interactions with caspase 1 and potentiate caspase 1 activation[67,68]. Gain-of-function mutations in pyrin exert their effects independently of NLRP3, suggesting that a distinct inflammasome may be the target of regulation by pyrin[69].

Although FMF has been traditionally thought of as an autosomal recessive disease, sequencing of the *MEFV* locus in patients with FMF revealed a substantial fraction of patients with only one mutated *MEFV* allele. This raises the possibility that FMF could also result from *MEFV* haploinsufficiency, a dominant-negative mechanism or a dose-dependent gain of function[70]. The fact that nearly all *MEFV* mutations are missense point mutations rather than nonsense mutations or deletions suggests that the mutant protein may have pro-inflammatory functions[70]. Indeed, mice engineered to express a human–mouse chimeric form of pyrin in

which the B30.2 domain of human pyrin had FMF-associated mutations developed spontaneous systemic inflammation similar to, but more severe than, human FMF. This phenotype occurred only when the mutant B30.2 domain was present in both pyrin alleles, suggesting a gain-of-function mechanism with a gene-dosage effect. Inflammation in these mice was dependent on bone marrow-derived cells expressing IL-1β and ASC, but interestingly not on NLRP3, suggesting that human mutant pyrin activates an NLRP3-independent inflammasome complex[69]. These data indicate that mutant pyrin enhances inflammation and the symptoms of FMF in a dominant manner that is highly dependent on gene dosage, although haploinsufficiency or a dominant-negative mechanism that inhibits an unknown inflammatory suppressive function remain conceptually possible.

The UPR in polygenic autoinflammatory diseases

HLA-B27 misfolding and spondyloarthritis. Spondyloarthropathies are a family of immune-mediated inflammatory diseases that are strongly associated with the MHC class I allele HLA-B27 (REF. 71). Although genetic predisposition for these conditions is complex, HLA-B27 is responsible for up to 30–40% of overall risk. In ankylosing spondylitis, the prototypical spondyloarthropathy, there is spinal inflammation with new bone formation, resulting in the fusion of sacroiliac and vertebral facet joints, together with syndesmophyte formation that links adjacent vertebral bodies. Despite years of intense investigation, the role of HLA-B27 in these disorders remains enigmatic, and a definitive role for particular peptides presented by HLA-B27 to $CD8^+$ T cells in the pathogenesis of these diseases has not been identified. There is increasing evidence that these diseases may be autoinflammatory rather than autoimmune in nature[72].

Recent evidence has suggested an alternative role for HLA-B27 in the pathogenesis of spondyloarthropathies that is independent of its role in antigen presentation. A tendency of the HLA-B27 heavy chain to misfold — characterized by prolonged ER retention, BIP binding and the formation of disulphide-linked heavy chain complexes — may provide clues to its role in disease[73]. In cells from transgenic rats, HLA-B27 misfolding results in ER stress and the activation of the UPR, particularly after the upregulation of HLA-B27 expression[74,75]. Occurrence of the UPR together with macrophage activation following exposure to TLR agonists (such as LPS) leads to the synergistic induction of certain cytokines, most notably interferon-β (IFNβ) and IL-23 (REFS 76,77). XBP1 is required for the effect of the UPR on IFNβ expression, whereas CHOP mediates IL-23 overproduction[78]. HLA-B27 may trigger the UPR more easily than other misfolded proteins (such as TNFR1 in TRAPS) because it is a relatively abundant cellular protein that can be substantially upregulated. This hypothesis is supported by the correlation between the upregulation of HLA-B27 by stimuli such as IFNs and the induction of the UPR[74,75].

In transgenic rats that express HLA-B27, macrophages in which the UPR is activated tend to produce more IL-23 relative to IL-12, suggesting that HLA-B27 misfolding could be a stimulus for the activation of T helper 17 (T_H17) cells[77]. Consistent with this, myeloid cells from the colon of these rats exhibit UPR activation and IL-23 upregulation, and $CD4^+$ T cells overexpress IL-17 concurrently with the development of inflammation[77]. These results provide a compelling model that links HLA-B27 misfolding to the pathogenesis of inflammation in a manner that is independent of its role in the presentation of antigens to $CD8^+$ T cells. Thus, although T cells have a role in pathogenesis, there are likely to be upstream autoinflammatory signals that may converge to activate cells expressing the IL-23 receptor (including T_H17 cells). It is of interest that polymorphisms in the *IL23R* gene influence susceptibility to ankylosing spondylitis[79].

