The unfolded protein response in immunity and inflammation

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Abstract

The unfolded protein response (UPR) is a highly conserved pathway that allows the cell to cope with endoplasmic reticulum (ER) stress imposed by the secretory demands associated with environmental forces. In this role, the UPR has increasingly been shown to have crucial functions in immunity and inflammation. In this Review, we discuss the importance of the UPR in the development and differentiation of immune cells, its role in immune cell function and its crucial role in the ability of immune cells to survive and thus meet the needs associated with an immune response. In addition, we review current insights into how the UPR is

involved in complex chronic inflammatory diseases and, through its role in immune regulation, anti-tumour responses.

Introduction

The endoplasmic reticulum (ER) is an extensive tubular-reticular network, separated from the surrounding cytosol by a single lipid bilayer, the ER membrane. It is a crucial site involved in maintaining Ca^{2+} homeostasis and its major function is the synthesis and folding of secreted and transmembrane proteins, which constitute approximately one third of all proteins made in the cell.[1,2] Following translation on ER membrane-associated ribosomes, proteins enter the ER lumen where chaperone-based folding occurs, together with complex protein modifications. These include N-linked glycosylation, disulfide bond formation and proline *cis-trans* isomerization, which are mediated by glycosyltransferases, oxido-reductases and peptidyl-prolyl *cis-trans* isomerases, respectively.[3-6]

Adequate folding and posttranslational modifications of proteins crucial for proper function; furthermore, in a dominant manner, misfolded and aggregated proteins can cause cellular stress and cell death, as exemplified by neurodegeneration and other protein misfolding diseases.[7,8] It is therefore of crucial importance that protein folding is subject to stringent quality control systems to allow a cell to carry out its necessary secretory functions. As an example, ER-associated degradation (ERAD) **[G]** ensures that mis- and unfolded proteins are removed from the ER lumen to the cytosol for subsequent degradation by the ubiquitin–proteasome system.[9]

Numerous environmental conditions, both endogenous and exogenous, can disrupt the ER protein-folding environment, and when protein folding requirements exceed the processing capacity of the ER, mis- and unfolded proteins accumulate in the ER lumen, triggering the unfolded protein response (UPR) **[G]**. The UPR is a sophisticated collection of intracellular signaling pathways that have evolved to respond to protein misfolding in the ER. In addition, it has become increasingly clear that UPR signaling has an important role in immunity and inflammation. In this Review, we discuss the role of UPR activation in the development of immune cells and how the UPR is involved in building efficient immune responses. In addition, we highlight causes of UPR activation that are directly linked to inflammation and review current insights into the downstream pathways by which UPR activation induces inflammation. Lastly, we discuss how the UPR is involved in various prevalent diseases, including inflammatory bowel disease, metabolic disease and cancer. Due to the breadth of this Review, we do not provide significant detail for individual sections but have attempted to provide an overview of the most relevant and recent findings.

[H1] The unfolded protein response pathway

In metazoans, the UPR is activated by the coordinate action of three ER transmembrane stress sensors: inositol-requiring enzyme 1α (IRE1α), PKR-like ER kinase (PERK) and activating transcription factor 6α (ATF6α). Under homeostatic conditions, the luminal domains of these ER stress sensors are retained in an inactive state through association with binding immunoglobulin protein (BiP; also

known as GRP78 and HSPA5). However, due to its higher affinity for misfolded proteins, BiP dissociates from the ER stress sensors as misfolded proteins accumulate, thereby releasing the stress sensors to permit downstream signaling **(Figure1)**.[10] In addition, it has been elegantly shown that, at least in *Saccharomyces cerevisiae,* basic and hydrophobic residues on unfolded proteins can bind directly to a putative peptide groove of IRE1α and direct binding of unfolded proteins is sufficient to induce the UPR.[11] At present it is unclear whether direct binding of unfolded proteins as a second mechanism of UPR activation is limited to IRE1α, or can also be observed with PERK and ATF6α. These mechanisms require further characterization.

The downstream transcriptional programmes of the UPR are primarily directed at restoring proteostasis, which is achieved by at least four different strategies. First, mRNA translation is transiently attenuated through PERK-dependent phosphorylation of the alpha subunit of eukaryotic translation initiation factor (eIF2α) which inhibits assembly of the eIF2-GTP-Met-tRNA ternary complexes (eIF2-TC), and thereby reduces the quantity of proteins that enter the ER, Second, entry of newly translated proteins into the ER is decreased by degradation of ER membrane-associated mRNAs by regulated IRE1α-dependent decay (RIDD).[12-14] Third, processes that eliminate unfolded proteins from the ER are induced, by increasing the transcription of ERAD- and autophagy-related proteins (see also **BOX 1**). Finally, genes are induced that increase protein folding capacity and expansion of the ER through increased biogenesis of ER and lipid components.[1,15]

However, when these attempts to restore proteostasis fail and ER stress is unabated, UPR signaling typically switches to a pro-apoptotic mode, a process that is also referred to as the terminal UPR [reviewed in REF 16].[16] The terminal UPR may have evolved to eliminate excessively damaged or pathogen-infected cells, for example.

*[H3] IRE1*α.

The cytoplasmic tail of the type I transmembrane protein IRE1α possesses two enzymatic activities: a serine/threonine kinase domain and an endoribonuclease (RNase) domain.[17] Upon release from BiP, dimerization of IRE1α elicits RNase activity to initiate the non-conventional splicing of a single mRNA encoding X-box binding protein 1 (XBP1) to produce a translational frameshift and create a potent transcriptional activator known as XBP1s ('s' for spliced). [18] [19] XBP1s translocates to the nucleus where it induces the transcription of a wide variety of ER-resident molecular chaperones and protein-folding enzymes that together expand ER size and function **(Figure 1A)**.[15,20] In addition to its specific endoribonuclease activity that splices *Xbp1*, IRE1α-dependent activation of RIDD is proposed to degrade ER-membrane-associated mRNAs to reduce the amount of protein entering the secretory pathway **(Figure 1A)**.[21] In addition, emerging evidence indicates that RIDD substrates encode proteins of diverse nature, which modulate cellular processes that are distinct from the control of ER homeostasis, including the

generation of inflammatory double-stranded RNA species through activation of intracellular nucleic acid sensors.[22,23]

During prolonged ER stress, the beneficial effects of IRE1α activation through the activity of XBP1s may be temporally and quantitatively impeded, while the PERK pathway becomes dominant.[24,25] In addition, chronic ER stress increases the oligomerization state of IRE1α to hyperactivate its cytosolic RNase domains, which leads to cleavage of many other RNAs besides *Xbp1*, including precursors of apoptosis-inhibitory microRNAs, which thus promotes apoptosis.[26-28] Therefore, prolonged ER stress may tend to tip the properties of IRE1α from being adaptive to promoting inflammation and cell death.

[H3] PERK.

