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Review article

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Control of immune cell function by the unfolded protein response

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Abstract

Initiating and maintaining optimal immune responses requires high levels of protein synthesis, folding, modification and trafficking in leukocytes, which are processes orchestrated by the endoplasmic reticulum. Importantly, diverse extracellular and intracellular conditions can compromise the protein-handling capacity of this organelle, inducing a state of 'endoplasmic reticulum stress' that activates the unfolded protein response (UPR). Emerging evidence shows that physiological or pathological activation of the UPR can have effects on immune cell survival, metabolism, function and fate. In this Review, we discuss the canonical role of the adaptive UPR in immune cells and how dysregulation of this pathway in leukocytes contributes to diverse pathologies such as cancer, autoimmunity and metabolic disorders. Furthermore, we provide an overview as to how pharmacological approaches that modulate the UPR could be harnessed to control or activate immune cell function in disease.

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Introduction

The correct folding of proteins is essential for their proper functionality. This process takes place within the endoplasmic reticulum (ER) and is controlled by the coordinated actions of molecular chaperones and by the protein disulfide isomerase, which catalyses the formation and breakage of disulfide bonds between cysteine residues within proteins as they fold. In addition to the amino acid sequence of a protein, glycosylation is also essential for both proper folding and functional maturation of proteins[1]. In 1977, the ER chaperone BiP (encoded by *GRP78*) was discovered to be highly induced in cells cultured without glucose[2], and later this increase was attributed to defective N-linked glycosylation in the absence of glucose resulting in the accumulation of misfolded proteins[3]. These findings led to the discovery of the unfolded protein response (UPR), an adaptive signalling cascade activated upon accumulation of unfolded or misfolded proteins in the ER that leads to a transcriptional programme with the aim of restoring ER homeostasis or inducing cell death if the damage is irreversible[4]. Three branches of the UPR have since been described: activation of inositol-requiring enzyme 1α (IRE1α) and the cleavage of messenger RNA (mRNA) encoding X-box protein 1 (XBP1) to generate the spliced transcription factor XBP1s; activation of the protein kinase RNA-like ER kinase (PERK), which phosphorylates eukaryotic translation initiation factor 2α (eIF2α) to attenuate general protein translation, while selectively inducing the translation of activating transcription factor 4 (ATF4); or activation of ATF6 (see Fig. 1 and Box 1 for further details of these pathways).

Since the initial discovery of the UPR, several extracellular and intracellular conditions have been shown to compromise the protein-folding capacity of the ER, leading to a cellular state of 'ER stress'[5]. Thus, in addition to defective glycosylation, disruption of protein homeostasis (proteostasis) and activation of the UPR are commonly induced by rapid increases in protein synthesis, hypoxia, acidosis, altered lipid metabolism and accumulation of reactive oxygen species (ROS)[6]. Furthermore, emerging evidence in the past decade has shown particular effects of the UPR on immune cell function and development. Here, we review recent advances in terms of the functional effects of the UPR in multiple immune compartments — both at steady state and in diverse pathological settings such as inflammatory diseases, cancer and infection — with the aim of providing examples that illustrate the variety of effects and affected cell types. Recent genetic and pharmacological evidence supports the modulation of UPR pathways as a novel strategy to control immune cell metabolism, function and fate. We discuss how targeting UPR signalling in immune cells could be used to develop novel therapeutic modalities for diverse human pathologies.

The UPR in innate immunity

The innate immune system is the frontline mechanism of defence against injury and pathogens. The high levels of translation that are required to respond effectively to these threats — for example, to synthesize cytokines, antimicrobial factors and granule proteins — can lead to the accumulation of misfolded proteins in the ER[6], and pathogens can also produce factors that directly alter the protein-folding capacity of the ER[7]. Both events can provoke ER stress and lead to activation of the UPR in innate immune cells. Here, we summarize seminal studies and highlight recent reports that provide examples of how the activation of the UPR in innate immune cells not only restores ER proteostasis but also controls major transcriptional and metabolic programmes in myeloid cells that shape diverse functional outputs (Fig. 2). We provide a global overview, through select examples, of how the UPR controls the production of inflammatory mediators in various myeloid cells and how it crosstalks with other signalling pathways to sculpt the functions of innate immune cells. In addition to controlling the functional attributes of multiple innate immune cell types, the UPR has also been implicated in the development of type 1 conventional dendritic cells (DCs) and eosinophils; these topics are reviewed elsewhere[8,9] and are not the focus of our discussion here.

Crosstalk with PRR signalling

Engagement of the microbial pattern recognition receptors (PRRs) Toll-like receptor 2 (TLR2) or TLR4 in bone-marrow-derived macrophages triggers the IRE1α–XBP1s pathway of the UPR[10,11]. Mechanistically, TLR4 activates TRAF6, which ubiquitylates IRE1α and promotes its phosphorylation by impeding its binding to the phosphatase PP2A. TLR4 also activates NADPH oxidase 2, leading to ROS production, which activates IRE1α, and this process is required for optimal expression of the genes encoding tumour necrosis factor (TNF), IL-1β and IL-6 in response to TLR4 ligation[10]. Of note, XBP1s was found to bind the *Il6* promoter to drive its rapid transcriptional induction upon TLR signalling[10]. Post-developmental ablation of XBP1s using Mx1-Cre mice resulted in increased bacterial burden upon infection with the intracellular pathogen *Francisella tularensis*; this was proposed to be caused by impaired macrophage function[10], although it has not been determined whether these effects are phenocopied in mice selectively lacking IRE1α or XBP1s in the macrophage compartment. Other studies suggest that IRE1α signalling to the PRRs nucleotide-binding oligomerization domain 1 (NOD1) and NOD2 induced by bacterial type IV secretion systems supports IL-6 induction in bone-marrow-derived macrophages, which is thought to be mediated by the kinase domain, but not the nuclease domain, of IRE1α[12]. Accordingly, a recent paper described that infection with *Citrobacter rodentium* induces IRE1α–NOD1 and/or IRE1α–NOD2 activation, promoting inflammation and bacterial clearance[13]. In addition, palmitate exposure — which induces the UPR by altering ER homeostasis and increasing mitochondrial ROS generation — was shown to stimulate IL-6 production through ATF4 (a component of the PERK branch of the UPR) in mouse peritoneal macrophages[14].

Hence, multiple branches of the UPR enable robust IL-6 production by macrophages in the context of PRR signalling, depending on their activation status and metabolic context. Similarly, in DCs, XBP1s has been reported to mediate potent production of interferon-β (IFNβ) upon TLR3 stimulation in an IRF3-dependent manner[15]. Overexpression of XBP1s enhanced IFNβ production in DCs and suppressed infection with vesicular stomatitis virus[15]. Recent studies have further shown that the link between the UPR and type I IFN responses is partly mediated by stimulator of interferon genes (STING)[16]. STING resides in the ER and senses bacteria-derived cyclic-di-AMP[17]. Upon infection of macrophages with Gram-positive bacteria, STING activates the PERK branch of the UPR and the induction of a selective form of autophagy (ER-phagy) that eliminates stressed ER membranes and leads to protective type I IFN production. Defective ER-phagy leads to unresolved ER stress and death of the infected phagocytes[16]. STING was also found to bind to and directly activate PERK to induce inflammatory and pro-survival translational programmes, revealing a novel crosstalk between STING and the UPR[18]. By contrast, however, another recent report showed that ER stress can dampen type I IFN production by human plasmacytoid DCs by rewiring glycolysis to serine biosynthesis. Specifically, ER-stressed plasmacytoid DCs activated through TLR7 or TLR9 had XBP1s-driven induction of phosphoglycerate dehydrogenase, which reduced pyruvate access to the tricarboxylic acid cycle and blunted the mitochondrial generation of ATP that is crucial for type I IFN responses[19].

