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REVIEW

The interplay between endoplasmic reticulum stress and inflammation

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Endoplasmic reticulum (ER) stress may be both a trigger and consequence of chronic inflammation. Chronic inflammation is often associated with diseases that arise because of primary misfolding mutations and ER stress. Similarly, ER stress and activation of the unfolded protein response (UPR) is a feature of many chronic inflammatory and autoimmune diseases. In this review, we describe how protein misfolding and the UPR trigger inflammation, how environmental ER stressors affect antigen presenting cells and immune effector cells, and present evidence that inflammatory factors exacerbate protein misfolding and ER stress. Examples from both animal models of disease and human diseases are used to illustrate the complex interactions between ER stress and inflammation, and opportunities for therapeutic targeting are discussed. Finally, recommendations are made for future research with respect to the interaction of ER stress and inflammation.
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Misfolding of some proteins occurs during biosynthesis, especially the complex secretory and transmembrane proteins assembled in the endoplasmic reticulum (ER). As part of normal cellular housekeeping, a complex molecular network has evolved to promote proper folding, and identify and degrade misfolded proteins. However, mutations predisposing to misfolding in both substrate and pathway chaperones, altered cellular metabolism, local factors, and environmental factors, including infection, can all promote increased protein misfolding. When this occurs in the ER it leads to a condition known as ER stress, which can result in inflammatory signalling by the stressed cells. Evidence is accumulating that ER stress occurs in chronic inflammatory and autoimmune disease and that in some cases ER stress may contribute to the initiation of these conditions. The purpose of this article is to explore how protein misfolding and ER stress may contribute to either the genesis or phenotype of chronic inflammation and autoimmune disease. Rather than provide a comprehensive review of all of the complex cell biology surrounding ER stress the major concepts and relevant pathways are introduced, and our emphasis is on consideration of how ER stress may trigger inflammation, and how inflammation itself can result in protein misfolding and ER stress. Animal models of inflammatory disease and human inflammatory and autoimmune disease are used to illustrate the potential importance of ER stress in chronic inflammation and autoimmune disease. Finally, the potential therapeutic opportunities and challenges for future research are identified and discussed.

ER stress has been implicated in chronic diseases involving inflammation including diabetes and obesity, neurodegenerative and neuromuscular inflammatory diseases, arthritis and spondyloarthropathies, multiple forms of respiratory inflammation and inflammatory bowel diseases (IBD).[1–6] Evidence for ER stress in these diseases is discussed in a later section of the review. However, one of the fundamental unanswered questions in many of these conditions is whether ER stress is a primary contributor to the genesis of disease or a consequence of the condition. We suggest that, in some circumstances, ER stress can initiate disease but that inflammation, in some cases owing to infection, is an important exacerbator of ER stress and can be the trigger for the onset of disease in a genetically susceptible individual. Alleviating ER stress has therapeutic potential regardless of whether ER stress is a primary, initiating event or a secondary perpetuator of chronic inflammation. We therefore argue that it is important to dissect the role of ER stress in each of these individual diseases in order to assess risks and implement appropriate therapeutic approaches. In considering the role of protein misfolding and ER stress in inflammatory and autoimmune disease, it is important to review the major drivers of protein misfolding and to consider why the ER stress associated molecular network is integrated with immunity.

The background rate of protein misfolding in any given cell type is dependent largely on the complexity of proteins being synthesised and the total protein production rates. Secretory cells that produce large amounts of complex proteins, for example, mucosal goblet cells, which produce the secreted mucus barrier to protect against microbes, or plasma cells, which secrete antibodies, experience relatively high rates of protein misfolding, but have well adapted responses to cope

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and ensure continuing protein production. A major evolutionary factor forging a link between ER stress and immunity is viral infection, because during viral infection cells are required to synthesise large amounts of viral proteins, which often misfold.[7] Another infection-related source of ER stress are toxins produced by pathogenic microbes, such as the Shiga toxins, which often reach the ER via retrograde transport in the secretory pathway.[8] Local environmental factors, which can be influenced by infection and inflammatory responses, can also modulate ER stress. For example, altered energy supply, disturbed intracellular calcium and the production of reactive oxygen species (ROS) can all induce or exacerbate ER stress. ATP levels and oxidative metabolism, and mitochondrial function, which can be altered during infection, can also contribute to ER stress. Therefore, the network of intracellular signalling and transcriptional changes known as the unfolded protein response (UPR) that ensues from ER stress has evolved, in some circumstances, to result in inflammatory signalling.