Similarly to IRE1α, dissociation of BiP from the ER luminal domain of PERK permits PERK homodimerization and autophosphorylation to activate the cytoplasmic kinase domain. In addition, it was shown that the lipid composition of the ER membrane can also activate PERK, which highlights the significance of lipid metabolism as a direct trigger of UPR activation.[29,30] Activated PERK phosphorylates eIF2α which inhibits eIF2-TC formation and thereby transiently attenuates global mRNA translation, enabling the cell to cope with temporary ER stress **(Figure 1B)**. Of note, eIF2α can be phosphorylated in mammals independently of ER stress, by three additional kinases: general control nonderepressible 2 (GCN2), which is induced by amino acid deprivation; the heme-regulated inhibitor kinase (HRI),

which is induced by oxidative stress or heme deprivation; and the protein kinase R (PKR), which is activated by double-stranded RNA as part of the interferon antiviral response.[31] Together, these additional mechanisms of eIF2α phosphorylation form the integrated stress response (ISR) **[G]** system, which allows the cell to integrate multiple stress stimuli into one common node, that being the general control of protein synthesis through phosphorylation of eIF2α.[31] As an example, both epithelial cells and dendritic cells activate GCN2 to phosphorylate eIF2α in response to amino acid deprivation, to induce autophagy, reduce oxidative stress and inhibit inflammasome activation.[32]

While translation of most RNAs is inhibited in ER stressed cells, translation of some species of mRNA is favoured under ER stress conditions, when eIF2α is phosphorylated and eIF2-TC availability is low (mechanisms reviewed in reference 33).[33,34] One important example is the mRNA encoding activating transcription factor 4 (ATF4; also known as CREB2), a crucial UPR mediator that transactivates genes involved in amino acid metabolism and oxidative stress resistance, as well as autophagy **(Figure 1B)**.[12] As a sustained translational block is not compatible with cell survival, ATF4 also induces *Ppp1r15a* expression, which encodes GADD34, a regulatory subunit of protein phosphatase 1 (PP1) that directs the dephosphorylation of eIF2α to restore mRNA translation **(Figure 1B)**. However, ATF4 also activates transcription of C/EBP homologous protein (CHOP),[12,35] which is involved in ER-stress-mediated apoptosis both *in vitro* and *in vivo.*[36,37] To allow the cell a chance to cope with the ER stress, several mechanisms suppress CHOP at

early times after ER stress, such as PERK-dependent miR-211 expression, which represses *CHOP* transcription through histone methylation.[38] In addition, CHOP is suppressed by Toll-like receptor (TLR) signaling during immune responses in macrophages by protein phosphatase 2A (PP2A)-mediated serine dephosphorylation of the eIF2Bε subunit.[39] Indeed, only strong and chronic activation of PERK increase steady-state levels of CHOP, due to the short half-life of both ATF4 and CHOP mRNAs and proteins, so that only excessive ER stress will promote the terminal UPR.[40] Studies of ATF4 and CHOP in cells experiencing chronic ER stress indicate that they function together as a heterodimer to induce apoptosis by increasing protein synthesis, thereby enhancing protein misfolding and oxidative stress and cell death.[41]

[H3] ATF6.

ATF6 is a type II transmembrane protein that contains a bZIP transcription factor within its cytosolic domain. Although there are two *ATF6* genes in the mammalian genome (*ATF6A* and *ATF6B*), only ATF6α is required to activate UPR gene expression.[42] Upon release from BiP, ATF6α transits to the Golgi compartment where it is processed by the Golgi enzymes site 1 protease (S1P) and S2P to produce a cytosolic p50 fragment that migrates to the nucleus. The p50 fragment activates expression of genes that encode functions to increase ER capacity and folding (including *BiP*, *Grp94*, *P58^{IPK}* and *Xbp1*) and the ERAD pathway **(Figure 1C)**.[19,42-44]

[H1] The physiological UPR in immune cell differentiation and function

Cells that have a large secretory demand as part of their function are particularly dependent on a well-developed and large ER and, consequently, UPR signaling. It is therefore not surprising that highly secretory immune cells are highly susceptible to environmental conditions that impose ER stress, either by directly targeting ER folding capacity (for example, microbial toxins)[45] or markedly increasing folding demand (for example, exposure to pathogens). Thus, inflammation *per se* is an important factor in ER stress induction, and UPR activation may be a sensitive hallmark of inflammation. In addition to the pathophysiological conditions that affect specific cell types, it is now well established that activation of the UPR has a role in a wide range of physiological events associated with immunologically important cell types.[46] Furthermore, although they have not been well studied, germline polymorphisms or, potentially, somatically generated mutations may affect the ability of the host to determine whether an appropriate UPR level is achieved based upon the demand. Understanding how the UPR affects specific functions at the cellular level and the host-related factors that affect this are therefore of great importance, as considered below.

[H3] B cells and plasma cells

Over the past 15 years, the role of the transcription factor XBP1 in B cell and plasma cell differentiation has become increasingly clear and has served as a paradigm for numerous secretory systems and their relationships to immune function and inflammation. Plasma cell differentiation is regulated by the transcription factors interferon-regulatory factor 4 (IRF4) and B lymphocyte-induced maturation protein

1 (BLIMP1).[47,48] In addition, induction of *Xbp1*, which is downstream of BLIMP1, is required for the marked expansion of the ER and increased protein synthesis that are necessary for high levels of antibody production and secretion during a physiological or pathological immune response **(Figure 2A)**.[49,50] Indeed, XBP1 deficiency in B cells leads to an absence of plasma cells and markedly reduces circulating antibody levels, but has no effect on B cell maturation or isotype switching.[51] Although it was initially assumed that XBP1 induction was caused by increased immunoglobulin synthesis and the accumulation of unfolded immunoglobulin heavy chains,[52] subsequent studies have shown that ER expansion is evident before the onset of immunoglobulin synthesis[53] and *Xbp1* is similarly spliced when IgM secretion is genetically abrogated.[54] This suggests that XBP1 induction is a differentiation-dependent event in plasma cells, rather than a response to increased immunoglobulin secretion. The factors that drive UPR activation early during plasma cell development are incompletely understood. Of note, part of the decrease in immunoglobulin production in XBP1-deficient B cells was later explained by IRE1α hyperactivation leading to increased RIDD and the subsequent degradation of immunoglobulin μ heavy-chain mRNAs.[55]

In addition to plasma cells, IRE1α activation is also observed in pro-B cells.[56] UPR activation in these early stages of B cell development might result from increased secretory demand caused by neo-expression of cell surface proteins associated with V(D)J antigen receptor rearrangements.[57] Indeed, a recent study shows that IRE1α and XBP1s are important during the pre-B cell stage when immunoglobulin heavy

chains are expressed for the first time and, importantly, that XBP1s in pre-B acute lymphoblastic leukemia (ALL) cells provides a survival benefit for tumour cells.[58]

[H3] T cells

Studies with XBP1s-GFP reporter mice have shown that IRE1α is activated in $CD4^+CD8^+$ thymic T cells and in $CD8^+$ splenic T cells, although the precise role of the UPR in early T cell development is not clear.[56] However, activation of the IRE1α–XBP1 axis in effector $CD8^+$ T cells was reported in response to acute infection with *Listeria monocytogenes*, which was associated with expression of high levels of killer cell lectin-like receptor G1 (KLRG1). This suggests that XBP1 is important for the terminal differentiation of effector $CD8^+$ T cells.[59]

[H3] Dendritic cells

Xbp1 is constitutively spliced in dendritic cells (DCs), and loss of XBP1 leads to significantly reduced numbers of both conventional and plasmacytoid DCs. **(Figure 2B, left panel)**.[60] In addition, XBP1-deficient DCs have increased rates of apoptosis and are resistant to survival signals associated with TLR engagement **(Figure 2B, left panel)**. Interestingly, ectopic expression of XBP1s in XBP1-deficient $FLT3^+$ hematopoietic progenitor cells can rescue this apoptotic phenotype.[60] Taken together, this study shows that XBP1 is important for DC development and survival.

Consistent with the findings above, constitutive activation of IRE1α is observed in DCs[61] using ER stress-activated indicator (ERAI) reporter mice.[62] Interestingly, although the highest levels of IRE1α activity are observed in $CD8\alpha^+$ DCs, deletion of

XBP1 in DCs using CD11c-directed *Cre* does not result in developmental and phenotypic abnormalities of CD8α⁺ DCs. This different result compared with the study of Iwakoshi *et al*.[60] may be explained by low levels of expression of CD11c, and hence *Cre* recombinase activity, in DC progenitor cells. Interestingly, however, XBP1-deficient CD8α⁺ DCs generated by CD11c-*Cre*-mediated deletion of *Xbp1* have RIDD-dependent defects in cross-presentation to OT-I T cells, owing to degradation of components of the cross-presentation machinery such as tapasin.[61] This study shows that the IRE1α–XBP1 arm of the UPR is crucially involved in DC function. **(Figure 2B, right panel)**.