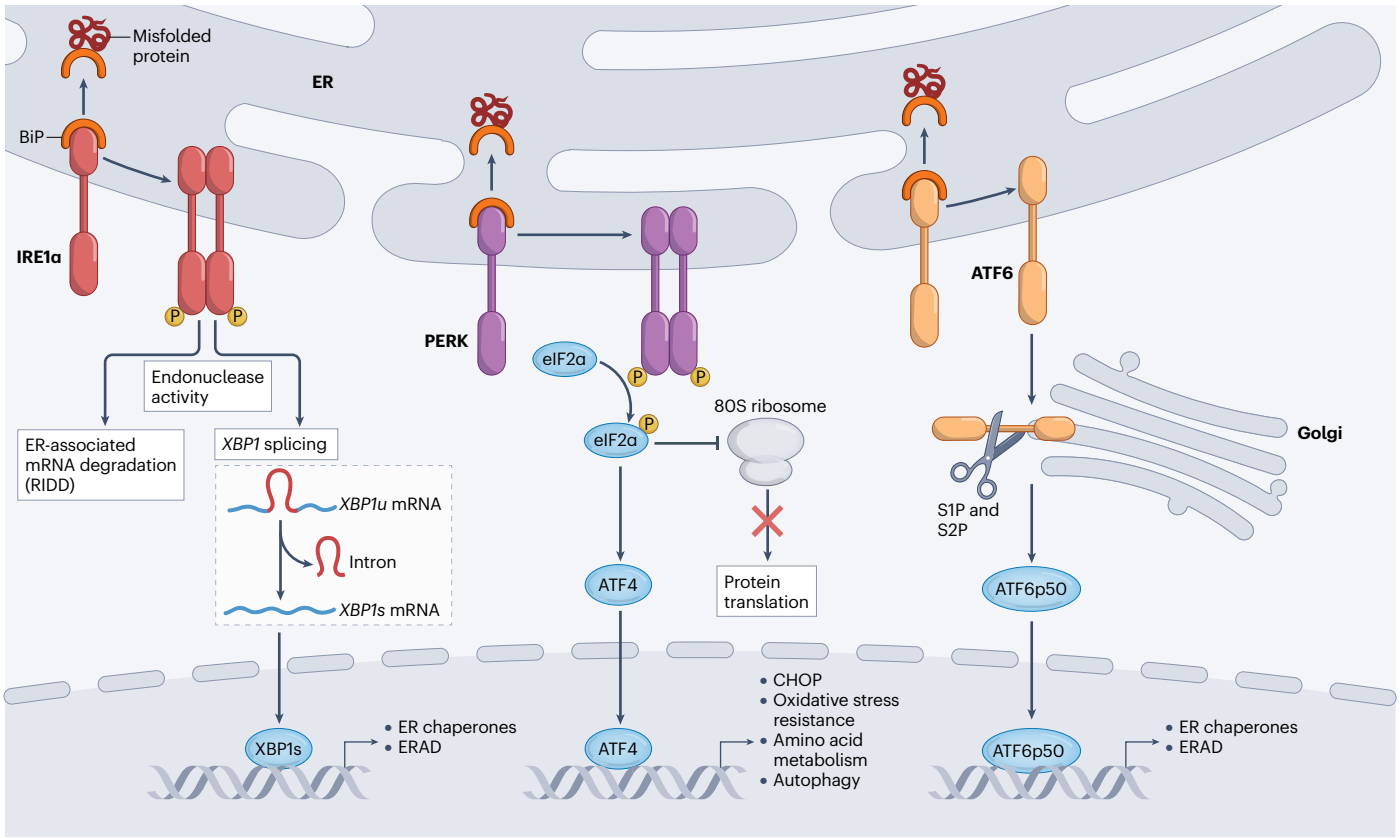

Fig. 1 | Overview of unfolded protein response signalling pathways. Under homeostatic conditions, the transmembrane proteins inositol-requiring enzyme 1α (IRE1α), protein kinase RNA-like ER kinase (PERK) and activating transcription factor 6 (ATF6) are bound by the endoplasmic reticulum (ER) chaperone BiP (encoded by *GPR78*) on their ER luminal side, which suppresses their activity. Under conditions of stress, the protein-folding capacity of the ER is exceeded and misfolded proteins accumulate in the ER, which leads to dissociation of BiP from IRE1α, PERK and ATF6 and the activation of downstream signalling cascades. IRE1α and PERK are activated by oligomerization and autophosphorylation upon release from BiP. The endonuclease domain of IRE1α cleaves messenger RNA (mRNA) encoding X-box protein 1 (XBP1) to generate XBP1s, a potent multitasking transcription factor. The activation of XBP1s leads to the upregulation of a subset of target genes involved in the unfolded protein response (UPR), including those encoding ER chaperones, foldases and components of ER-associated protein degradation (ERAD). The endonuclease activity of IRE1α also mediates ER-associated mRNA degradation, known as regulated IRE1α-dependent decay (RIDD). Activated PERK phosphorylates eukaryotic translation initiation factor 2α (eIF2α), which inhibits the assembly of functional 80S ribosomes and hence attenuates general protein translation, while selectively inducing the translation of ATF4. ATF4 activates the transcription of UPR genes, including the gene encoding pro-apoptotic DNA damage-inducible transcript 3 (commonly known as CHOP) and multiple genes involved in the regulation of antioxidant responses, amino acid metabolism and autophagy. ATF6 translocates to the Golgi apparatus upon release from BiP, where its luminal domain is removed by site 1 protease (S1P) and S2P. The remaining N-terminal fraction (ATF6p50) functions as a transcription factor in the nucleus to activate the transcription of UPR target genes, including those encoding XBP1, ERAD pathway components and ER chaperones to regulate protein folding and degradation in the ER.

Regulation of cytokine production

The expression of IL-23 — a pro-inflammatory cytokine implicated in diverse human pathologies — can be regulated by multiple branches of the UPR in myeloid cells. For example, pro-apoptotic DNA damage-inducible transcript 3 (DDIT3; commonly known as CHOP), which is normally induced through PERK–ATF4 signalling, was found to enhance IL-23 production in human monocyte-derived DCs stimulated through diverse PRRs, such as TLR2, TLR3, TLR4, TLR9, dectin 2, NOD1 or NOD2. Mechanistically, CHOP binds to the promoter of *IL23A*, which encodes the p19 subunit of IL-23 (ref. [20]), presumably transactivating its expression. ER stress caused by the hexokinase 2 inhibitor 2-DG, which halts the hexosamine biosynthetic pathway and inhibits N-linked glycosylation of proteins, also increases IL-23 production through the IRE1α–XBP1s branch of the UPR in human monocyte-derived DCs stimulated with the TLR4 ligand lipopolysaccharide (LPS) or with the fungal β-glucan zymosan (which binds to dectin and NOD receptors)[21]. Moreover, both PERK–CHOP and IRE1α–XBP1s signal axes are reported to increase IL-23 expression in TLR8-activated mouse bone-marrow-derived dendritic cells (BMDCs) exposed to palmitate[22]. Hence, the type of UPR stressor, the engagement of specific PRRs and the metabolic alterations elicited by the combination of both stimuli seem to determine the branch of the UPR that is involved in regulating IL-23 production by myeloid cells.

The sigma-1 receptor, an ER-resident protein, has been reported to suppress the RNase activity of IRE1α in LPS-stimulated bone-marrow-derived macrophages[23]. Notably, mice with genetic ablation

of sigma-1 receptor have increased IRE1α–XBP1s activation that exacerbates inflammatory responses in preclinical models of septic shock[23]. Together, these studies show that UPR components can boost inflammatory cytokine production in myeloid cells, but the context-dependent, global transcriptomic and metabolic programmes induced by ER stress that regulate this inflammatory response require further investigation. In addition, although the regulation of cytokine production by the UPR seems to be predominantly controlled at the transcriptional level, it is possible that the UPR might also alter cytokine folding and/or secretion in innate immune cells. Furthermore, the IRE1α–p38 axis has been proposed to control the stability of mRNAs encoding diverse pro-inflammatory cytokines in activated invariant natural killer T cells[24], which adds another layer of complexity to the immunomodulatory effects of IRE1α activation in this specific cell type.

Crosstalk with metabolic pathways

Beyond mediating rapid cytokine production, the UPR has also been shown to orchestrate metabolic pathways that are involved in the synthesis of inflammatory lipid mediators. IRE1α-deficient or XBP1-deficient DCs, macrophages and neutrophils undergoing canonical ER stress or stimulated by plasma membrane-bound TLRs showed reduced expression of gene programmes involved in eicosanoid metabolism and production of the pain-causing lipid mediator prostaglandin E_2 (PGE_2)[25]. IRE1α-activated XBP1s was found to facilitate the conversion of arachidonic acid to PGE_2 by transactivating the promoters of *COX2* and *PTGES*, which encode prostaglandin-endoperoxide synthase (also known as cyclooxygenase 2) and prostaglandin E synthase, respectively[26]. Accordingly, selective deficiency of IRE1α or XBP1 in leukocytes reduced behavioural pain responses in mouse models of PGE_2-driven inflammatory and post-surgical pain[25]. These findings reveal an unexpected physiological role for the IRE1α–XBP1s branch of the UPR as a central driver of prostanoid metabolism in myeloid cells.

Crosstalk with cytokine receptor signalling

Signalling through cytokine receptors has been shown to activate the UPR in innate immune cells. Concomitant stimulation with IL-4 and IL-6 triggers IRE1α–XBP1s signalling in bone-marrow-derived macrophages through activation of the transcription factors STAT6 and STAT3, respectively, and this process promotes IRE1α-dependent expression of cathepsin proteases that have been shown to facilitate cancer cell migration[27]. However, the mechanisms by which STAT3 and STAT6 trigger IRE1α activation are unknown and it is not clear whether this process is mediated by canonical ER stress. IRE1α–XBP1s activation is also induced by cytokine signalling in natural killer (NK) cells. Concurrent stimulation of primary NK cells with IL-12 and IL-18 triggered IRE1α–XBP1s signalling, partly through the STAT4 and mTORC1 pathways[28]. Although this branch of the UPR was dispensable for NK cell survival, NK cells deficient for IRE1α or XBP1s had reduced homeostatic and inducible proliferation, mainly because XBP1s was required for optimal transactivation of *cMyc*. Indeed, NK cells devoid of IRE1α–XBP1s signalling had mitochondrial and proliferative defects, similar to those resulting from cMYC deficiency[28].

Box 1

Signalling pathways of the unfolded protein response

Dysregulation of protein homeostasis (proteostasis) in the endoplasmic reticulum (ER) is associated with infection, malignancy and ageing. ER proteostasis surveillance is mediated by the unfolded protein response (UPR), which initiates a signal transduction cascade that results in the general arrest of protein translation but induces the expression of a specific set of genes for restoring and/or promoting efficient protein folding and trafficking at the ER lumen. The basic UPR signalling cascade was initially characterized in yeast almost 30 years ago, in which a linear pathway is controlled by one stress sensor, inositol-requiring protein 1 (Ire1), and a downstream transcription factor, Hac1 (which is a homologue of activating transcription factor (ATF) and CREB in mammals)[141–143]. In vertebrates, however, the UPR has evolved into a more complex network of signalling cascades, the three branches of which are primarily driven by inositol-requiring enzyme 1α (IRE1α), protein kinase RNA-like ER kinase (PERK) and ATF6, all of which have an ER luminal domain that enables them to detect the build-up of multiple cellular stress signals[6].

IRE1α comprises two enzymatic domains: a serine/threonine kinase domain and an endoribonuclease domain[144,145]. Upon activation, IRE1α initiates unconventional splicing of the messenger RNA encoding X-box protein 1 (XBP1) to excise a 26-nucleotide intron. This generates a spliced variant (XBP1s) that subsequently can translocate to the nucleus and induce the transcription of genes associated with physical expansion of the ER and ER function[146,147]. Although most of the signalling events downstream of IRE1α–XBP1s activation are associated with the induction of pro-survival pathways, IRE1α can also degrade non-*Xbp1* messenger RNA targets through regulated IRE1α-dependent decay, which is pro-apoptotic under conditions of severe ER stress[148,149].

PERK is a type I serine–threonine transmembrane protein kinase, which, upon release from the ER chaperone BiP, dimerizes and promotes its autophosphorylation. Phosphorylated PERK then phosphorylates and hence activates eukaryotic translation initiation factor 2α (eIF2α)[150]. Phosphorylated eIF2α inhibits general translation by interfering with 5′-cap assembly, resulting in the transient attenuation of protein synthesis and thereby relieving the stressed ER from the influx of newly synthesized proteins. Phosphorylation of eIF2α also allows for the cap-independent translation of ATF4 (ref. [151]). ATF4 is a stress-inducible factor that transcriptionally upregulates the expression of pro-apoptotic DNA damage-inducible transcript 3 (commonly known as CHOP) to upregulate the levels of genes that are involved in redox homeostasis, apoptosis, amino acid metabolism and autophagy[152,153].

ATF6, which is also freed from BiP upon ER stress, translocates to the Golgi apparatus, where it is cleaved intramembranously by site 1 and site 2 proteases to liberate an active, soluble amino-terminal fraction ATF6p50. ATF6p50 migrates to the nucleus, where it functions as a transcription factor to regulate the expression of XBP1, molecular chaperones and components of the ER-associated protein degradation machinery, which increase ER function and protein degradation[154,155].