THE UPR AS A TRIGGER FOR INFLAMMATION

ER stress initiates a molecular cascade involving coordinated activation of specific enzymes and transcription factors that act to alter conditions in the ER to restore homeostasis, but which can induce inflammatory signalling and/or apoptosis if ER stress is chronic or severe.[9] Within the ER there are numerous chaperones which associate with proteins during folding to promote correct folding, sense misfolding and prevent protein aggregation. The central factor in initiation of the UPR is a heat shock protein family chaperone GRP78, which is also known as BiP and encoded by the *HSPA5* gene. In addition to associating with proteins during folding, GRP78 binds three major ER transmembrane molecules, inositol requiring enzyme 1 (IRE1α/β), protein kinase RNA-like ER kinase (PERK) and activating transcription factor 6 (ATF6α/β), each of which initiates downstream effectors of the UPR (see Figure 1). When protein misfolding increases, GRP78 accumulates with the misfolded proteins and disassociates from the UPR initiating molecules, triggering their activation and downstream UPR signalling.[9] UPR signalling is complex and results in inhibition of translation to relieve protein synthesis load in the ER, expansion of ER, increased production of chaperones and other molecules involved in protein folding, activation of multiple elements of ER-associated degradation (ERAD, which senses and removes misfolded proteins from the ER for ubiquitination and degradation), as well as interaction with non-ER related cellular pathways. *HSPA5* mRNA and GRP78 protein are substantially increased by the UPR and regarded as reliable ways to measure ER stress in experiments and in human disease. UPR signalling is comprehensively reviewed elsewhere, so our focus will be on UPR initiated inflammatory signalling.

The multiple mechanisms by which ER stress and UPR signalling influence inflammation are depicted in Figure 1. IRE1 is an endoribonuclease, which undergoes aggregation and autophosphorylation when GRP78 disengages and/or by direct recognition of misfolded protein complexes by its luminal domain.[9,10] Although the major function of IRE1 is specific splicing of the X-box-binding protein 1 (*XBP1*) mRNA resulting in the coding of a transcription factor, which induces UPR target genes;[11,12] there is evidence that IRE1β degrades ER-localised mRNAs perhaps targeting major secreted