UPR activation in inflammatory myositis. Idiopathic inflammatory myopathies (IIMs) are a group of inflammatory muscle diseases that include juvenile and adult dermatomyositis, polymyositis and inclusion body myositis. Chronic skeletal muscle inflammation and muscle fibre damage are characteristic features of these diseases. Although myositis has been thought of as an autoimmune disease, it has recently become clear that the innate immune system also has a key role in the pathogenesis of this condition. Type I IFNs may be particularly important in dermatomyositis, in which an IFN-induced gene signature has been found in muscle tissue[80,81]. The cellular source of the type I IFNs has been difficult to elucidate. However, recent evidence suggests that, in addition to plasmacytoid dendritic cells, immature myocytes expressing TLR3 are a local source of IFNβ[82]. The IFNβ may help to drive the inappropriate high level of MHC class I expression that is seen in these cells. Interestingly, UPR activation is prominent in certain forms of IIM in association with increased MHC class I expression. In a mouse model, enforced conditional expression of MHC class I ($H\text{-}2K^b$) molecules in muscle cells initiates the UPR and causes muscle inflammation that is thought to be sustained through UPR-induced apoptosis of myocytes[83]. However, the ability of spliced XBP1 to promote the induction of IFNβ by TLR3 or TLR4 agonists[76] suggests that myocytes in which the UPR is activated could be a significant source of type I IFNs. These studies demonstrate another potential link between UPR activation and a disease that is associated with autoantibodies but may nevertheless have a strong autoinflammatory component.

Dysregulation of the immunoproteasome and the UPR in autoinflammation. In spondyloarthropathies and inflammatory myositis, inflammation may arise in the context of activation of the UPR. However, the UPR also restores homeostasis in cells, in part by mediating a transient block in protein synthesis. When the UPR is dysfunctional, inflammation can arise as a consequence of the inability of cells to respond to

Table 2 | **Pathogenic mechanisms in autoinflammatory diseases**

Molecular abnormality*	Mechanism	Disease(s)‡
Pyrin mutation	Highly gene-dosage dependent; ASC-dependent, NLRP3-independent inflammasome activation	Familial Mediterranean fever
NLRP3 mutation	Abnormal oligomerization; spontaneous activation of caspase 1 activity	FCAS, MWS and NOMID
TNFR1 mutation	Abnormal aggregation and intracellular accumulation of TNFR1; ligand-independent signalling; mitochondrial ROS; MAPK activation	TRAPS
HLA-B27	Abnormal protein accumulation; ER stress	Spondyloarthropathies
PSMB8 deficiency	Abnormal accumulation of ubiquitylated protein aggregates	JMP syndrome, CANDLE syndrome
XBP1 deficiency	Global disruption of ER stress response; protein overload; cell death	Inflammatory bowel disease in XBP1-deficient mice

CANDLE, chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature; ER, endoplasmic reticulum; FCAS, familial cold autoinflammatory syndrome; JMP, joint contractures, muscle atrophy, microcytic anaemia and panniculitis-induced childhood-onset lipodystrophy; MAPK, mitogen-activated protein kinase; MWS, Muckle–Wells syndrome; NLRP3, NOD-, LRR- and pyrin domain-containing 3; NOMID, neonatal-onset multisystem inflammatory disease; ROS, reactive oxygen species; TNFR1, tumour necrosis factor receptor 1; TRAPS, tumour necrosis factor-associated periodic syndrome; XBP1, X-box-binding protein 1. *The abnormal folding and accumulation of proteins regulating inflammation can lead to different degrees of cellular stress. The effect of mutations in pyrin and NLRP3 is primarily local; HLA-B27 and XBP1 deficiency can activate the unfolded-protein response associated with toxicity and enhanced inflammatory responses. ‡Diseases are listed in order of an association with increasing levels of cellular stress.

unfolded proteins[84]. Conditional knockouts of *Xbp1* in intestinal epithelial cells make mice hypersensitive to colitis induced by dextran sulphate sodium (DSS), and also cause spontaneous ileal inflammation that is probably due to the loss of secretory Paneth cells and goblet cells in the intestinal epithelia[85]. Paneth cells secrete antimicrobial peptides and IL-1 in response to bacteria, and the loss of these cells may impair host defence against commensal organisms and result in inflammatory bowel disease (IBD). *XBP1* polymorphisms were found to be a genetic risk factor for ulcerative colitis and Crohn's disease, suggesting that these findings may be relevant for human IBD[85]. However, deletion of *Xbp1* in macrophages reduces their ability to produce sustained levels of pro-inflammatory cytokines in response to TLR agonists, and thus prevents them from clearing bacterial infection[13]. Similarly, in B cells, an intact UPR is required for plasma cell differentiation, and XBP1-deficient B cells die when stimulated to become plasma cells[86].