Potential roles of ATF6 and PERK signalling in NK cell biology remain to be defined, as does any involvement of the UPR in controlling the activity of innate lymphoid cells.

Antigen cross-presentation

In addition to cytokine-mediated and PRR-driven activation of UPR sensors, recent studies have highlighted a complex regulatory role for IRE1α signalling in antigen cross-presentation by BMDCs. An early study reported that pulsing BMDCs with melanoma cell lysates as a source of antigens triggered IRE1α–XBP1s signalling without inducing regulated IRE1α-dependent decay (RIDD) of mRNAs through the endonuclease activity of IRE1α and that IRE1α inhibition in this context impaired cross-presentation to $CD8^+$ T cells[29]. By contrast, a subsequent study found that IRE1α could be directly activated by peptides derived from exogenous antigens that are transported into the ER by the TAP1 protein, which were postulated to mimic the action of misfolded proteins[30]. In this setting, IRE1α activation degraded mRNAs encoding MHC class I heavy chain through RIDD, hence blunting antigen cross-presentation by BMDCs to T cells[30]. Preventing IRE1α activation consequently enhanced the T cell-activating capacity of BMDCs pulsed with antigens. The mechanism by which antigenic peptides trigger IRE1α activation in this context requires further investigation, and it is likely that the source of the antigen (whole cancer cell lysate versus purified peptides) might have distinct effects on IRE1α activation in

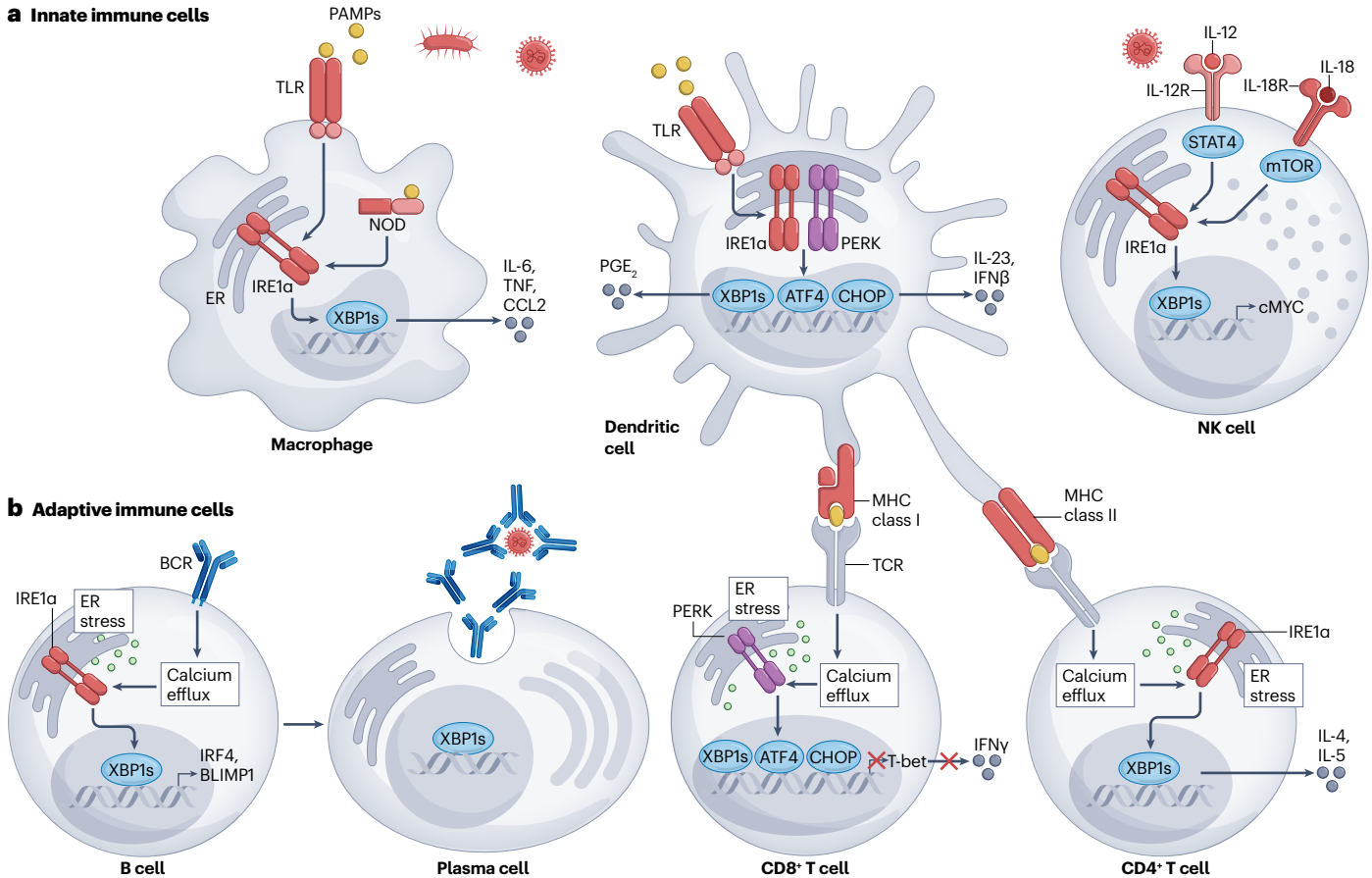

Fig. 2 | Intrinsic activation of unfolded protein response sensors during physiological immune responses. a, Innate immune cells. Pathogen recognition by dendritic cells and macrophages occurs through binding of pathogen-associated molecular patterns (PAMPs) to pattern recognition receptors such as Toll-like receptors (TLRs) and intracellular nucleotide-binding oligomerization domain (NOD) receptors. Both TLR and NOD signalling can lead to the activation of IRE1α–XBP1s and/or PERK–ATF4–CHOP pathways of the unfolded protein response, which lead to the transcription of pro-inflammatory cytokines and production of prostaglandin E_2 (PGE_2) by facilitating transcription networks and signalling cascades. In natural killer (NK) cells, the pro-inflammatory cytokines IL-12 and IL-18 activate STAT4 and mTOR, which induce IRE1α–XBP1s signalling and promote cMYC activation. **b**, Adaptive immune cells. In activated B cells and T cells, B cell receptor (BCR) and T cell receptor (TCR) signalling trigger calcium efflux from the endoplasmic reticulum (ER), which triggers mild ER stress that contributes to the physiological activation and function of these cells through the coordination of calcium signalling cascades and XBP1s-based transcriptional networks. Activation of XBP1s in B cells and expression of interferon-regulatory factor 4 (IRF4) and B lymphocyte-induced maturation protein 1 (BLIMP1) are also essential for their differentiation to plasma cells, in which XBP1s is required for efficient, high-level antibody production. In $CD8^+$ T cells, XBP1s activation ensures the differentiation of antigen-experienced effector cells. However, activation of PERK–ATF4–CHOP represses T-bet expression and inhibits interferon-γ (IFNγ) production. In $CD4^+$ T cells, IRE1α–XBP1s activation promotes expression of IL-4 and IL-5, favouring differentiation towards T helper 2 cells. ATF4, activating transcription 4; CCL2, CC-chemokine ligand 2; CHOP, also known as DNA damage-inducible transcript 3; IRE1α, inositol-requiring enzyme 1α; mTOR, mechanistic target of rapamycin; PERK, protein kinase RNA-like ER kinase; STAT4, signal transducer and activator of transcription 4; TNF, tumour necrosis factor; XBP1s, spliced X-box protein 1.

BMDCs that account for the different functional outputs in the in vitro settings examined by these studies.

The UPR in adaptive immunity

Adaptive immune responses are orchestrated by T cells and B cells that can provide durable, antigen-specific protection against pathogens and neoplasms. UPR pathways have been shown to be activated in lymphocytes in various ways. IRE1α–XBP1s activation has been documented during the early stages of lymphocyte development[31]. Furthermore, upon antigen encounter, engagement of T cell receptors and B cell receptors induces calcium efflux from the ER to the cytosol[32,33], which may alter the function of calcium-binding protein chaperones in this organelle and thus induce ER stress[34] (Fig. 2). Activated T cells and B cells produce and secrete large amounts of effector proteins (such as cytokines and antibodies), which require a highly functional ER[35]. These secreted proteins are glycosylated in the ER using uridine diphosphate *N*-acetylglucosamine generated via the hexosamine biosynthetic pathway, which in turn is regulated by IRE1α–XBP1s signalling[36]. Here, we provide some seminal examples that illustrate how UPR sensors and components of the UPR are closely linked with pathways mediating adaptive immunity.

T cells

IRE1α–XBP1s signalling is rapidly triggered in naive T cells stimulated through CD3 and CD28 (refs. [37–40]). Activation of this pathway was initially documented during the early stages of T cell development[31] as well as during the expansion of antigen-specific $CD8^+$ T cell populations in response to bacterial or viral infection[39]. However, ablation of XBP1s in haematopoietic stem cells did not compromise T cell development[41], and subsequent analyses using Cd4-Cre mice confirmed that selective loss of XBP1s in T cells did not alter their development, reconstitution, proliferation or cell cycle progression in naive mice[37]. It remains to be determined whether XBP1s is necessary for T cell-dependent control of pathogens.

The UPR has also been implicated in the control of T cell polarization. Pharmacological targeting of IRE1α–XBP1s signalling using 4μ8c (an inhibitor of the IRE1α RNase domain) blunted T helper 2 (T_H2) cell differentiation in vitro by suppressing the expression and secretion of IL-4 and IL-5 (ref. [40]). In addition, metabolic stress induced by hypoxia or nutrient restriction was found to trigger IRE1α–XBP1s signalling in activated $CD4^+$ T cells that facilitated their differentiation towards T_H17 cells even in the absence of transforming growth factor-β[42]. Deletion of *Xbp1* in the lymphoid compartment using Rag1-Cre mice reduced the severity of experimental autoimmune encephalomyelitis (EAE) in these mice, a pathology that is normally driven by T_H17 cells[42]; however, it should be noted that RAG1 is expressed in multiple lymphocytes, including B cells and T cells, so confirmatory experiments selectively deleting XBP1 in the T cell compartment are warranted. In addition, optimal glucose availability is required to maintain ER proteostasis; blocking glucose uptake or utilization not only curtails glycolysis but also the hexosamine biosynthetic pathway that is required for N-linked protein glycosylation in the ER[43]. Hence, in activated $CD4^+$ T cells, glucose restriction provoked sustained ER stress and maladaptive IRE1α–XBP1s signalling that suppressed the mitochondrial respiration and effector function of $CD4^+$ T cells[37].