Consistent with a role for ER stress in regulating antigen presentation, several other studies have shown that ER stress affects the surface expression of MHC class I molecules by mechanisms that are not fully elucidated as yet. [63,64] In ovarian cancer, impaired MHC class I-restricted antigen presentation to CD8⁺ T cells has been linked to reactive oxygen species (ROS)-induced ER stress and increased lipid metabolism in DCs induced by an unknown transmissible stress factor in the tumor microenvironment. While the concept of lipid accumulation-dependent dysfunction of DC antigen presentation had been described previously,[65] this study links ER stress-induced *Xbp1* splicing to lipid accumulation in DCs, resulting in DC antigen presentation defects **(Figure 2B, right panel)**.[66]. **[Note: we felt that this pathway should also be stressed in Figure 2, as it shows that also increased XBP1s can result in cross presentation defects]** In accordance with this, deletion of XBP1 in DCs abolished the accumulation of lipid in DCs and increased T cell anti-tumour immunity, resulting in decreased tumour burden and improved survival.[66]

[H3] Granulocytes

Granulocytes are represented by neutrophils, basophils and eosinophils. Eosinophils are typically associated with type II immune responses, allergy and parasitic infections.[67] Recently, it has also been shown that eosinophils might have a role in maintaining immune homeostasis in the intestine by promoting IgA class switching in Peyer's patches and controlling the pool of $CD103^+$ T cells and DCs.[68] Interestingly, amongst all types of granulocyte, eosinophils are uniquely dependent upon XBP1 in that haematopoietic deletion of *Xbp1* (*Xbp1*^{Vav1} mice) leads to a loss of fully mature eosinophils. In addition, deletion of *Xbp1* specifically in eosinophils using *Epx*-Cre-mediated *Xbp1* deletion results in a significantly smaller bone marrow eosinophil pool, which indicates that XBP1 is also needed to sustain the viability of eosinophil-committed progenitor cells.[69]

[H3] Macrophages

In macrophages, TLR signaling induces ER stress, and ER stress, in turn, amplifies the response to TLR ligation. For example, TLR2 and TLR4 ligands Pam_3CSK_4 and lipopolysaccharide (LPS), respectively, induce IRE1α activation in mouse J774 macrophages. Furthermore, treatment with TLR agonists increases *Xbp1* splicing, which is dependent on TNF receptor-associated factor 6 (TRAF6) recruitment to IREα and requires the NAPDH oxidase NOX2.[70] Importantly, IRE1α-induced *Xbp1* splicing in response to TLR ligation has been shown to be crucial for cytokine production as macrophage-specific deficiency of XBP1 impairs the production of

interleukin-6 (IL-6), tumor necrosis factor (TNF) and interferon-β (IFNβ) **(Figure 3A)**.[70]

TLR signaling also amplifies IRE1α signaling by modulating its phosphorylation status. In the absence of TLR signaling, phosphorylated IRE1α is dephosphorylated and thus inactivated by PP2A. Upon TLR ligation, however TRAF6, interaction with IRE1α catalyzes its ubiquitylation, which prevents the interaction of IRE1α with PP2A through its adaptor receptor for activated C kinase 1 (RACK1) to avoid IRE1α dephosphorylation and inactivation **(Figure 3B)**.[71,72] By contrast, induction of ER stress in bone marrow-derived macrophages strongly potentiates LPS-induced pro-inflammatory signaling, including the induction of genes encoding CXCL1, CXCL2, TNF, IL-1α and IL-6, in a pathway dependent on receptor-interacting serine/threonine-protein kinase 1 (RIPK1).[73]

The IRE1α–XBP1 pathway has also been linked to increased IL-1β production through IRE1α-dependent activation of glycogen synthase kinase 3β (GSK3β). In addition, GSK3β inhibits further *Xbp1* slicing and thereby *Tnf* transcription, which modulates the inflammatory response to ER stress and potentially TLR signaling **(Figure 3C)**.[74]

The PERK pathway has been shown to induce inflammation through direct binding of ATF4 to the *Il6* promotor.[75] In macrophages, however, TLR signaling inhibits translation of *Atf4* mRNA and thereby its downstream target CHOP.[76] Similarly, CHOP is further suppressed by TLR–TRIF signaling in macrophages through PP2A-mediated serine dephosphorylation of the eIF2Bε subunit.[39] These mechanisms of

CHOP suppression are required for macrophage survival during an immune responses. This demonstrates that specific control of the different arms of the UPR is crucial for innate immune function **(Figure 3A)**.[70]

[H3] Paneth cells.

Conditional deletion of *Xbp1* in intestinal epithelial cells (IECs) has shown that Paneth cells **[G]**, which are an important source of anti-microbial peptides and stem cell survival signals,[77] and to a lesser extent goblet cells that are dedicated to mucus production, are highly dependent on this UPR transcription factor.[78] Paneth cells in mice with specific deletion of XBP1 in IECs are severely hypomorphic. The resultant decreased antimicrobial function has been shown to be associated with attenuated killing of *L. monocytogenes* and increased bacterial translocation to the liver. In addition, IRE1α hyperactivation leading to phosphorylation of JNK is also observed in XBP1-deficient intestinal epithelium, resulting in spontaneous superficial small intestinal enteritis **(Figure 4)** .[78] Moreover, deletion of *Xbp1* specifically in Paneth cells is sufficient to induce small intestinal enteritis.[79] As the spontaneous inflammation observed in the setting of IEC-specific deletion of *Xbp1* is reversed under germ-free conditions, these studies indicate that the UPR in IECs and, particularly, in Paneth cells is crucial to the maintenance of intestinal homeostasis in the presence of microbial commensalism. Whether XBP1 has a crucial role in the development of Paneth cells, as in highly secretory haematopoietic cells, is not fully clear.

[H1] 'Pathological' causes of UPR activation

It is clear that the UPR has a crucial function under physiological conditions, particularly during periods of high secretory demand, as shown by the loss of numerous haematopoietic cells (such as dendritic cells, eosinophils and plasma cells) and parenchymal cells (such as hepatocytes, pancreatic β cells, acinar cells of the salivary glands and Paneth cells) when critical elements of the UPR are absent.[80] In addition to this physiological UPR, ER stress can further result from various intrinsic and environmental factors, such as inadequate energy supply (as observed during hypoxia and nutrient deprivation) and increased secretory demand during exposure to pathogens and inflammatory stimuli.[81] These environmental challenges induce UPR activation and thereby contribute to inflammation through various mechanisms.

[H3] Hypoxia

Hypoxia inhibits oxygen-dependent protein folding processes, which directly affects disulfide bond formation during post-translational folding of proteins or *cis-trans* prolyl isomerization in the ER,[82] leading to ER stress. Hypoxia can occur in adipose tissue in the setting of obesity, probably as a result of the diffusion limit of oxygen imposed by the hypertrophic adipocytes. Adipocyte hypoxia has been shown to be associated with the induction of ER stress and increased expression of CHOP, which is at least partly responsible for the reduced expression of adipocytokines that provide important anti-inflammatory functions.[83] This raises the possibility that hypoxia-induced ER stress may be linked to obesity-related metabolic diseases.

Hypoxia, due to inefficient vascularization, is also a hallmark of the tumour microenvironment and is one of the factors that accounts for UPR activation in solid tumours. UPR activation in tumours triggers protective responses that enable the tumour to cope with conditions of low oxygen and nutrient supply.[84] Splicing of *XBP1* mRNA was recently shown to provide a survival benefit for highly aggressive triple negative breast cancer cells. Inhibition of *XBP1* in these tumour cells using RNA interference decreased tumour growth and particularly affected angiogenesis, which links XBP1 to tumour hypoxia. Moreover, it was shown that XBP1 directly interacts with the central mediator of the cellular response to hypoxia, hypoxia-inducing factor 1α (HIF1α), and assists in regulating the expression of HIF1α target genes.[85] As HIF1α also has a prominent role in the anti-tumour immune response by inhibiting the effector functions of tumour-infiltrating lymphocytes,[86] targeting the UPR and HIF1α as connected pathways in solid tumours might provide a therapeutic benefit in boosting anti-tumour immune responses.