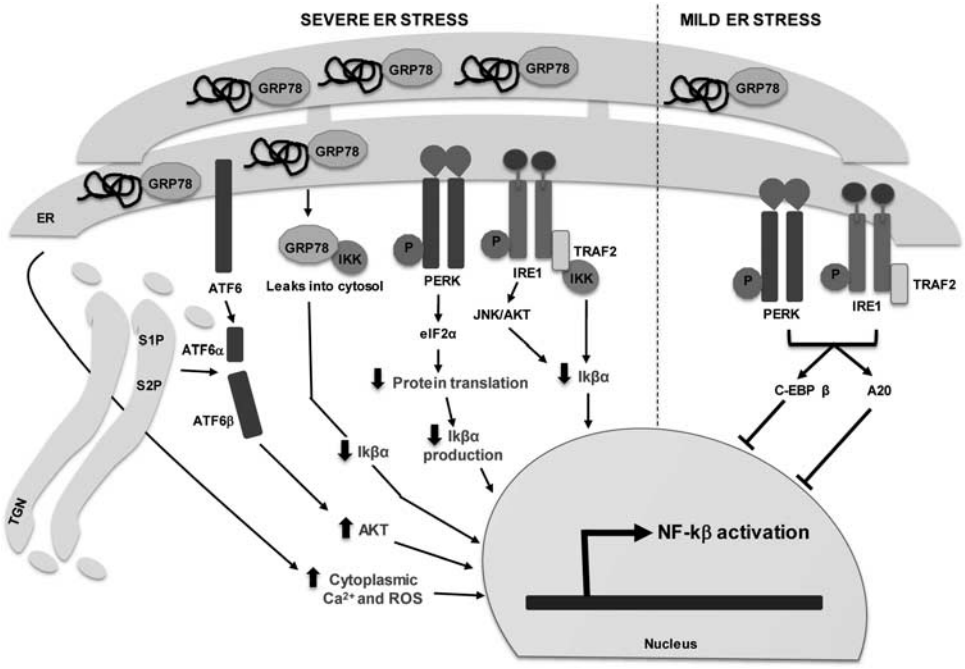


Figure 1 ER stress, UPR signalling and NF-κB activation. The three branches of the UPR, activating transcription factor 6 (ATF6), protein kinase RNA-like ER kinase (PERK) and inositol requiring enzyme 1 (IRE1), are activated when the chaperone GRP78 that is usually bound to these factors is recruited to misfolded proteins accumulating in the ER. ATF6 activation requires its migration to the trans Golgi network (TGN) where it's cleaved by site 1/2 proteases (S1P/S2P) leading to interaction with AKT and NF-κB activation. Autophosphorylation of PERK results in the phosphorylation of eIF2α, which can inhibit protein translation and lead to decreased IκBα production and thereby, induce NF-κB transcription. Phosphorylated IRE1 binds the adaptor protein TNF-receptor activating factor 2 (TRAF2), which can activate the JNK/AKT pathway, and phosphorylate NF-κB protein IκB kinase (IKK) leading to cleavage of Ikβα and activation of NF-κB. UPR-independent Ca^{2+} and ROS release and GRP78 that leaks into the cytosol have also been proposed to activate NF-κB to induce inflammation. In contrast, during mild ER stress IRE1- and PERK-dependent production of Ccaat-enhancer binding proteins (C-EBP) and A20 can inhibit activation of NF-κB in response to inflammatory stimuli, including microbial proteins and inflammatory cytokines. A full colour version of this figure is available at the *Immunology and Cell Biology* journal online.

proteins.[13,14] Additionally, quite separate from its endonuclease function, the cytoplasmic domain of activated IRE1α complexes with the NF-κB protein IκB kinase via the adaptor protein TNF-receptor activating factor 2, leading to degradation of IκBα,[15] providing one of several direct links between ER stress and NF-κB activation. Via TNF-receptor activating factor 2, IRE1 can also induce activation of AKT and JNK connecting ER stress with other major signalling pathways. PERK, like IRE1, is activated by autophosphorylation. Its major functions are to reduce protein translation by phosphorylating eIF2α, which is a component of the translation initiation complex,[16] and to modulate transcription by phosphorylating activating transcription factor 4.[12,13] The PERK-initiated inhibition of translation in ER-stressed cells results in decreased translation of IκBα, therefore leading to greater translocation of NF-κB transcription factors to the nucleus.[17,18] In response to protein misfolding ATF6α and ATF6β translocate from the ER to the Golgi where they are cleaved by the S1P and S2P (site 1 and site 2) proteases, resulting in the release and translocation to the nucleus of the active transcription factors.[19] Following exposure to the bacterial subtilase cytotoxin the ATF6 arm of the UPR also appears to activate NF-κB via phosphorylation of AKT, independently of the IRE1 or PERK pathways.[20] Although AKT has been shown to locate to the ER during ER stress, the mechanism by which ATF6 leads to AKT phosphorylation is unknown. There is also some evidence that ER overload, even in the absence of significant misfolding, can result in NF-κB activation in a classical UPR-independent, but Ca^{2+}- and ROS-dependent, manner.[21] Another less well-established proposed mechanism by which ER stress activates NF-κB involves leakage of GRP78 into the cytoplasm during stress leading to a direct interaction between cytoplasmic GRP78 and the NF-κB protein IκB kinase complex.[22]

Although all of the above evidence suggests ER stress activates NF-κB, there is an emerging body of evidence that, at least in some cell types, chronic low level ER stress can, conversely, make cells refractory to NF-κB activation and inflammatory stimulation. Preconditioning with ER stress by prior exposure to ER stressors like tunicamycin or thapsigargin, decreases disease severity in models of renal inflammation, including Heymann nephritis and mesangio-proliferative glomerulonephritis.[23,24] Preconditioned mesangial cells show decreased NF-κB activation in response to LPS, which is mediated by a PERK and IRE1-dependent increase in Ccaat-enhancer binding proteins proteins, particularly Ccaat-enhancer binding proteinβ, which are known inhibitors of activation of NF-κB in response to inflammatory cytokines.[25,26] In a similar fashion ER stress preconditioning in endothelial cells inhibits TNFα-induced NF-κB activation. Inhibition of NF-κB in these cells is dependent on a negative feedback loop involving a XBP1 transcription factor-dependent decrease in IRE1 activation by a yet to be determined mechanism.[27] The subtilase cytotoxin-induced ER stress-mediated activation of NF-κB described above also results in an increase in the A20 protein, which is a NF-κB inhibitor. Thus, A20 appears to also contribute to the ER stress 'pre-conditioning' suppression of inflammatory signalling.[28]