PERK signalling has also been implicated in regulating T cell function. CHOP induction driven by the PERK–ATF4 pathway was found to repress T-bet expression in $CD8^+$ T cells, thereby reducing their effector function and capacity to secrete IFNγ[38]. In addition, oxidizing environments and low availability of extracellular amino acids were reported to trigger ATF4 activation in $CD4^+$ T cells, which induced metabolic reprogramming leading to enhanced glycolysis, glutaminolysis and oxidative phosphorylation necessary to tolerate these forms of stress[44]. Hence, signalling through the IRE1α or PERK branches of the UPR can regulate the differentiation and activity of T cells in a dynamic manner, depending on nutrient availability and/or metabolic status. The role of the ATF6 branch of the UPR in T cell biology remains elusive, as does the role of the UPR in regulatory T (T_{reg}) cell responses. Pharmacological ER stressors were reported to induce *IL10* transcription in human T_{reg} cell clones, probably through eIF2α activation downstream of PERK[45], but additional research is needed to define the potential role of the UPR in the differentiation and functional attributes of this important T cell subset.

B cells

Although the IRE1α–XBP1s branch of the UPR is robustly activated upon B cell activation, various studies showed that selective loss of XBP1s in the B cell compartment did not affect the development, maturation, isotype switching or memory lineage commitment of these cells[46]. Nonetheless, IRE1α–XBP1s activation has been postulated to operate as a pre-emptive mechanism that enables B cells to rapidly respond to alterations in ER homeostasis while preparing them for optimal antibody production and secretion[46]. Indeed, the potent capacity of plasma cells, which have an enlarged ER, to produce and secrete antibodies was proposed to induce basal levels of ER stress that sustain constitutive UPR activation[47]. In this setting, XBP1s, but not PERK or ATF6, was found to be necessary for the differentiation of B cells towards plasma cells[47–51]. The accumulation of misfolded IgM in the ER of XBP1s-deficient B cells with a compromised UPR was speculated to account for their inability to generate plasma cells, although normal protein folding was observed in another study of plasmablasts devoid of XBP1s[52]. In addition, abrogating IgM production did not alter XBP1s induction[53], which suggests that increased antibody generation per se is unlikely to elicit the IRE1α–XBP1s branch of the UPR in plasma cells. Additional research identified that loss of XBP1s in B cells compromises normal B cell receptor signalling, leading to aberrant expression of IRF4 and of B lymphocyte-induced maturation protein 1 (BLIMP1)[53], a key transcriptional repressor that controls the terminal differentiation of antibody-secreting cells[35]. B cells lacking XBP1s were also found to undergo RIDD of the transcript encoding secretory Igμ heavy chain, hence reducing the levels of soluble IgM produced by these cells[54]. Nonetheless, a potential role for RIDD in controlling the function of XBP1s-competent B cells remains to be fully characterized.

The UPR in cancer

As illustrated by the aforementioned examples, UPR signalling can have effects on the activation and functions of both innate and adaptive immune cells, and perturbations of tissue homeostasis can trigger UPR-mediated responses that contribute to host protection and the restoration of homeostasis (Box 2). However, in the context of some infections or chronic and/or age-related diseases with an inflammatory component, such as cancer, metabolic disease and autoimmune disease, prolonged perturbations of tissue homeostasis and tissue damage can lead to dysregulated, hyperactive UPR pathways that induce pathological immune responses and/or deregulated immune activation. Differences between these disease states and normal physiology in terms of the pro-inflammatory or anti-inflammatory effects of the UPR also highlight that nutrient availability and the metabolic status of immune cells in specific tissues and/or specific contexts can

guide the engagement of either adaptive (protective) or maladaptive (pathological) UPR signalling. In the following sections, we summarize how protective and pathological UPR pathways are engaged in immune cells in different pathologies and their contribution to disease progression.

Cancer tissues are characterized by the coexistence of several cell types in a tumour microenvironment (TME) often dominated by nutrient deprivation, hypoxia and acidosis[55,56]. These conditions disrupt ER proteostasis and induce sustained activation of the UPR in tumour-infiltrating leukocytes, a process that alters their transcriptional, metabolic and functional programmes. Here, we summarize how activation of the UPR can affect antitumour immunity by providing examples of effects on DCs, T cells and myeloid cells in the TME.

Tumour-infiltrating DCs

In general, tumours restrain the normal antigen-presenting activity of DCs to evade adaptive immune control, and several pieces of evidence indicate a role for pathological activation of the UPR in this modulation of tumour-associated DCs. In one study, dysfunctional DCs at tumour sites in mice with metastatic ovarian cancer were shown to have IRE1α–XBP1s hyperactivation driven by ROS overproduction and the generation of lipid peroxidation by-products that directly induced ER stress[57]. In this pathological setting, XBP1s induced a lipid biosynthetic programme leading to the abnormal accumulation of cytosolic lipid droplets in intratumoural DCs, which reduced their capacity to present tumour antigens to infiltrating T cells[57]. Accordingly, disabling the IRE1α–XBP1s axis in DCs was found to enhance their local antigen-presenting capacity, eliciting T cell-dependent antitumour immunity[57] (Fig. 3). Subsequent studies in independent cancer models also showed that controlling ROS accumulation and targeting IRE1α pharmacologically improved the functions of intratumoural DCs and enhanced the effects of DC-based immunotherapy, inducing protective anticancer responses[58]. In another study, BAT3 — an ER-resident protein chaperone involved in the quality control of newly synthesized proteins — was found to be downregulated in DCs isolated from mice with cancer compared with control mice[59]. Loss of BAT3 in DCs caused overactivation of the UPR that resulted in IRE1α-driven metabolic reprogramming leading to increased expression of immunosuppressive glucocorticoids, which ultimately hindered the development of antitumour T cell responses[59]. However, in contrast to these results suggesting that IRE1α activation hinders intratumoural DC function, other groups have reported that IRE1α signalling in DCs outside the tumour has an important role in promoting the cross-presentation of melanoma-derived antigens and in priming anticancer T cell responses[29,60]. A plausible explanation for this apparent discrepancy is that the IRE1α–XBP1s branch of the UPR integrates diverse pathological stress signals

Box 2

Inducers of the unfolded protein response in immune cells

The endoplasmic reticulum (ER) is a crucial organelle for many immunological processes, including peptide loading on MHC class I and class II molecules, the activation of the inflammasome and the production of cytokines and antibodies[156,157]. In keeping with the crucial immunological role of the ER, microbial pathogens hijack several steps of the antigen presentation pathway or cytokine production occurring in the ER of infected cells to evade the immune response. For example, hepatitis C virus escapes the interferon response by binding and inhibiting protein kinase RNA-like ER kinase (PERK) through the viral proteins E2 and NS5A. In the context of PERK inhibition, the unfolded protein response (UPR) in infected cells then transitions from an adaptive, homeostatic response towards a pathological stress response. Another example of this transition from physiological to pathological UPR is represented by the recent discovery that mutation in the coat protein I (COPI) complex, which promotes the retrograde transport of proteins from Golgi to ER necessary for ER proteostasis, results in strongly impaired B cell and T cell responses. Mice with whole-body COPI homozygous mutations develop combined immunodeficiency[158]. Activated B cells and T cells from these mice have exacerbated spliced XBP1 (XBP1s)-mediated ER stress responses that lead to impaired functions and cell death[158]. Another example of the cell-intrinsic effects of a UPR sensor is the role of XBP1 in B cells and plasma cells, in which XBP1s is an essential transcription factor regulating the expression of MHC class II proteins in B cells[159]. Genetic depletion of XBP1 in B cells led to impaired plasma cell differentiation and defects in antibody production[48]. Together, these studies highlight the importance of a balanced response of UPR sensors to the physiological functions of immune cells.

In addition to UPR signalling triggered by intracellular components, hypoxia is also associated with induction of the UPR as oxygen is needed for protein folding and isomerization. Prolyl hydroxylases and hypoxia-inducible factors have been shown to trigger ER stress, by regulating the expression of ER chaperones[160], activating PERK and activating transcription factor 4 (ATF4) and inducing the production of XBP1s[161–163]. Functionally, activation of the UPR not only deals with the accumulation of misfolded proteins but also upregulates the expression of antioxidant response elements in cells. Nutrient deprivation also drives ER stress responses. In the tumour microenvironment, highly proliferating cancer cells and immune cells rapidly consume nutrients to support their energetic needs, creating a nutrient-deprived environment. In this context, the limited amounts of glucose and glutamine have been shown to drive ER stress in both cancer cells and immune cells by impeding the production of ATP and uridine diphosphate-*N*-acetylglucosamine, two substrates required for protein folding and glycosylation[43]. ER membranes are composed of phosphatidylcholine, phosphatidylethanolamine and cholesterol in a balanced ratio to ensure proper functionality of all processes occurring in the ER[164,165]; changes in the lipid composition of the ER and the saturation status of the lipids in response to changes in extracellular lipids can be sensed by PERK and inositol-requiring enzyme 1α (IRE1α) through their transmembrane domains[166]. In this context, lipid droplets can form from the ER membrane not only to store excess triglycerides but also to remove oxidized lipids from the ER[167]. In diseases such as obesity and atherosclerosis, where the lipid composition of the microenvironment is altered, ER stress increases in immune cells and worsens disease progression.