[H3] Reactive oxygen species

The oxidizing environment of the ER is optimized for disulfide bond formation and the redox status of the ER is tightly regulated in mammalian cells.[87] This environment is primarily derived from ROS generated by endoplasmic reticulum oxireductin 1 (ERO1), with protein disulfide isomerase being responsible for disulfide bond formation. However, cellular ROS production increases significantly and to supraphysiological levels under ER stress and inflammatory conditions. These conditions are closely connected in a reciprocal manner such that they induce

self-reinforcing pathways that may further promote inflammation.[4,88] ROS can be induced by a wide variety of immunological signals. These include, for example, TLR and TNF receptor signaling, which further trigger inflammatory responses by enhancing the phosphorylation of IκB to induce nuclear factor-κB (NF-κB) signaling.[89] Conversely, it has been shown that extracellular sources of ROS can induce ER stress, possibly by disrupting Ca^{2+} retention in the ER.[90] Once they are produced, ROS can also promote NLRP3 inflammasome activation[91] and regulate lymphocyte function[89] and, as discussed above, NOX2-derived ROS in macrophages are required for XBP1-dependent cytokine production upon TLR2 and TLR4 ligation.

In mouse models of diabetes, ER stress and ROS production have recently been linked to inflammasome activation and pancreatic β-cell death in a pathway involving the induction of thioredoxin-interacting protein (TXNIP). This protein interacts with thioredoxin and reduces its anti-oxidant effect. Two recent studies have shown that ER stress-induced inflammasome activation and pancreatic β-cell apoptosis were preceded by increased *Txnip* mRNA expression through a pathway involving IRE1α[27] and/or PERK.[92] As a consequence, genetic deletion of TXNIP partly protects against diabetes progression.[27]

[H3] Pathogens

Many pathogens interfere with the function of host ER as part of their infectious life cycle, and therefore can activate distinct arms of the UPR. Pathogen-induced UPR

activation can be beneficial to the host by functioning to enable an innate immune response directed against invading pathogens. However, invading pathogens can selectively modulate UPR pathways to promote their own survival. [93]

Viral replication requires the host ER for the production of viral structural and non-structural proteins and as such interferes with the host protein synthesis machinery.[94] Unsurprisingly, viral infection can trigger the UPR (excellently reviewed in REF 95).[95] The IRE1α-mediated arm of the UPR can block viral replication in the case of respiratory syncytial virus (RSV), probably through RIDD-dependent viral RNA degradation, as XBP1 is not required for defence against RSV. [96] However, some viruses, such as Japanese encephalitis virus, use RIDD to their advantage to degrade host RNAs without affecting viral RNA.[97] Similarly, many viruses have evolved mechanisms to circumvent the disadvantageous consequences of the UPR; selective activation of the ATF6α- and IRE1α-mediated arms of the UPR sustains viral replication by increasing the production of ER chaperones, while at the same time viruses block PERK-mediated UPR activation to circumvent a translational block and/or ER stress-induced apoptosis. For example, herpes simplex virus type 1 selectively blocks activation of the PERK-mediated arm of the UPR by association of viral glycoprotein gB with the luminal domain of PERK.[98] Also, whereas hepatitis C virus (HCV) induces IRE1α and ATF6α activation, the virus suppresses the downstream effects of XBP1s to prevent activation of ERAD, which enables HCV replication and contributes to persistence of the virus in infected hepatocytes.[99,100]

An important example of how the UPR protects the host from bacterial infection comes from studies in *Caenorhabditis elegans*. In this nematode, infection with *Pseudomonas aeruginosa* induces a p38 MAPK-driven innate immune response, which in turn triggers the IRE1α–XBP1 pathway of the UPR. XBP1 activation is protective for the host as *xbp1*-mutant larvae exhibit ER disruption and increased lethality in response to infection. Interestingly, lethality could also be prevented by loss of p38 MAPK, which shows that it is not the infection itself but rather the innate immune response that is lethal in the absence of a simultaneous protective XBP1-dependent UPR response.[101]

A recent study in primary bronchial epithelial cells further supports the link between an innate immune response and the UPR by showing that virulence factors derived from *P. aeruginosa* strongly induce UPR activation in a p38 MAPK-dependent manner, as evidenced by *Xbp1* splicing and the induction of BiP and CHOP. However, induction of GADD34 occurred through activation of the ISR, which was associated with improved survival of host cells.[102]

L. monocytogenes has a well-documented ability to induce the UPR, which was recently shown to occur without active infection of cells by secretion of the cytolysin listeriolysin O. This toxin induces all branches of the UPR, possibly by altering intracellular Ca^{2+} homeostasis, which leads ultimately to apoptosis.[103] Similarly, the AB_5 subtilase cytotoxin from Shiga-toxigenic *Escherichia coli* cleaves BiP, thus inducing all three arms of the UPR and resulting in ER stress-induced cell death.[104]

Pathogen associated molecular patterns may also activate specific arms of the UPR. As an example, *Streptomyces* sp. produce a toxin (trierixin) that directly inhibits

Xbp1 splicing.[45] By contrast, TLR2 and TLR4 ligands trigger the IRE1α–XBP1 arm of the UPR in macrophages while suppressing ATF4–CHOP signaling to promote macrophage survival during infections.[70,76] Thus, pathogens can either subvert or induce the UPR during their life cycles, which in the latter case may alert the immune system to their presence.

[H3] Cell damage and immunogenic cell death

Dying cells can elicit inflammatory responses through the induction of cell surface receptors that are recognized by immune cells, such as the ER-resident protein calreticulin (CRT), or through secreted factors including high mobility group box 1 (HMGB1) and ATP.[105,106] HMGB1 activates all arms of the UPR in endothelial cells, resulting in increased expression of intercellular adhesion molecule 1 (ICAM1) and P-selectin, which probably function to attract immune cells to dying cells.[107] In splenic DCs, HMGB1 exposure induces UPR activation with increased levels of BiP and *Xbp1* splicing; this pathway seems to be important in mounting an immune response, as silencing *Xbp1* is associated with downregulation of the costimulatory cell surface receptors CD80 and CD86, decreased MHC class II expression and, subsequently, impaired stimulation of T cells in co-culture systems.[108]

Even before cells die and intracellular components such as HMGB1 are released to elicit inflammation, stressed cells induce immune responses; for example, the cell surface expression of CRT is a recognition signal for engulfment by antigen-presenting cells. Human bladder carcinoma T24 cells exposed to photodynamic therapy, which causes ROS-mediated induction of ER stress, increase their cell

surface expression of CRT and secretion of ATP through PERK-orchestrated pathways.[109] Interestingly, this was associated with increased engulfment of cancer cells by DCs and increased DC expression of CD80, CD86 and MHC class II, as well as increased IL-1β and nitric oxide production, all of which are important for an effective antitumor $CD8^+$ T cell response.[109] Increased cell surface expression of CRT also occurs in a PERK-dependent manner in cancer cells that have nonphysiological increases in chromosome content (known as 'hyperploidy', which occurs in the early stages of various cancers), and is an important immunosurveillance system against hyperploidy in cancers.[110]

Whether UPR activation can drive the specific upregulation of ligands recognized by innate immune cells, including natural killer cells, requires further research. Interestingly, CHOP has been linked to cell death in HCT116 cells through upregulation of death receptor 5 (DR5), a receptor for TNF-related apoptosis-inducing ligand (TRAIL). However in this setting, DR5 drives apoptosis in a ligand-independent manner through activation of caspase-8..[111] In addition, it is important to recognize that although UPR activation in tumour cells can sometimes increase their immunogenicity and is therefore beneficial for the host, some tumours also take advantage of the UPR to prevent anti-tumour immune responses. **[BOX 2]**. Elucidating the crosstalk between UPR activation in tumour cells, the release of damage-associated molecules, cell surface receptor expression and immune activation is of great importance to improve anti-tumour immune responses.