ER stress also contributes to inflammation by NF-κB-independent mechanisms. Although severe ER stress can trigger apoptosis, via the transcription factor CCAAT/enhancer-binding protein homologous protein (CHOP) and other mechanisms, apoptosis in the absence of danger signals should not trigger immune activation. However, there is evidence that ER stress-induced apoptosis can provide danger signals to antigen presenting cells (APCs). ER stress induced by thapsigargin, which interferes with ER Ca^{2+} transport, results in increased cell surface expression of calreticulin, which is an ER chaperone for glycoproteins. Increased cell surface calreticulin-expression in cells experiencing ER stress results in increased phagocytosis of those cells by APCs, and increased production of inflammatory cytokines by the APCs when co-exposed to Toll-like receptor (TLR)-ligand.[29] Yet to be identified soluble factors produced by ER stressed tumour cells also appear to 'transmit' ER stress to macrophages resulting in UPR activation and increased production of IL-6 and IL-23, which is accentuated when the macrophages are also exposed to LPS.[30] ER stress in the APCs themselves results in increased ER retention of antigen (antigen processing during ER stress is discussed in detail below), increased production of IL-23 and enhanced T-cell stimulation with increased production of IFNγ and TNFα by T cells.[31,32] Another example of ER stress contributing to inflammation is the deposition of hyaluronan into the extracellular matrix by cultured colonial[33] and respiratory[34] smooth muscle cells and respiratory epithelial cells[33,34] during ER stress by a mechanism not yet understood, which results in increasing local recruitment of inflammatory leucocytes. In the muscle cells hyaluronan deposition was also seen following poly(I,C) exposure/TLR3 signalling, but this was not seen in poly(I,C)-exposed epithelial cells, which are the major targets of respiratory viral infection.[34] ER stress in the liver can induce systemic inflammation by inducing release of the acute phase proteins serum amyloid P-component and C-reactive protein into the circulation.[35] Transcription of serum amyloid P-component and C-reactive protein in hepatocytes is mediated by the activation of an ER resident pro-transcription factor, cAMP-responsive element-binding protein 3-like protein 3 (CREBH), which translocates from the ER to Golgi and is cleaved by Golgi-resident proteases to form the active transcription factor. Systemic LPS or pro-inflammatory cytokines can via this mechanism result in hepatic ER stress, CREBH activation and release of serum amyloid P-component and C-reactive protein.[35] Clearly there are multiple mechanisms by which ER stress can promote inflammation, many of which may be dependent on the nature of the ER stressor and the differentiated characteristics of the ER stress affected cells. Another consideration is that immune effector cells often produce large amounts of secretory proteins and can experience ER stress, and that a functional UPR is important for their competence. For example, B cells require XBP1 to continue appropriate antibody production[36] and macrophages require XBP1 for cytokine production,[37] which will be discussed later in the review.

ER STRESS AND AUTOPHAGY

Autophagy is a process in which unwanted organelles or intracellular pathogenic microbes are surrounded by a membrane for fusion with lysosomes and degradation.[38] Several links between autophagy and ER stress via UPR signalling have been described. However, although ER stress has been shown to induce macroautophagy, there is limited conclusive evidence for autophagy of misfolded protein aggregates from the ER.[39–41] IRE1, via phosphorylation of JNK,[40] and PERK, via phosphorylation of eIF2α,[42] can induce autophagy in response to ER stress. Release of ER Ca^{2+} stores during stress leads to activation of 5′-AMP-activated protein kinase via calcium-activated calmodulin-dependent kinase kinase-β.[43] 5′-AMP-activated protein kinase in turn modulates the kinase, which is the central regulator of autophagy, mammalian target of rapamycin.[38] Autophagy is important for processing intracellular pathogens and presentation of microbial antigens on MHC Class II, but can also be a pathway for MHC Class I presentation.[44] Defects in autophagy have been linked with intestinal inflammation possibly related to inappropriate presentation of antigen from intracellular pathogens.[45–47] Thus, ER stress could affect processing of intracellular pathogens and the efficiency of MHC Class I and II antigen presentation by modulating autophagy and in turn affect the inflammatory response as discussed below.

ER STRESS AND ANTIGEN PRESENTATION

MHC Class I antigen presentation is fundamentally connected to the ER because peptides for loading onto Class I are generated from both cytosolic and ER-derived proteins, and once generated by the proteasome are returned to the ER for loading onto Class I molecules, which themselves are synthesised in the ER. Protein misfolding and ER stress would be predicted to affect Class I antigen presentation in several different ways, however, there are somewhat contradictory experimental data on the effect of ER stress on antigen presentation. Misfolded proteins that can be denatured and removed from the ER by ERAD should be more likely to be degraded in the proteasome and presented by Class I. However, while it is clear that increased degradation of cytoplasmic proteins leads to increased Class I presentation,[48] at least in some model systems misfolding of ER proteins does not lead to increased presentation.[49] One important consideration is that the PERK-induced inhibition of translation will reduce ER protein biosynthesis. One experimental study provides evidence for lowered cell surface Class I presentation during ER stress with some contribution via reduced production of Class I itself, but a larger contribution from reduced peptide loading resulting in ER retention of Class I.[50] In contrast, another study shows increasing Class I presentation of the tyrosinase antigen in melanoma cells with increasing misfolding of tyrosinase.[51] This is an important area for further research as genetic or environmentally induced protein misfolding could lead to a higher likelihood of induction and expansion of autoreactive T cells in an immunologically susceptible individual. Potential influences of ER stress on antigen presentation are depicted in Figure 2.