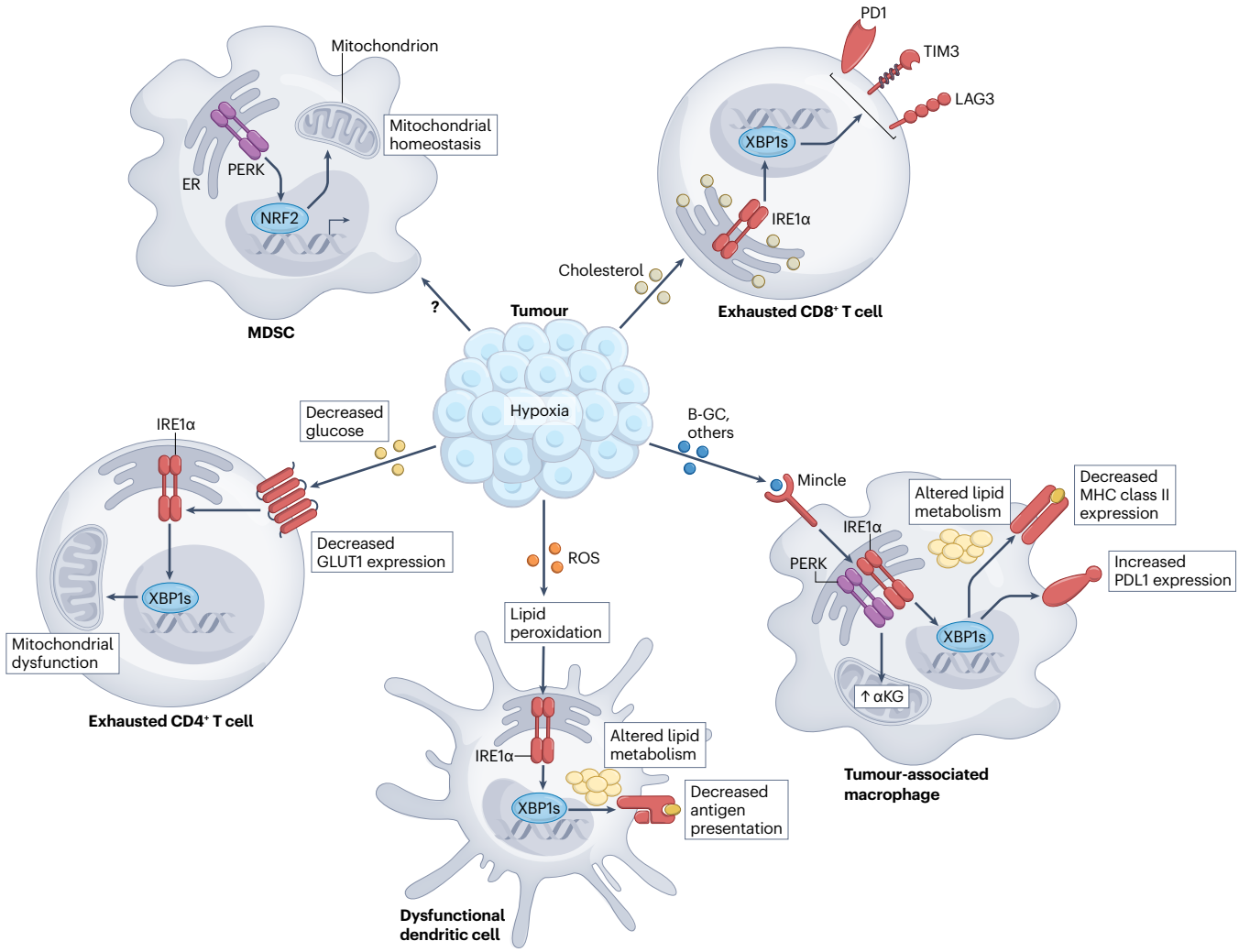

Fig. 3 | Pathological unfolded protein response signalling in immune cells in cancer. Changes to the concentrations of extracellular lipids, nutrient deprivation and hypoxia are microenvironmental features of chronic inflammatory diseases that perturb endoplasmic reticulum (ER) homeostasis and lead to unfolded protein response signalling. The tumour microenvironment (TME) is enriched in β-glucosylceramide (B-GC) produced by cancer cells that binds the pattern recognition receptor Mincle on tumour-associated macrophages. Signalling downstream of Mincle activation leads to the activation of an XBP1s-mediated ER stress response and drives imbalanced lipid composition in the ER membrane, which induces the transcription of an immunosuppressive phenotype characterized by high levels of expression of PDL1 and low levels of expression of MHC class II. PERK is also activated in tumour-associated macrophages where it promotes oxidative phosphorylation and the synthesis of increased levels of α-ketoglutarate (αKG), which is a cofactor for immunosuppressive epigenetic modification. Cholesterol in the TME is taken up by $CD8^+$ T cells, inducing an XBP1s-mediated ER stress response and the upregulation of expression of checkpoint inhibitors such as PD1, TIM3 and LAG3. Intratumoural dendritic cells are characterized by XBP1s expression triggered by the accumulation of lipid peroxides in response to reactive oxygen species (ROS) in the TME, which perturbs MHC class I expression and antigen presentation. Glucose deprivation in the TME and decreased levels of expression of the glucose transporter GLUT1 activate IRE1α–XBP1s in intratumoural $CD4^+$ T cells, which affects mitochondrial metabolism and the antitumour response. A combination of different microenvironmental features of the TME, including cytokines and disturbed lipid metabolism, activate PERK in myeloid-derived suppressor cells (MDSCs) and sustain their metabolic fitness through nuclear factor erythroid 2-related factor 2 (NRF2) activation. IRE1α, inositol-requiring enzyme 1α; PERK, protein kinase RNA-like ER kinase; XBP1s, spliced X-box protein 1.

that are specifically enriched in the TME, causing context-dependent metabolic alterations in infiltrating DCs, such as uncontrolled lipogenesis or overexpression of glucocorticoids that ultimately inhibit the development of adaptive antitumour immune responses. Hence, a careful consideration of the specific drivers of IRE1α–XBP1s activation is needed to properly understand the functional effects of this pathway in DCs. Importantly, whether the PERK and/or ATF6 branches of the UPR are also involved in regulating the activity of tumour-associated DCs remains to be determined.

Intratumoural T cells

Sustained UPR signalling also promotes the dysfunction of intratumoural T cells[61,62]. For example, in patients with ovarian cancer, intratumoural T cells had low levels of expression of the glucose

transporter GLUT1, and cell-free supernatants from malignant ascites decreased expression of GLUT1 in activated T cells from cancer-free women, which suggests that soluble factors in the TME can actively suppress expression of GLUT1 by T cells. Decreased GLUT1 expression blocked normal glucose uptake by T cells and hindered N-linked protein glycosylation, triggering aberrant IRE1α–XBP1s signalling[37]. Hyperactive XBP1s decreased the abundance of glutamine transporters on T cells, thereby limiting the uptake of glutamine that is used as an alternative carbon source for mitochondrial respiration under conditions of glucose restriction[37] (Fig. 3). Hence, intratumoural T cells have evidence of IRE1α–XBP1s-dependent mitochondrial dysfunction that limits their antitumour effector capacity. Of note, in B16 melanoma models, intratumoural $CD8^+$ T cells were found to accumulate cholesterol derived from the tumour, which induced expression of the inhibitory molecules PD1 and 2B4 on T cells and promoted T cell exhaustion in an XBP1s-dependent manner[63]. Importantly, both studies showed that XBP1 deletion specifically in T cells increased their antitumour activity and prolonged host survival compared with XBP1-sufficient T cells[37,63].

Activation of the PERK–CHOP branch of the UPR in intratumoural T cells further curtails their function in hosts with cancer. CHOP overexpression in tumour-infiltrating $CD8^+$ T cells correlated with poor clinical outcomes in patients with ovarian cancer[38], and deletion of CHOP in $CD8^+$ T cells enhanced antitumour immunity in mice through derepression of the transcription factor T-bet[38]. PERK activation has also been associated with decreased mitochondrial fitness in tumour antigen-specific T cells[64]. Disabling PERK genetically or pharmacologically in T cells prevented the mitochondrial overproduction of ROS and improved tumour control in mouse models of sarcoma and melanoma[64]. Whether UPR signalling modulates T_{reg} cell differentiation or function in cancer in addition to these effects on $CD8^+$ T cells remains to be established, although some reports do suggest that ER stress might impact T_{reg} cell biology. For example, exposure to pharmacological ER stressors increased the expression of transforming growth factor-β and IL-10 by T_{reg} cells, which was mitigated upon inhibition of PERK or its downstream substrate eIF2α[45,65]. Thus, it is possible that UPR dysregulation may also promote the immunosuppressive functions of T_{reg} cells in the TME.

Macrophages and myeloid-derived suppressor cells

Recent evidence shows that the UPR is strongly associated with local immune dysregulation in the TME[66]. Notably, the expression of UPR pathways is highly enriched in immunosuppressive tumour-associated macrophages (TAMs) in different types of human cancer[67–70]. One recent study showed that tumour-derived glucosylceramides can bind to the PRR Mincle on TAMs to induce the IRE1α–XBP1s pathway and, in parallel, activate IRE1α–STAT3 signalling to facilitate lipid remodelling of the ER and enforce the expression of immunosuppressive genes that skew TAMs towards a pro-tumoural phenotype. Disrupting the release of tumour-derived lipids or targeting the IRE1α–XBP1s and IRE1α–STAT3 axes by genetic and pharmacological strategies resulted in fewer pro-tumorigenic TAMs and delayed tumour progression[68]. In another study, Kaposi's sarcoma-associated herpesvirus, which is associated with the development of various human cancers, was found to activate STAT3 and trigger IRE1α–XBP1s signalling in macrophages, resulting in a pro-tumorigenic phenotype[71] (Fig. 3). Accordingly, other studies have shown activation of IRE1α–XBP1s in TAMs isolated from murine melanoma or human colorectal cancer, and pharmacological or genetic depletion of this pathway in TAMs resulted in improved tumour control[67,70].

In addition to the IRE1α–XBP1s pathway, a recent study has uncovered a previously undescribed role for the PERK branch of the UPR as a crucial cellular hub for reprogramming metabolic circuits and gene expression that favour an immunosuppressive TAM phenotype[69]. TAMs have robust PERK activation, which promotes mitochondrial function and amino acid metabolism by activating ATF4. Induced PERK–ATF4 signalling in TAMs is linked to the upregulation of cell-intrinsic serine biosynthesis and results in increased levels of α-ketoglutarate, a crucial metabolite for the mitochondrial tricarboxylic acid cycle and a cofactor for epigenetic modifications in immunosuppressive macrophages[69,72]. Genetic or pharmacological targeting of PERK suppressed ATF4 activity, which disrupted cellular serine synthesis and α-ketoglutarate generation (Fig. 3). In turn, this impaired mitochondrial homeostasis and histone demethylation, leading to the subsequent induction of antitumour immune responses capable of enhancing the efficacy of immune checkpoint blockade for the treatment of melanoma[69].