[H1] The UPR as an inflammatory nidus

ER stress is implicated in various chronic pathological conditions involving inflammation (such as metabolic diseases, inflammatory bowel diseases, atherosclerosis and neurodegenerative diseases, amongst others). Investigation of the pathogenic mechanisms involved reveals a reciprocal regulation between ER stress and inflammation — whereby ER stress can directly initiate inflammatory pathways and, in turn, pro-inflammatory stimuli such as ROS, TLR ligands and cytokines trigger ER stress — such that the resulting UPR activation can further amplify inflammatory responses.[112]

[H3] ER stress-induced inflammatory signaling

NF-κB, a master transcriptional regulator of proinflammatory pathways, is activated by the interaction of IRE1α with TRAF2 in response to ER stress, which leads to the recruitment of IκB kinase (IKK) and the phosphorylation and subsequent degradation of IκB. This releases NF-κB for translocation to the nucleus **(Figure 5)**.[113] The PERK–eIF2α- and ATF6-mediated branches of the UPR activate NF-κB through different mechanisms to IRE1α. Engaging the PERK–eIF2α signaling pathway in response to ER stress halts overall protein synthesis and increases the ratio of NF-κB to IκB, owing to the shorter half-life of IκB, thereby favoring NF-κB-dependent transcription **(Figure 5)**.[114,115] ATF6α activation through exposure to the bacterial subtilase cytotoxin can induce phosphorylation of AKT to activate NF-κB **(Figure 5)**.[116,117]

JNK, together with p38 and ERK, is one of the stress-inducible MAPKs that mediate a wide variety of responses, including but not limited to proliferation, autophagy and

inflammation. The IRE1α–TRAF2 complex can, in addition to the activation of NF-κB, recruit apoptosis signal-regulating kinase 1 and subsequently activate JNK, leading to the increased expression of proinflammatory genes through enhanced activator protein 1 activity **(Figure 5)**.[118,119]Loss of XBP1 in small IECs results in hyperactivation of IRE1α, which drives inflammatory responses, including cytokine production, that are in part mediated by phosphorylation of JNK.[78] ER stress in human cancer cell lines has also been shown to trigger ERK activation through PI3K, which was associated with increased resistance to ER stress-induced cell death[120]

UPR activation in immune cells and various stromal cells leads to the induction and secretion of various cytokines, such as IL-6 and TNF.[121,122] In macrophages, XBP1s directly binds the *Tnf* and *Il6* promoters to regulate their expression,[70] and ATF4, downstream of PERK activation, functions as a transcription factor for *Il6* **(Figure 5)**.[75] ER stress in pancreatic β cells can trigger activation of the NLRP3 inflammasome and IL-1β secretion through IRE1α- and PERK-mediated induction of TXNIP **(Figure 5)**.[27]

Conversely, cytokines themselves can directly regulate the UPR. Interleukin 10, for example, can inhibit inflammation-induced ER stress by blocking shuttling of ATF6p50 to the nucleus in a pathway that involves p38 MAPK.[123] However, circulating pro-inflammatory cytokines including IL-1, IL-6, CXCL8 (also known as IL-8) and TNF, can trigger UPR activation in the liver and thereby stimulate the release of products of the acute phase response (APR) **[G]**, which amplify inflammatory responses to eliminate infection and restore tissue homeostasis. Upon ER stress in hepatocytes, ATF6α and CREBH traffic to the Golgi to undergo S1P- and

S2P-mediated cleavage; the cytosolic active transcription factors that are released induce expression of APR genes including C-reactive protein and serum amyloid P component **(Figure 5)**.[112]

[H1] The 'pathological' UPR in inflammatory disease

UPR-associated inflammatory pathways are increasingly recognized to be involved in a variety of complex inflammatory diseases. In the sections below, we discuss several examples of autoimmune, metabolic and neoplastic conditions in which the UPR may play a significant role.

[H3] Inflammatory bowel disease

Over the past decade, genome wide association studies (GWAS) have identified various susceptibility loci for Crohn disease **[G]** and ulcerative colitis **[G]**, which are chronic inflammatory diseases of the gastrointestinal tract, collectively known as inflammatory bowel disease (IBD).[124, 125] Many of these risk genes encode proteins that have an important role in proteostasis. Orosomucoid-like 3 (*ORMDL3*), a risk locus for both Crohn disease[126] and ulcerative colitis[127] (as well as interestingly asthma[128]), encodes an ER trans-membrane protein that in lung epithelial cells activates ATF6α and induces expression of SERCA2B (also known as ATP2A2), which might be associated with airway remodeling.[128] In addition, ORMDL3 represses serine palmitoyltransferase activity and thereby decreases ceramide levels, which might protect from apoptosis.[129] However, the mechanism by which ORMDL3 is involved in IBD pathogenesis is unstudied. Candidate gene approaches have also identified anterior gradient 2 (*AGR2*)[130] and *XBP1* as risk loci for Crohn

disease and ulcerative colitis. AGR2 is a member of the protein disulfide isomerase family and is particularly highly expressed by secretory cells of the intestinal tract. In line with this, *Agr2*$^{-/-}$ mice have decreased expression of mucin 2 (MUC2) in goblet cells and abnormal localization of Paneth cells in the small intestine, which coincides with UPR activation and the development of spontaneous ileocolitis.[131]

Conditional deletion of *Xbp1* in IECs causes ER stress and results in spontaneous inflammation of the small intestine and increased susceptibility to dextran sodium sulfate (DSS)-induced colitis.[78] As for AGR2 deficiency, deletion of XBP1 in IECs particularly affects secretory cells, as *Xbp1*$^{\Delta IEC}$ mice have decreased numbers of goblet cells and severely hypomorphic Paneth cells with decreased antimicrobial peptide production, which is associated with increased susceptibility to infection with *L. monocytogenes.*[78] It is not fully clear exactly how UPR activation in the AGR2-deficient and *Xbp1*$^{\Delta IEC}$ mice eventually causes intestinal inflammation. *Xbp1*$^{\Delta IEC}$ mice show signs of IRE1α-dependent JNK phosphorylation and NF-κB activation, and blockade of NF-κB activation or genetic deletion of IRE1α in IECs or systemic deletion of TNF receptor 1 protects *Xbp1*$^{\Delta IEC}$ mice from spontaneous enteritis.[79,124] Thus, IRE1α–NF-κB signaling, in a pathway that is driven by TNF, has a crucial role in the development of inflammation upon deletion of XBP1 in IECs.

As autophagy compensates for ER stress **[BOX 1]**, genetic deficiency of autophagy in *Xbp1*$^{\Delta IEC}$ mice is associated with even more severe spontaneous enteritis that extends transmurally.[79] Indeed, signs of ER stress have been detected in Paneth cells in patients with Crohn disease carrying the risk allele of autophagy-related 16-like 1

(*ATG16L1*^{T300A}).[132] Moreover, as the *ATG16L1*^{T300A} risk allele encodes a protein that is otherwise functionally intact but has increased sensitivity to caspase 3-mediated cleavage,[133,134] and as XBP1 deletion in IECs leads to caspase 3 activation,[78] it is possible that in humans environmentally and/or genetically determined ER stress can reveal the phenotypic manifestations of this common risk variant.[135] The epithelium of the small intestine may be particularly sensitive to ER stress-induced caspase 3 activation and *ATG16L1*^{T300A} cleavage as there is evidence for ER stress even under baseline, non-inflammatory conditions.[136]

In addition to IRE1α, the intestinal and lung epithelia express IRE1β (encoded by *Ern2*), particularly in goblet cells. *Ern2*$^{-/-}$ mice have increased basal levels of BiP in the intestinal epithelium, aberrant MUC2 accumulation in the ER of goblet cells[137] and increased sensitivity to DSS-induced colitis.[138]

Various other perturbations in UPR signaling have been associated with intestinal inflammation. Mice lacking CREB3L1 (also known as OASIS),[139,140] ATF6α or p58IPK (also known as DNAJC3) are more susceptible to DSS-induced colitis.[138] Furthermore, nonphosphorylatable Ser51Ala eIF2α-mutant mice have defective UPR signaling in IECs, leading to secretory dysfunction of Paneth cells and increased sensitivity to oral *Salmonella* infection and DSS-induced colitis.[141]

Forward genetic approaches using N-ethyl-N-nitrosourea mutagenesis have yielded mouse strains (named *Winnie* and *Eeyore*) that carry single missense mutations in *Muc2.* These mutations cause misfolding of MUC2 protein, which results in strong UPR activation and spontaneous ulcerative colitis-like colitis, characterized by

inflammatory responses that involve both innate and adaptive immunity (including the IL-23–T helper 17 cell inflammatory axis).[142,143]

Together, these studies show that the UPR is important in maintaining intestinal epithelial homeostasis in response to a highly complex intestinal luminal environment challenged by microrganisms and other external factors. However, intestinal inflammation can also be promoted by ER stress in the haematopoietic system of mucosal tissues, as shown by studies in HLA-B27 transgenic rats,[144] and the contributions of UPR activation in distinct cell types to intestinal inflammation requires further research.