Although a complete deficiency of proteasome or UPR-associated proteins is likely to be lethal in humans, loss-of-function mutations in *PSMB8* — which encodes a subunit of the immunoproteasome — have been recently identified as the cause of a spectrum of systemic autoinflammatory conditions that are associated with lipodystrophy[19–22]. In addition to its role in antigen presentation, the immunoproteasome has recently been shown to mediate the decay of short-lived proteins in the secretory pathway[87]. The mechanism by which this occurs is not clear, although the incomplete clearance of proteins by the immunoproteasome that occurs when PSMB8 is absent results in the intracellular accumulation of ubiquitylated proteins, which enhances inflammatory responses.

Implications for therapy

Taken together, the molecular abnormalities that connect protein misfolding to autoinflammatory disease can be seen as a spectrum of disorders, in which increasing levels of cellular stress trigger commensurate pathological consequences (TABLE 2). In CAPS, *NLRP3* mutations lead to hyperactivation of the inflammasome and the production of IL-1, probably because of enhanced oligomerization of NLRP3. In TRAPS, mutations more severely affect the TNFR1 protein, causing trafficking abnormalities and abrogating the normal signalling function of TNFR1 as a TNF receptor. In diseases that are linked to the abundantly expressed MHC class I genes, misfolded proteins may accumulate over the threshold to trigger the UPR and the induction of chaperone gene expression. In spondyloarthropathies, the UPR can cause the dysregulation of cytokine production. In the extreme case of XBP1 deficiency, pathology results from the consequences of a lack of appropriate cellular responses to ER stress.

Therapeutic strategies aimed at the problems of protein misfolding and its consequences for disease are being actively pursued. Chemical chaperones and drugs that stimulate autophagy may be of some benefit in this regard. One of the consequences of misfolding of certain ER-synthesized proteins is activation of the UPR. Pharmacological targeting of the signalling molecules that mediate this response is possible, and enhancing UPR activation might be a viable strategy to improve protein homeostasis. However, the UPR is a proverbial double-edged sword in that its activation in innate immune cells may exacerbate inflammation and disease manifestations.

Targeting cytokines that are produced in genetic autoinflammatory syndromes has been a very fruitful area of clinical investigation. The degree of success with these therapies has shed light on the importance

of the targeted cytokines in the pathological process. For example, therapies targeting IL-1 have been highly effective in suppressing most symptoms and halting the progression of organ damage in patients with CAPS[88,89,90], whereas TNF blockade is only partially effective in patients with TRAPS[57]. These successes have inspired therapeutic trials in more common diseases, particularly for IL-1 blockade[91]. In diseases such as gout in which there is a clear role for the NLRP3 inflammasome in disease pathogenesis, therapeutic trials of IL-1β inhibition combined with uric acid-lowering therapy have been successful in reducing symptoms in refractory disease and preventing flares[92,93]. In type 2 diabetes, the efficacy of IL-1 blockade has sparked interest in the role of the inflammasome in pancreatic β-cell dysfunction[94,95]. Remarkably, in both gout and type 2 diabetes, the anti-inflammatory effects of IL-1 blockade appeared to persist long after the withdrawal of therapy. In systemic-onset juvenille inflammatory arthritis, analyses of the profiles of gene expression and cytokine production have suggested a role for IL-1β, and pilot trials of IL-1 blockade have been moderately successful in this paediatric inflammatory syndrome[96,97]. Gene expression profiling and analyses of the cytokines produced by peripheral blood cells may allow for the identification of particular pro-inflammatory cytokines that drive other, more common diseases. It is likely that this will lead to more therapeutic trials of cytokine blockade, shed light on the pathogenesis of other inflammatory diseases and hopefully lead to better treatments. With a large number of anti-cytokine biological therapies and small molecules targeting Janus kinases (JAKs) approved for clinical use and in late-stage trials, testing the role of specific cytokines in human disease through clinical investigational trials will become an increasingly common way of determining the relevance of autoinflammation to human disease.

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Competing interests statement
The authors declare no competing financial interests.

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