The UPR has also been shown to have an important role in regulating the immunoinhibitory functions of myeloid-derived suppressor cells (MDSCs) in cancer, a topic that has been reviewed elsewhere[73] and so only a few recent examples are summarized here. PERK activation supports immunosuppression mediated by tumour-infiltrating MDSCs by activating nuclear factor erythroid 2-related factor 2 (NRF2), which maintains mitochondrial homeostasis of MDSCs in the TME. PERK deficiency impairs NRF2 signalling and triggers STING-dependent production of type I IFNs to promote antitumour immunity[74]. IRE1α–XBP1s signalling has been shown to contribute to the development and lipid metabolism of the polymorphonuclear (PMN)-MDSC subset from patients with cancer[75]. Recently, it has been further illustrated that IRE1α–XBP1s and ATF6 are essential for the acquisition of immunosuppressive function by PMN-MDSCs in the TME but, surprisingly, these factors are not required for the function of monocytic MDSCs[76]. Tumour progression is associated with induction of the UPR in PMN-MDSCs; deletion of IRE1α and ATF6 in PMN-MDSCs led to enhanced antigen-specific responses and delayed tumour growth in animals[76]. Together, these new findings further indicate that the TME profoundly influences UPR pathways in tumour-associated myeloid cells, in which activated UPR signalling rewires cellular metabolic circuits and modulates gene expression to promote tolerogenic, immunosuppressive functions. Additional research is necessary to investigate the interplay between the ATF6 branch of the UPR and metabolic networks and whether this interaction further instructs the immunological activity of intratumoural myeloid cells, including TAMs, to affect adaptive antitumour immune responses and tumorigenesis.

The UPR in metabolic diseases and ageing

The ER is the main organelle involved in controlling lipid biosynthesis and lipid metabolic processes. XBP1s induces the expression of genes controlling cholesterol and phospholipid metabolism, and increased XBP1s expression drives the increased formation of ER membranes and compartments that help cells to cope with unfolded proteins and return to homeostasis. However, lipid accumulation or disbalance can itself trigger ER stress. Metabolic diseases, including obesity, atherosclerosis, non-alcoholic steatohepatitis (NASH) and type 2 diabetes, have been shown to result in disrupted lipid metabolism and ER stress in immune cells infiltrating inflamed tissues. A classic example is provided by foam cells, which are lipid-loaded macrophages with a foamy appearance that infiltrate atherosclerotic plaques. Foam cells are characterized by ER stress that often causes cell death, which increases the necrotic

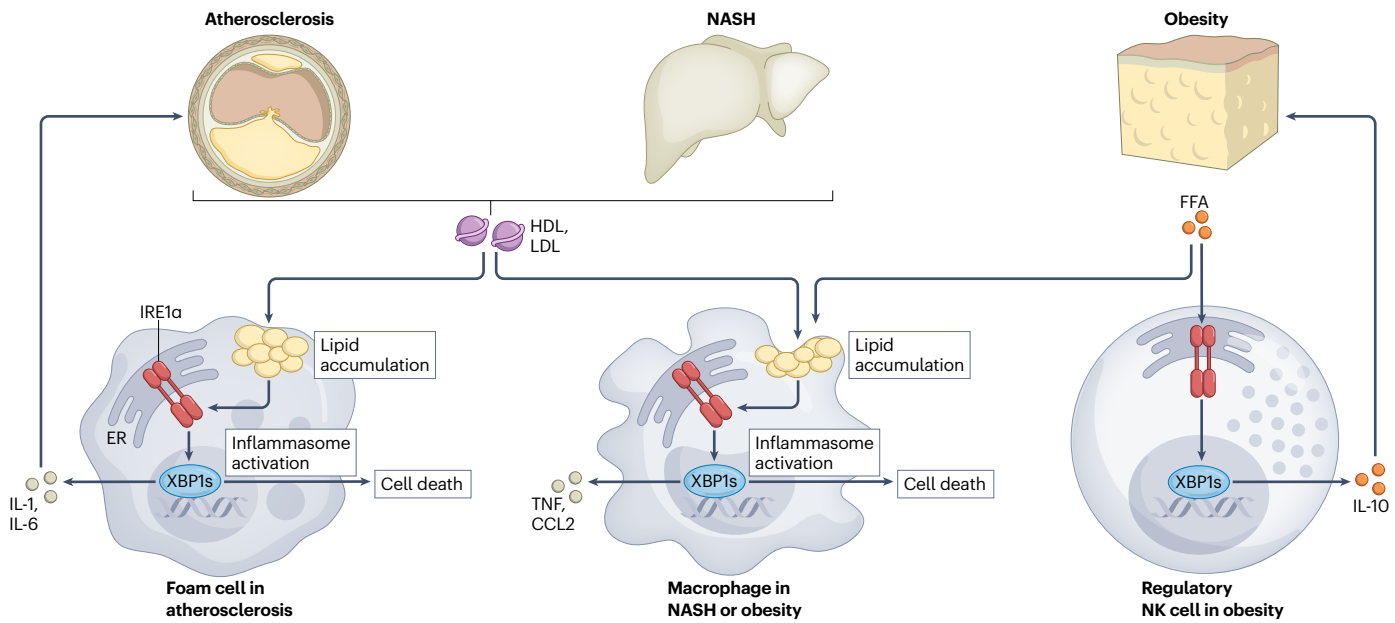

Fig. 4 | Pathological unfolded protein response signalling in immune cells in metabolic disease. In atherosclerosis and non-alcoholic steatohepatitis (NASH), lipid-loaded macrophages (known as foam cells in the case of atherosclerosis) have high levels of endoplasmic reticulum (ER) stress that can trigger inflammasome activation and cell death. In both conditions, inositol-requiring enzyme 1α (IRE1α) activation induced by lipid accumulation also drives the expression of pro-inflammatory cytokines such as IL-1β and IL-6, or tumour necrosis factor (TNF) and CC-chemokine ligand 2 (CCL2). In contrast to the pro-inflammatory role of unfolded protein response signalling in macrophages, free fatty acids (FFAs) in adipose tissue induce IRE1α activation in natural killer (NK) cells that induces a regulatory phenotype characterized by high levels of expression of IL-10. HDL, high-density lipoprotein; LDL, low-density lipoprotein; XBP1s, spliced X-box protein 1.

area of plaques and promotes inflammation, thereby contributing to disease progression[77,78]. Administration of 4-phenylbutyrate (4-PBA), a chemical chaperone used as an ER stress inhibitor[79], prevented PERK-mediated ER stress in macrophages and hence their death, which alleviated pathological features of atherosclerosis in a mouse model[80]. In addition, pharmacological inhibition of IRE1α by STF083010 or 4μ8c reduced the size of plaques in mice with atherosclerosis by decreasing macrophage infiltration and inhibiting the release of pro-inflammatory IL-1β and CCL2 (ref. [81]). Similarly, in high-fat diet-induced obesity in mice, genetic depletion of IRE1α in macrophages drives the switch from a pro-inflammatory phenotype to an anti-inflammatory phenotype, thereby alleviating pathological symptoms of insulin resistance and adipose tissue energy imbalance in obesity[82]. In a model of high-fat diet-induced NASH and hepatocellular carcinoma, administration of 4-PBA and another ER stress inhibitor taurine-conjugated tursodeoxycholic acid (TUDCA) improved pathological outcomes by inhibiting the release of TNF by inflammatory macrophages[83]. Genetic ablation of phosphocholine cytidylyltransferase A has also been used to ameliorate lipid-driven ER stress and prevent inflammation in adipose tissue-infiltrating macrophages[84]. Genetic depletion of IRE1α in myeloid cells reduced the development of diabetes-induced NASH by dampening the expression of pro-inflammatory genes[85]. These studies highlight that the UPR can promote metabolic diseases in a cell-type-dependent and context-dependent manner. Interestingly, in contrast to the pro-inflammatory role of UPR signalling in macrophages described by these studies, in invariant NK cells in adipose tissue, free fatty acids stimulate IRE1α–XBP1s signalling to induce a regulatory phenotype characterized by high levels of expression of IL-10. Adoptive transfer of these regulatory invariant NK cells to mice that are obese resulted in reduced inflammation and restoration of metabolic functions[86] (Fig. 4). This study is also in keeping with the apparently conflicting data showing that IRE1α activation in TAMs promotes an anti-inflammatory phenotype, whereas the same pathway induces a pro-inflammatory phenotype in adipose tissue macrophages. Of relevance to the anti-inflammatory effects of UPR signalling is the interaction of IRE1α–XBP1s with the transcription factor STAT3 that has been extensively reported[68,87,88]. STAT3 can promote *IL10* transcription and IL-10 can activate STAT3 in a positive-feedback loop that promotes an anti-inflammatory response[89,90]. The IRE1α–STAT3 interaction has been shown to have a role both in physiological conditions[91] and in cancer[27], but the exact molecular mechanism of this interaction is still unknown. It is possible that the UPR pathways contain a control mechanism that enables them to switch off pro-inflammatory pathways (such as those that are active in metabolic disease) and promote a regulatory, anti-inflammatory phenotype when needed (for example, in the context of the TME). Therefore, depending on the cytokine and metabolic milieu, the activation of IRE1α–XBP1s signalling might lead to different outcomes.

Similar to metabolic disease, ageing is also associated with low-grade inflammation, metabolic dysfunction and impaired proteostasis. Moreover, aged individuals have a progressive loss of ER chaperone activity and ER size[92]. However, it should be noted that most studies of ER stress and ageing have been carried out in invertebrates, and thus some of the concepts discussed remain to be confirmed in mammals. Studies in *Caenorhabditis elegans* have shown that ageing leads to an inability to respond to ER stress and that overexpression of XBP1s in neurons can rescue this defect in a non-cell autonomous manner, by

alleviating ER stress in distal organs and prolonging lifespan[93]. Similarly, providing N-glycan as a precursor substrate for the hexosamine biosynthetic pathway improves ER proteostasis and prolongs the lifespan of cultured nematodes[94]. Mechanistically, induction of the UPR in invertebrates promotes inflammation and aids the response to pathogen infection. In a model of UPR induction driven by lipoprotein accumulation, silencing of *ire-1* and *xbp-1* increased susceptibility to infection and decreased lifespan in *C. elegans*[95]. In humans, ageing-associated neurodegenerative diseases such as Alzheimer disease and Parkinson disease are associated with loss of ER proteostasis, which can result from metabolic perturbations and accumulation of misfolded proteins, and with low levels of chronic inflammation[96]. Although the role of the UPR in circulating immune cells has not been directly studied in these diseases, evidence from microglia strongly supports the need for further research in this area. For example, post-mortem analysis of brain tissue of patients with Alzheimer disease revealed the presence of UPR activation in the microglia[97], and a recent paper described a role for TREM2 expression by microglia in controlling inflammation in Alzheimer disease[98]. In this study, *Trem2*-knockout mice developed Alzheimer disease-like pathology, and *Trem2*-knockout macrophages were shown to be particularly susceptible to ER stress[98] (Fig. 5). It is known that TREM2 senses damage-associated lipids and mediates myelin phagocytosis to support microglial function in the brain[99]. Studies have shown that TREM2-deficient microglia fail to clear myelin cholesterol causing pathogenic lipid accumulation, which results in their transition towards a disease-associated microglial phenotype during ageing[100]. It is possible that the lipid-loaded microglia undergo ER stress, as occurs for TAMs in cancer or foam cells in atherosclerosis, and thus lipid accumulation could explain the increased susceptibility of TREM2-deficient microglia to ER stress[100]. In contrast, however, it has also been shown that mild ER stress in microglia favours protection against neuroinflammation[101], which further highlights the importance of balanced UPR signalling to ensure homeostasis.