[H3] Diabetes mellitus

Pancreatic β-cells rapidly increase protein synthesis during acute and chronic stimulation and are therefore dependent on a well-developed ER and protein quality control mechanisms. β-cell-specific deletion of IRE1α or XBP1 results in impaired β-cell proliferation, defective proinsulin synthesis and processing, and decreased insulin secretion,[145] Similarly, genetic deletion of PERK leads to ER stress-induced loss of pancreatic β-cells and a progressive decline in endocrine (but also exocrine) pancreatic function, with hyperglycaemia developing within 4 weeks,[146] which further emphasizes the central role of the UPR in pancreatic β-cell survival and diabetes. In Akita mice, the oxidative folding of proinsulin by ER-resident oxireductases, which is required to obtain the native shape of proinsulin and allow its trafficking from the ER further down the secretory pathway, is impeded by the

expression of a mutant form of proinsulin (Ins2 C96Y). This results in the accumulation of misfolded proinsulin proteins in the ER, which triggers UPR activation, β-cell inflammation and eventually β-cell death, and leads to diabetes as early as 4 weeks of age,[147,148] similarly to PERK-deficient mice.[149] Importantly, IRE1α oligomer formation, which is indicative of high and chronic ER stress, is a crucial propagator of the phenotype of Akita mice, as pharmacological inhibition of IRE1α kinase activity alleviates the disease phenotype.[26]

ER stress-induced JNK activation occurs in adipose and liver tissue of obese mice, whether induced by high fat diet or genetically through leptin deficiency (*ob/ob* mice). Obese mice develop insulin resistance through ER stress-mediated JNK-induced phosphorylation of insulin receptor substrate 1, which impairs insulin action and causes insulin resistance. However, overexpression of spliced XBP1 protected these mice from insulin resistance.[150] Importantly, lower levels of spliced XBP1 in livers of obese mice were recently shown to result from inducible nitric oxide synthase (iNOS)-dependent — and thus inflammation-dependent — S-nitrosylation of the RNase domain of IRE1α, while the kinase domain was unaffected; this resulted in decreased levels of protective XBP1s but maintained the levels of pro-inflammatory phosphorylated JNK. This study thereby demonstrates how obesity-induced inflammation can alter UPR signaling and provides a new link between inflammation, UPR activation and metabolic dysfunction.[151]

ROS production secondary to ER stress can be another trigger for inflammation as it activates the NLRP3 inflammasome and IL-1β secretion in β-cells through increased levels of TXNIP, either as a result of RIDD-dependent decay of the *Txnip*-suppressing

microRNA miR-17 or through the PERK pathway, ultimately leading to β-cell apoptosis and diabetes.[27,92] Indeed, suppression of JNK activity protects β-cells from oxidative stress and ameliorates glucose tolerance.[152] Although this is a field of extensive research, much still needs to be learned about the interplay between ER stress, inflammation and the development of metabolic diseases such as diabetes, as the UPR could be an attractive signaling pathway for therapeutic intervention.

[H3] Non-alcoholic steatohepatitis

The crucial role of the UPR in the liver was first discovered in 2000, when it was shown that XBP1 was required for liver development.[153] In the liver, the ER and UPR are highly important for lipid synthesis and metabolism, in addition to their well-known role in protein quality control.[154] UPR activation in the liver has been extensively studied in light of the increasing prevalence of non-alcoholic fatty liver disease (NAFLD) **[G]**, which is the foremost cause of non-alcoholic and non-viral liver–associated illness and death in the United States.[155] Hepatic steatosis, the mildest form of NAFLD, can progress to non-alcoholic steatohepatitis (NASH), through a process that involves free fatty acid and lipid accumulation in hepatocytes followed by a series of innate immune responses in leukocytes including liver-resident macrophages known as Kupffer cells.[156] It has been shown that ER stress can induce liver steatosis through effects on lipid synthesis and inflammation, but high fat feeding-induced steatosis itself can also trigger ER stress, thereby providing a positive feedback loop that amplifies liver inflammation and injury.[157] In turn, UPR activation can modulate inflammatory signaling to induce liver inflammation, in part

through JNK activation, and IkB phosphorylation leading to NF-kB activation,[113,114] which is an important mediator of methionine- and choline- deficient (MCD) diet-dependent development of NASH.[158] In addition, the accumulation of lipids in hepatocytes can lead to mitochondrial dysfunction with increased ROS levels, which further triggers downstream inflammatory responses. The importance of ROS in the development of NASH had specifically been shown in mice deficient in NRF2, a protein that is crucially involved in the antioxidant response and can be induced by PERK; *Nrf2*$^{-/-}$ mice develop NASH more rapidly than wild-type mice when fed high-fat, high-cholesterol or MCD diets.[159]

[H3] Cancer

Activation of the UPR in cancers can initiate transcriptional programmes that allow them to combat harsh environmental conditions such as hypoxia, oxidative stress and low nutrient availability. In addition, these transcriptional programmes can actively shape a tumourigenic proinflammatory milieu. For example, UPR activation in prostate cancer cells results in transcriptional upregulation of *IL6* and *TNF*,[160] the promotors of which contain functional binding sites for XBP1s,[70] proinflammatory mediators that may promote inflammation-induced malignancies.[161,162] Moreover, both the PERK-[163] and IRE1α-mediated[164] arms of the UPR in tumour cells can increase transcription of genes encoding pro-angiogenic mediators such as vascular endothelial growth factor A, fibroblast growth factor 2 and IL-6.[163-165]

Intriguingly, UPR activation in cancer cells can also affect the anti-tumour immune response. Soluble factors secreted by ER-stressed tumour cells, but not non-stressed

tumour cells, can upregulate pro-inflammatory cytokine expression in macrophages, including the tumorigenic cytokines IL-6, IL-23p19 and TNF.[166] These soluble factors are also involved in dampening the anti-tumour immune response by inhibiting antigen presentation by APCs to cytotoxic $CD8^+$ T cells.[66,167] The mechanism by which this occurs is only partly elucidated but may include ROS-induced ER stress in DCs, transmitted through a yet to be defined factor, which increases lipid synthesis through XBP1 activation and thereby disrupts antigen presentation.[66]

[H1] Conclusions

Much has been learned about the functions of the UPR beyond being simply a means to cope with ER stress. The UPR has now also been recognized for its role in immune cell differentiation and function, and in regulating immune and inflammatory responses, including those associated with infections, tumours and autoimmune responses. It is clear that the UPR, and thus a certain level of ER stress, is crucial for cellular and consequently tissue homeostasis (the 'eustress' response). Therefore, understanding how the balance tips towards a pathophysiological UPR (the 'distress' response) that is associated with disease, and how this balance can be manipulated to enable appropriate immune responses and restore homeostasis, are important future research directions.

With the development of therapeutic agents that enhance proteostasis or interfere with specific components of the UPR **[BOX 3]**, used either alone or together, the hope is that an improved understanding of the UPR in immunity and inflammation

will eventually lead to the development of novel therapeutic strategies for chronic immune-mediated diseases.