In addition to neurological disorders, ageing is also associated with perturbance of intestinal functions. In the gut, T_{reg} cells have an essential role in preventing unwanted inflammation and T cell activation. The ubiquitin ligase HRD1 induces the degradation of unfolded proteins in T_{reg} cells through the ER-associated protein degradation system and thus promotes T_{reg} cell stability. Accordingly, HRD1 depletion leads to excessive inflammation in the small intestine and accelerated ageing in mice. Mechanistically, HRD1-deficient T_{reg} cells activate IRE1α and mediate ER stress-induced intestinal pathology. In mice with T_{reg} cell-specific genetic ablation of *Hrd1*, pharmacological inhibition of IRE1α restores T_{reg} cell stability and alleviates the pathology[102] (Fig. 5). These findings highlight the contribution of ER stress to ageing-related immune dysfunction, but further research is needed to directly investigate the role of ER stress in age-related immunosenescence and its contribution to the ageing process.

The UPR in autoimmunity

ER stress has been associated with many autoimmune diseases, including rheumatoid arthritis, vitiligo, inflammatory bowel disease (IBD) and multiple sclerosis. Alterations of ER proteostasis and induction of the UPR could contribute to autoantigen generation, uncontrolled cytokine production and formation of autoreactive T cells[103], all of which might contribute to the development or progression of autoimmune disease. Although the regulatory mechanisms are unclear and causal relationships not fully established, several reports have shown the involvement of ER-stressed immune cells in the pathogenesis of various autoimmune diseases. For example, $CD4^+$ T cells isolated from patients with rheumatoid arthritis have an enlarged ER compartment, evidence of ER stress and decreased mitochondrial membrane potential, which is indicative of decreased mitochondrial activity. Mechanistically, reduced mitochondrial aspartate results in a low NAD^+-to-NADH ratio that causes ADP de-ribosylation of the ER chaperone BiP and its release from IRE1α. The resulting activation of IRE1α triggers excessive production of TNF and contributes to disease progression[104]. In addition to $CD4^+$ T cells, macrophages isolated from patients with rheumatoid arthritis have increased levels of IRE1α that contribute to cytokine production in a TRAF6-dependent manner, and myeloid-specific genetic ablation of IRE1α protects mice from disease development[11].

In a recent study of patients with vitiligo — a skin disease characterized by autoimmunity against melanocytes — it has been shown that levels of XBP1s in peripheral blood mononuclear cells were higher than in the control population; this suggests that ER stress in immune cells could contribute to the pathology, although the causality and potential mechanisms remain to be determined[105]. Furthermore, dysregulated UPR signalling in intestinal epithelial cells has been implicated in the pathogenesis of IBD, with the upregulation of ER stress in intestinal epithelial cells inducing a pro-inflammatory response in gut innate immune cells[106,107]. However, a direct role of ER stress in myeloid cells has not so far been shown in IBD. Nevertheless, an elegant study has shown that macrophages isolated from patients carrying the IBD-associated risk variant of laccase domain containing 1 have defects in bacterial clearance resulting from perturbed UPR signalling induced by the PRR NOD2 (ref. [108]) (Fig. 5). This study suggests that maintaining a functional UPR in gut-associated macrophages is essential to clear bacteria and protect from IBD progression. In the EAE model of multiple sclerosis, it has been shown that the differentiation of pathogenic T_H17 cells under stress conditions is partly mediated by XBP1s and that pharmacological or genetic inhibition of XBP1 perturbs T_H17 cell differentiation and delays disease onset[42]. The UPR has also been shown to be upregulated in microglia in humans with multiple sclerosis[109], but the effects of the UPR in immune cells and its role in the aetiology or progression of multiple sclerosis remain unknown.

Therapeutic targeting of UPR pathways

Inflammation can cause considerable tissue damage and cellular stress, including ER stress. The induction of UPR signalling by ER stress can engage pro-survival or pro-apoptotic cellular processes depending on the balance of signalling. As discussed earlier, although the UPR is generally a protective, homeostatic response, it can lead to pathology when dysregulated or chronically stimulated. Thus, modulating UPR signalling or targeting the dysregulated activity of ER stress sensors could be used for therapeutic purposes — for example, to alleviate the effects of protein misfolding or aggregation in ageing-associated neurodegenerative diseases or to promote cell apoptosis and antitumour immune responses in cancer. Drugs that modulate UPR pathways have been extensively reviewed elsewhere[110]. Here, we focus on the small pharmacological molecules that have shown most promise in terms of modulating UPR activity and improving outcomes in animal models of disease, although it should be noted that the beneficial effects of these drugs may result from their actions in multiple cell types when used systemically in mice rather than specific effects on immune cells. Moreover, the use of stress sensor inhibitors may be limited by their potential toxicities in patients. Thus, we provide some examples of pharmacological means of targeting the UPR that we suggest are the most likely to proceed towards clinical use.

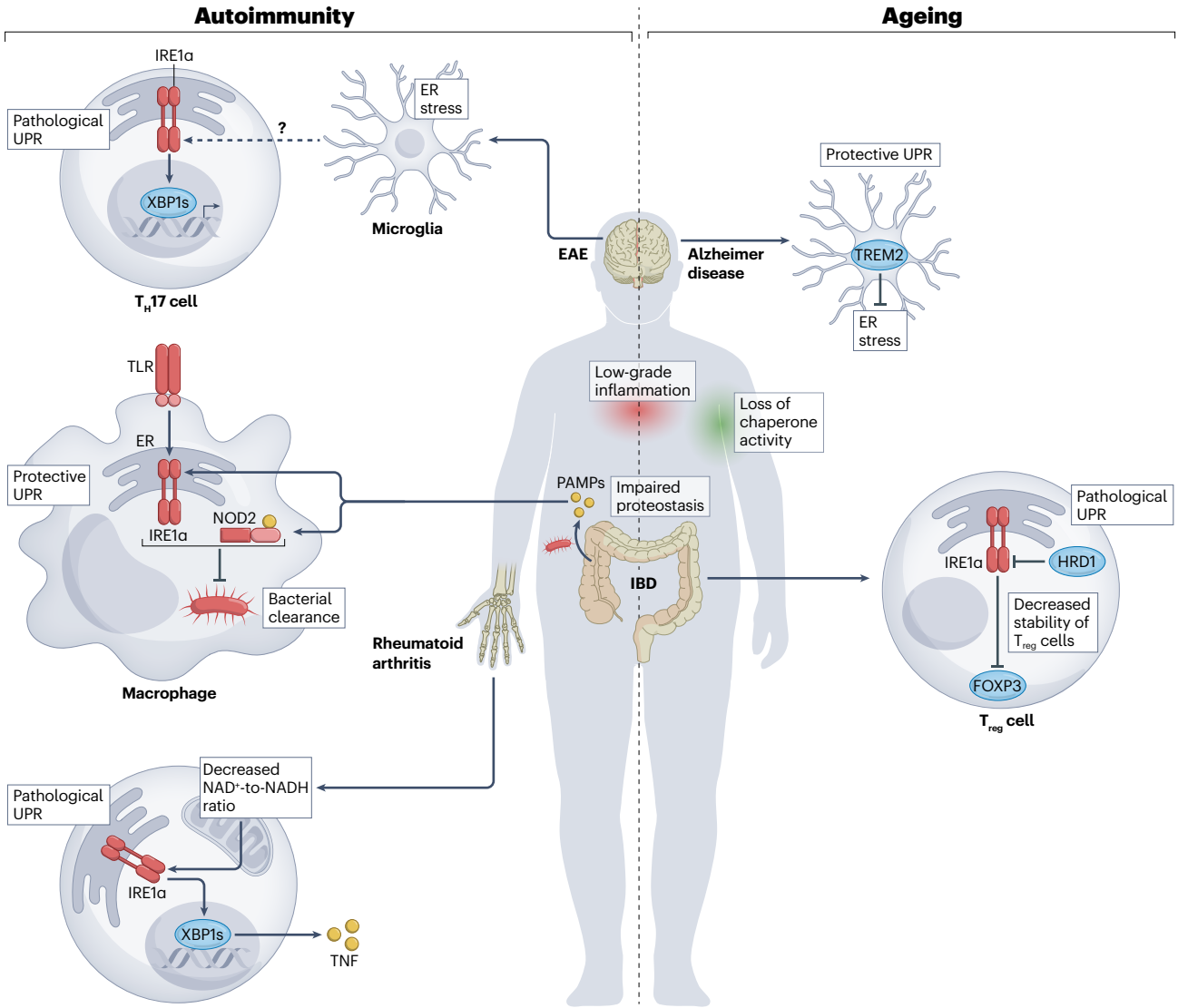

Fig. 5 | Pathological and protective unfolded protein response signalling in immune cells in autoimmune disease and ageing. In models of experimental autoimmune encephalomyelitis (EAE), endoplasmic reticulum (ER) stress has been detected in microglia, and increased IRE1α–XBP1s signalling in $CD4^+$ T cells promotes the differentiation of pathological T helper 17 (T_H17) cells. In inflammatory bowel disease (IBD), pathogen-associated molecular patterns (PAMPs) derived from the microbiota induce NOD2 and IRE1α activation in resident macrophages. This favours bacterial clearance, controlling disease progression. In rheumatoid arthritis, a perturbed NAD^+-to-NADH ratio downstream of ER stress induces ADP-deribosylation of the ER chaperone BiP and causes its release from IRE1α, which results in tumour necrosis factor (TNF) production and disease progression. In patients with Alzheimer disease, mild ER stress in microglia was shown to be protective. However, TREM2-deficient microglia, which are associated with disease progression, have increased susceptibility to ER stress, suggesting a potential pathological role for the unfolded protein response (UPR) in this setting. Finally, in ageing and IBD, the ERAD-associated E3 ubiquitin ligase HRD1 inhibits IRE1α activation, which results in reduced suppressive functions of regulatory T (T_{reg}) cells by destabilizing FOXP3. IRE1α, inositol-requiring enzyme 1α; NOD2, nucleotide-binding oligomerization domain 2; XBP1s, spliced X-box protein 1; TLR, Toll-like receptor.