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BOX 1 | Autophagy as a compensation strategy for ER stress

In response to the challenge of misfolded proteins, autophagy has a crucial function as an adaptive, "self-eating" process in which cellular components are encapsulated within autophagosomes and degraded. Similarly to the unfolded protein response (UPR), autophagy can result in cell survival or cell death.[168] [169] The mechanisms by which the UPR induces autophagy are incompletely understood, but probably involve PERK–eIF2α and IRE1α signaling.[170]

ATF4 and CHOP function both independently and together to induce a large array of autophagy genes.[171] In addition, eIF2α phosphorylation, in response to polyQ72 aggregate-induced ER stress, is associated with autophagosome formation and protection against neuronal cell death.[43] In another example, using unfolded dysferlin as a model of muscular dystrophy, XBP1s was shown to be crucial for autophagy induction and protection from neurodegeneration.[172] Furthermore, UPR pathways can activate AMPK, which attenuates AKT–mTOR signaling to enhance autophagy.[173]

A direct demonstration that autophagy can compensate for ER stress derives from studies of ER stress-induced small intestinal inflammation,[78] whereby concomitant deletion of epithelial XBP1 and of epithelial associated autophagy related protein 7 (ATG7) or ATG16L1 results in increased ER stress and severe transmural Crohn disease-like enteritis, compared with deletion of XBP1 alone, which only induces mild superficial inflammation **(Figure 4C)**. In this model, the induction of autophagy depends on phosphorylation of eIF2α.[79] In the case of intestinal epithelial stress associated with inflammatory bowel disease, autophagy probably functions to

selectively remove inflammatory ER membranes (*vide infra*). In the setting of cancer, ER stress induces activation of the PERK–eIF2α–ATF4 signaling pathway to increase tumour cell survival through the induction of autophagy.[174] [175] The precise mechanism by which autophagy relaxes ER stress remains unclear. Autophagy was previously considered to be a non-specific process (bulk macro-autophagy), but selective autophagy processes that precisely target organelles such as mitochondria and peroxisomes into autophagosomes have now been described. Autophagy of the ER (ER-phagy) has been described in yeast,[176] and more recently also in mammalian cells. In mammalian cells, ER-phagy depends on the FAM134 reticulon family of proteins, which function as ER transmembrane receptors binding LC3 and GABARAP, thereby initiating ER degradation by autophagy.[177] It remains to be determined whether autophagy directly degrades stressed ER membranes containing misfolded proteins, and whether FAM134 proteins or the selective autophagy receptors NBR1, optineurin, p62 or NDP52 are involved.

BOX 2 | Transmission of ER stress

Can immune cells sense ER stress in other cells? Intriguing studies have shown that activation of the unfolded protein response (UPR) in tumour cells can, through an unknown transmissible factor, induce ER stress in macrophages involving upregulation of *BiP*, *Chop* and *Gadd34* mRNAs and increased splicing of *Xbp1*. This occurs in a TLR4-dependent manner, which suggests that a TLR4 ligand is the transmissible factor. Importantly, ER stress-induced cell death was not responsible for the observed transmissible ER stress, which indicates that there is active

secretion of the transmissible factor rather than passive leakage of damage-associated molecules from dying cells.[166] Macrophages that were stimulated by neighboring ER-stressed tumour cells had increased inflammatory responses, which is in keeping with previous observations that TLR4 ligation amplifies pro-inflammatory cytokine signaling and may be the responsible signaling moiety.[70] Apart from macrophages, dendritic cells (DCs) are also susceptible to an ER stress-transmissible factor secreted by tumour cells, which interferes with DC-mediated cross-presentation to $CD8^+$ T cells and results in a less effective anti-tumor immune response.[167] It was recently shown that XBP1s-induced increases in lipid metabolism in DCs could be the underlying mechanism of impaired MHC class I-mediated antigen presentation to cytotoxic T cells.[66]

It is not clear whether the unidentified transmissible factor is a byproduct of ER stress in tumour cells or is actively secreted by these cells to modulate the anti-tumour response. Identification of the ER stress-transmissible factor would greatly enhance our understanding of how tissue-specific ER stress can become systemic in nature. In addition, this could provide possible new therapeutic targets to improve the anti-tumour immune response.

BOX 3 | Therapeutic opportunities

Tauro-ursodeoxycholic acid (TUDCA) and 4-phenyl butyrate (PBA) are small molecule chaperones that contribute to proper protein folding in the ER; they have proved successful in alleviating ER stress-induced hyperglycaemia, restoring insulin sensitivity and ameliorating fatty liver disease in obese mice.[178] TUDCA and PBA

have also been shown to reduce ER stress in the intestinal epithelium and thereby decrease the severity of DSS-induced colitis.[179] In addition, LPS-induced lung inflammation was reduced by PBA, through decreasing ER stress and modulating NF-κB and HIF1α signaling pathways.[180]

In haematological malignancies that produce large amounts of protein, such as multiple myeloma, blocking the 26S proteasome with bortezomib induces activation of the PERK-mediated unfolded protein response (UPR) pathway, which increases ATF4 and CHOP activity and sensitizes multiple myeloma cells to apoptosis.[181] In addition, blockade of IRE1α endonuclease activity with the small molecule inhibitor MKC-3946 increases multiple myeloma cell toxicity in response to the proteasome inhibitor bortezomib.[182] In line with this, $CD138^+$ plasma cells from patients with multiple myeloma are highly prone to cell death after treatment with STF-083010, another specific IRE1α RNase domain inhibitor, which further illustrates the importance of XBP1 in plasma cells. In addition, STF-083010 has potent cytotoxic effects in multiple myeloma cell lines and xenograft models.[183]

Sunitinib is a receptor tyrosine kinase inhibitor that targets the receptors for platelet-derived growth factor and vascular endothelial growth factor and thereby affects tumour angiogenesis and tumor cell proliferation. It has been FDA approved for the treatment of renal cell carcinoma and gastrointestinal stromal tumours. However, sunitinib also seems to influence the kinase activity of IRE1α[184] and PKR-dependent phosphorylation of eIF2α.[185] Therefore, sunitinib is potentially beneficial for the treatment of UPR-prone cancers, but it has also been shown to have negative

effects on the anti-viral immune response, as measured by decreased levels of IFN-β in response to infection with encephalomyocarditis virus, which was dependent on decreased RNase L activity.[185] This study exemplifies that broadly targeting the UPR might have important side effects that must be considered.

Lastly, given the recent reports showing that UPR activation in cancers can modulate the tumour immune microenvironment, antigen presentation by dendritic cells and thereby anti-tumour responses, targeting the UPR in cancers may improve tumour recognition by the immune system or, synergistically, improve the efficacy of immunotherapy in cancer.

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Figure legends

Figure 1: Accumulation of unfolded proteins in the ER lumen induces the three arms of the UPR. A) Dissociation of BiP from IRE1α, or direct binding of misfolded proteins to IRE1α, activates the endoribonuclease domain of IRE1α, which non-conventionally splices *Xbp1u* mRNA to produce a translational frameshift and create a potent transcriptional activator XBP1s. XBP1s activates the transcription of genes encoding proteins that are important for increasing the protein folding capacity of the ER and for the degradation of misfolded proteins via ERAD (see inset box). In addition, the entry of newly synthesized proteins into the ER is limited by the degradation of mRNA through regulated IRE1α-dependent decay of mRNA (RIDD). **B)** PERK-dependent phosphorylation of eIF2α inhibits ribosome assembly, which results in a translational block and allows the cell to cope with temporary ER stress. ATF4 escapes translation inhibition and induces the transcription of genes involved in downstream pro-survival pathways, including compensatory autophagy. Once ER stress is resolved, eIF2α is dephosphorylated by GADD34–PP1 to restore protein translation. However, if ER stress-induced damage is irreversible, the terminal UPR is activated to induce apoptosis, mainly through CHOP. **C)** Upon BiP dissociation from ATF6α during ER stress, ATF6α travels to the Golgi compartment where it is processed by the Golgi enzymes S1P and S2P to produce a cytosolic p50 fragment. ATF6p50 functions as a transcription factor that activates transcriptional programmes that increase ER capacity and protein folding, and that remove misfolded proteins from the ER for degradation (ERAD; see inset box).