IRE1α modulators

Most small-molecule antagonists of IRE1α target the catalytic activity of the RNase domain of IRE1α. IRE1α inhibitors from the salicylaldehyde family include STF-083010, ManKindCorp (MKC) analogues and B-I09[111]. Inhibition of IRE1α activity by administration of STF-083010 has been shown to counteract tissue inflammation and alleviate atherosclerosis in hyperlipidaemic mice[81] and to lessen brain injury and improve neurological behaviour in neonatal rats with hypoxic–ischaemic brain injury[112]. Moreover, treatment with STF-083010 suppresses IRE1α signalling and reprogrammes cholesterol metabolism in intratumoural $CD8^+$ T cells to promote antitumour activity in mouse melanoma models[63]. STF-083010, MKC-3946 and MKC-8866 inhibited tumour progression in mouse models of multiple myeloma, prostate cancer and lymphoma[113–116]. MKC-3946 and MKC-8866 also had synergistic effects in combination with the use of clinically approved agents in multiple myeloma[114] and prostate cancer[116], respectively. B-I09 reduced the growth of human chronic lymphocytic

leukaemia cells and promoted tumour regression in vivo[115]. In addition, ORIN1001, another IRE1α inhibitor with a good safety profile, is undergoing clinical trials in patients with advanced solid tumours and metastatic breast cancer (ClinicalTrials.gov ID: NCT03950570).

Other IRE1α modulators interact with the ATP-binding pocket of IRE1α to inhibit the active form of the kinase domain — including sunitinib, kinase-inhibiting RNase attenuator 6 (KIRA6) and KIRA8 (also known as compound 18). Sunitinib is an approved drug for patients with renal carcinoma that potentially antagonizes activity of the IRE1α kinase domain but also allosterically activates the IRE1α RNase domain in cultured insulinoma cells in vitro[117]; however, the role of sunitinib in animal models of ER stress is still unclear. KIRA6 has been shown to impede IRE1α-dependent inflammation and hence prevent the clearance of *C. rodentium* infection in mice[13]. KIRA6 also has non-canonical effects, such as inhibiting the phosphorylation of p38 in human phagocytes[118] and effects on HSP60 and the NF-κB axis that modulate inflammatory responses independently of IRE1α[119]. KIRA8 attenuates IRE1α kinase activity to protect ER-stressed pancreatic β cells from cell death, thereby reversing autoimmune diabetes in mice[120]. KIRA8 also disrupts the IRE1α-dependent growth of multiple myeloma in mice and has increased efficacy in combination with the proteasome inhibitor bortezomib or the immunomodulatory agent lenalidomide[121].

Recently, IXA4, an IRE1α activator that selectively promotes XBP1s signalling, has been identified by high-throughput screening and has shown promising therapeutic efficacy for obesity-induced metabolic dysfunction in mouse models[122] and amyloid precursor protein-triggered mitochondrial toxicity in a cellular model[123].

PERK modulators

Phosphorylation of PERK is prevented by the small molecules GSK2606414 and GSK2656157, which have been shown to impair tumour growth in various models[38,124]. Animals treated with PERK inhibitors had a marked decrease in blood vessel density and vascular perfusion of tumours. Interestingly, recent studies also indicated that the administration of GSK2606414 and GSK2656157 restricted the immunosuppressive properties and intrinsic cellular metabolism of MDSCs and TAMs, which led to increased numbers of tumour-infiltrating T cells mediating antitumour responses in animal models of lung carcinoma and melanoma, respectively[69,74]. Increased PERK-mediated stress signalling is known to inhibit the effector functions of $CD8^+$ T cells in the TME[38], and it has been shown that treatment with GSK2606414 can reactivate T cell-mediated immunity and enhance the efficacy of anti-PD1 immune checkpoint therapy in a mouse sarcoma model[64]. GSK2656157 sensitized colorectal cancer cells to cytotoxicity induced by treatment with 5-fluorouracil[125]. In addition, administration of GSK2606414 or GSK2656157 was found to be neuroprotective during brain injury or neurodegeneration in mice[126–129]. However, these effects may not only involve PERK inhibition as GSK2606414 and GSK2656157 have a potential off-target effect by interacting with receptor-interacting serine/threonine protein kinase 1 (RIPK1) to protect cells from TNF-mediated cell death[130]. They also cause toxicity in the pancreas, leading to mild hyperglycaemia in vivo[126,131], which could restrict their clinical use.

Unlike GSK2606414 and GSK2656157, AMG44 selectively inhibits the activation of PERK[132]. AMG44 markedly delayed tumour growth in mice, without alterations in glucose level or insulin secretion by pancreatic islets, by impairing the immunoregulatory activity of MDSCs in the TME to induce antitumour responses by tumour-infiltrating $CD8^+$ T cells that synergize with anti-PDL1 therapy[74]. Moreover, inhibition of PERK phosphorylation by AMG44 was shown to normalize the calcium homeostasis and large-conductance potassium channel physiology of neurons to alleviate pain in mice with EAE[133]. Two other PERK inhibitors, HC-5404-FU and NMS-03597812, are undergoing clinical trials for the treatment of solid tumours (ClinicalTrials.gov IDs: NCT04834778 and NCT05027594). In addition, ISRIB has been identified as a potent small-molecule antagonist of eIF2α phosphorylation downstream of the PERK signalling axis, with effects on the UPR[134]. ISRIB treatment had no overall obvious toxicity in animals; it could improve learning and memory in animal models[134] and it has been found to inhibit the immunosuppressive function of macrophages[69] and to promote tumour regression and survival in tumour-bearing immunodeficient NOD/SCID mice (lacking T cells, B cells and NK cells)[135]. Together, these preclinical and early clinical studies suggest that inhibition of PERK signalling has promising applications for the therapy of inflammatory diseases including cancer and neurological disorders.

Restoring ER proteostasis

It has been recognized that strategies to increase the adaptive capacity of UPR signalling pathways can ameliorate pathogenic effects in several human diseases by restoring cellular homeostasis. For example, disruptions to ER proteostasis arising from protein misfolding or defects in protein degradation may have crucial roles in several human metabolic disorders. The small molecules TUDCA and 4-PBA enhance ER protein-folding capacity to reduce ER stress, and they have been approved for use in patients with diabetes and obesity to reverse leptin resistance and hyperglycaemia and restore insulin sensitivity[136–138]. Administration of 4-PBA to animal models of brain injury mitigated ER stress and pathology by inhibiting cell apoptosis and inflammation in neurons[139,140]. Furthermore, it has been suggested that increasing IRE1α activity offers a unique opportunity to attenuate pathological

Box 3

Key outstanding questions

- When one branch of the unfolded protein response (UPR) is perturbed by genetic or pharmacological means, what are the effects on parallel signalling arms of the UPR in relation to immune outcomes?
- How does adaptation of the endoplasmic reticulum to chronic stress and its crosstalk with other organelles (such as mitochondria and Golgi) influence the inflammatory response?
- Do the protein complexes formed by inositol-requiring enzyme 1α (IRE1α), protein kinase RNA-like endoplasmic reticulum kinase (PERK) and activating transcription factor 6 (ATF6) differ in different cell types or in response to the specific types of stress to which immune cells are exposed?
- Does the activation of UPR signalling have tissue-specific imprinting as a source of phenotypic and functional heterogeneity of immune cells in different human diseases?
- Is UPR signalling co-opted by cell-type-specific transcription factors within the inflammatory milieu?
- How do sensors of the UPR integrate information regarding the intensity and duration of environmental challenges and/or cellular conditions to determine immune cell fate?

imbalances in neurodegenerative diseases. IXA4, which selectively promotes IRE1α-dependent XBP1s signalling, improved ER proteostasis of destabilized variants of amyloid precursor protein in vitro[123]. Interestingly, treatment with IXA4 also improved systemic glucose homeostasis, insulin secretion and liver and pancreas function in mouse models of obesity[122].

In summary, agents that target specific components of the UPR have been used to modulate and alleviate tissue inflammation in a wide range of disease models. However, most of these agents have not yet been extensively assessed for their immunological properties in relevant clinical models in vivo. It is apparent that ER stress occurs early during the onset of pathogenic insults and that the initial activation of the UPR is an adaptive and protective mechanism aiming to restore homeostasis. By contrast, excessive ER stress leading to a maladaptive UPR occurs mostly in the advanced stages of disease and contributes to the worsening of tissue damage and inflammation. Thus, inhibiting maladaptive UPR signalling by pharmacological means could be useful to ameliorate pathogenesis, but the timing and kinetics of any therapeutic intervention will need to be carefully considered. In addition, another major challenge is the identification and characterization of the functional points of integration between the disease-promoting UPR and the cellular signalling mechanisms in immune cells that mediate tissue homeostasis and inflammation. Although targeting the UPR alone can mitigate some aggressive features of diseases such as cancer, this may not lead to greater therapeutic efficacy than the current standard of care for advanced-stage disease. Thus, more sophisticated studies are needed to understand the effects of targeting UPR signalling earlier during disease progression and to define its global interaction network in different immune cells in different tissues, which could help to uncover potent combination treatments that have durable effects on the immune response in human inflammatory diseases.

Conclusions

Recent research progress has provided substantial insight into how UPR signalling contributes to regulating immune responses. However, many pieces of information are still missing (Box 3). We have learned that ER stress is a pathological factor in many diseases. However, we also know that short-term or low levels of ER stress are important for immune cells to function efficiently and to survive. Therefore, when thinking about targeting ER stress in pathology, care must be taken to specifically target the aberrant functions of UPR pathways while preserving their physiological functions. The role of the ATF6 branch of the UPR, in particular, is still largely unexplored owing to the lack of technical tools. As the three branches of the UPR seem to have specific and non-overlapping functions, further study of ATF6 signalling in immune cells will be crucial to gaining a more complete picture of inflammatory disease processes and their safe manipulation.

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The authors contributed equally to all aspects of the article.

Competing interests
G.D.C. is employed at iOnctura SA. P.-C.H. serves as a scientific advisor for Elixiron Immunotherapeutics and is a co-founder of Pilatus Biosciences. J.R.C.-R. serves as scientific consultant for NextRNA Therapeutics, Inc., Immagene, B.V. and Autoimmunity Biologic Solutions, Inc. J.R.C.-R. holds patents on the targeting of ER stress responses for the treatment of disease. S.C.-C.H. declares no competing interests.

Additional information
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