Figure 2: XBP1 has a crucial role in plasma cell differentiation and dendritic cell differentiation and function. A: B cell receptor (BCR) ligation induces phosphorylation of B cell lymphoma 6 (BCL6) and its subsequent ubiquitylation and degradation. BCL6 degradation de-represses *Blimp1* in naïve B cells to activate cellular programmes that are crucial for the development of plasma cells. These include activation of the unfolded protein response (UPR) and *Xbp1* splicing, which is required for the expansion of the ER and increased protein (immunoglobulin) synthesis involved in plasma cell differentiation. **B:** Left panel: XBP1 is crucial for the differentiation of conventional DCs and plasmacytoid DCs from immature progenitors. Loss of XBP1 in progenitor cells abrogates maturation and decreases DC survival. Right panel, top: XBP1 deletion in mature CD11c-expressing DCs results in IRE1α hyperactivation leading to RIDD-dependent degradation of components of the MHC class I-mediated antigen cross-presentation machinery and, as such, ER stress in DCs interferes with their function. Right panel, bottom: In addition, increased XBP1s in response to ROS production in tumour DCs leads to augmented lipid biogenesis which is associated with disruption of MHC class I-mediated cross-presentation.

Figure 3: TLR signaling and the UPR coordinate immune responses in macrophages. A: Upon TLR ligation, the IRE1α–XBP1 arm of the unfolded protein response (UPR) is activated through a mechanism that requires TRAF6 recruitment to the TLR and ROS production by the NADPH oxidase NOX2. XBP1s functions as a

transcription factor to induce the transcription of pro-inflammatory cytokines *Il6* and *Tnf*α. In a separate pathway, translation of *Atf4* mRNA is inhibited by TLR4 ligation, which decreases CHOP levels and apoptosis in activated macrophages and thereby facilitates the immune response by favoring macrophage survival. **B:** In the absence of TLR signaling, phosphorylated IRE1α is subject to protein phosphatase 2A (PP2A)-mediated dephosphorylation and inactivation (left panel). Upon TLR ligation however, TRAF6 interacts with IRE1α and catalyzes the ubiquitylation of IRE1α, which prevents PP2A-mediated dephosphorylation and inactivation of IRE1α, thereby amplifying inflammation (right panel). **C:** IRE1α activation can induce *Il1*β transcription through GSK3β, which at the same time inhibits *Xbp1* splicing and thereby transcription of XBP1s target genes including *Tnf*.

Figure 4: A) Intestinal epithelial cells, particularly Paneth cells, have a well developed ER to cope with the high secretory demands, including production of antimicrobial proteins such as lysozyme and defensins. **B)** Deletion of XBP1 in the small intestinal epithelium (*Xbp1*$^{\Delta IEC}$ mice) induces ER stress, resulting in hypomorphic Paneth cells that have signs of IRE1α hyperactivation and downstream activation of NF-κB and JNK. Induction of autophagy through the PERK–eIF2α–ATF4 axis of the UPR alleviates ER stress. **C)** Simultaneous deletion of XBP1 and ATG16L1 in the small intestinal epithelium therefore further increases ER stress and ER stress-induced inflammation. Hypothetically, ER stress, which induces caspase 3, could be an important pathway responsible for the degradation of ATG16L1 in

patients carrying the Crohn disease risk allele *Atg16L1*^{T300A} which is prone to caspase 3-mediated cleavage**.**

Figure 5: the UPR as an inflammatory nidus. A) IRE1α activation and subsequent splicing of *Xbp1* produces the transcription factor XBP1s that directly binds the promoters of *Tnf* and *Il6*. **B)** RIDD-dependent degradation of miR-17, which in unstressed conditions represses *Txnip*, allows for increased TXNIP levels and NRLP3 inflammasome activation with upregulation of IL-1β. In addition, *Txnip* can be induced through the PERK–ATF5 pathway to induce inflammasome activation. **C**) Activated IRE1α forms a complex with TRAF2 to induce phosphorylation of JNK and upregulation of pro-inflammatory genes through AP1-activated transcription. **D**) In addition, the IRE1α–TRAF2 complex recruits IKK and subsequent phosphorylation of IκB leads to its degradation, thereby freeing NF-κB for nuclear translocation. **E**) Translation attenuation by PERK-dependent phosphorylation of eIF2α results in decreased IκB and NFκB translation. However, owing to the shorter half-life of IκB, the result is an increased NFκB to IκB ratio, which promotes inflammation. **F**) ATF4 directly binds the *Il6* promotor. **G**) ATF6α induces NFκB signaling via AKT phosphorylation. **H,I**) S1P- and S2P-mediated cleavage of ATF6α and CREBH allows their cleavage fragments to translocate to the nucleus and induce genes of the acute phase response (APR).

Glossary

ER-associated degradation

(ERAD). A pathway that removes terminally misfolded proteins from the ER through their retrotranslocation to the cytosol and that targets them for degradation by the ubiquitin–proteasome system.

Unfolded protein response

(UPR). A highly conserved pathway that regulates the balance between folding capacity of the endoplasmic reticulum (ER) and protein synthesis.

Integrated stress response

(ISR). An ancient stress response that modulates protein biosynthesis by integrating various types of stress signal, including ER stress, amino acid deprivation, virus infection and oxidative stress.

Paneth cells

Highly specialized small intestinal epithelial cells that shape the composition of the microbiota by the secretion of antimicrobial proteins and that sustain and modulate epithelial stem cells by the secretion of niche factors.

Acute phase response

(APR). A group of systemic and innate physiological processes in the early response to infection or injury.

Crohn disease

An inflammatory disease of the small and large intestines that is thought to arise from an inappropriate immune response towards the intestinal microbiota in a genetically susceptible host.

Ulcerative colitis

A chronic disease of the colon with unknown aetiology, characterized by inflammation and ulceration of the colon.

Non-alcoholic fatty liver disease

(NAFLD). Liver disease characterized by the accumulation of fat (steatosis) in the liver, which is often associated with obesity. Although NAFLD is benign, it can progress towards steatohepatitis and even cirrhosis.

Key points

1. The unfolded protein response (UPR) has an important role in the differentiation and maturation of various immune cells and is crucial for immune cell function, such as cytokine production by macrophages and cross-presentation of dendritic cells, for example.
2. Innate immune signaling differentially affects the three arms of the UPR to optimize inflammatory responses, while at the same time inhibiting the activation of the terminal UPR, which is associated with cell death. This allows the cell to survive and cope with temporary increases in protein production during immune responses to pathogens.
3. In complex autoimmune diseases, chronic activation of the UPR can function as the nidus for the development of inflammation.
4. UPR activation triggers inflammatory responses mainly through NF-kB activation, phosphorylation of JNK, activation of the inflammasome or direct

interaction of downstream UPR targets with the promoters of inflammatory cytokine genes.

5. UPR activation in cancer cells may interfere with anti-tumour immunity, which indicates that manipulating UPR signaling could boost anti-tumor immune responses.
6. The UPR is amenable to therapeutic manipulation to either promote its beneficial homeostasis-inducing properties and/or inhibit its inflammation-inducing activities in the setting of unresolved ER stress.

Biographies

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Randal J. Kaufman, a founding scientist at Genetics Institute Inc. (presently, Pfizer Inc., Cambridge, Massachusetts, USA), was an HHMI Investigator at the University of Michigan, USA, for 18 years, where he studied the steps that limit protein secretion, leading to the discovery of the unfolded protein response (UPR). In 2011, he became Program Director at the Sanford-Burnham Medical Research Institute, La Jolla, California, USA, where he studies how protein misfolding in the endoplasmic reticulum (ER) causes degenerative disease.

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Competing interests statement
The authors declare no competing interests

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