INFLAMMATORY FACTORS AS ER STRESS MODULATORS

Thus, far we have considered how ER stress may modulate inflammation, but another important consideration is how inflammation and infection-related factors in the microenvironment affect protein folding, ER stress and the UPR. There are surprisingly few direct studies of how individual inflammatory factors affect protein folding and ER stress. Oxidative stress is well known to increase protein misfolding and inflammatory cytokines can induce oxidative stress, and activated granulocytes and macrophages release oxidative stressors. In fibrosarcoma cells TNFα induces intracellular ROS which in turn induces ER stress, but preconditioning of these cells to ER stress by inducing misfolding was protective against the deleterious effects of ROS.[52] NO produced during an inflammatory setting can also activate the UPR by inhibiting the production of protein disulphide isomerases, resulting in the accumulation of proteins within the ER.[53] In pancreatic beta cells IL-1β, and to a lesser extent TNFα and IFNγ, increase ER stress in a nitric oxide dependent manner.[54] Another way in which inflammatory cytokines may induce ER stress is by driving increased synthesis of secretory proteins, many of which, such as the molecules produced in mucosal defence, are complex proteins that are likely to be susceptible to misfolding. IL-10, which has a key role in maintaining intestinal homeostasis, appears to modulate the UPR by inhibiting nuclear translocation of ATF6 in a p38-mediated fashion.[22] A very limited study in colon cancer cells suggests that a combination of the inflammatory cytokines IFNγ and TNFα induces ER stress.[55] Although these experiments suggest that inflammatory factors can modulate ER stress, much more comprehensive studies are required before we can understand how the diverse combinations of inflammatory factors produced during infection and sterile inflammatory disease impact on ER stress.

MICROBIAL MODULATORS OF ER STRESS AND THE UPR

During infection local inflammation occurs in the context of exposure to microbial molecules and TLR, NOD and inflammasome signalling, and it is therefore not surprising that responses to microbial factors appear to modulate the UPR. As described above, some microbial toxins enter the ER and directly induce ER stress and UPR signalling. ER stress-induced production of the transcription factor CHOP is suppressed by TLR3 or TLR4 ligands by inhibiting eIF2α induction of activating transcription factor 4 in a TRIF-dependent manner.[56] In a somewhat surprising finding, a recent study has shown that both TLR2 and TLR4 signalling in macrophages results in what is claimed to be ER stress-independent activation of IRE1α and splicing of XBP1 resulting in transcriptional changes somewhat different to those following ER-stress-induced XBP1 splicing.[37] Additionally, by using XBP1 null cells it was shown that XBP1 increases cytokine production, particularly IL-6, in response to TLR2 and TLR4 ligands and intracellular bacteria. Although these effects are claimed to be independent of ER stress, they are mediated via activation of NAPDH oxidase and production of ROS, which are known to induce ER stress.[37] In non-sterile inflammation the contribution of microbial factors to UPR signalling clearly needs to be given consideration.

Viruses have also evolved mechanisms to modulate the UPR to their advantage to ensure continued production of viral glycoproteins in the ER. For example, the hepatitis C virus NS4B protein activates IRE1 without upregulating the EDEM proteins involved in ERAD, favouring continued production of viral proteins in the face of misfolding.[57] *In vivo*, hepatitis C virus induces prolonged ER stress and UPR activation and the hepatocytes become non-responsive to further ER stress in a mechanism controlled by the virus.[58] West Nile virus uses similar non-structural proteins to stimulate IRE1 and ATF6 while suppressing the PERK/eIF2α pathway, therefore promoting chaperones via IRE1 and ATF6 that will help folding while avoiding the translation suppression mediated by PERK.[59] Coronaviruses utilise components of ERAD, which usually form membranes around misfolded protein aggregates, to derive cellular membranes for their own replication.[60] These sorts of effects on regulation of ER folding, coupled with inflammatory responses to the virus, may explain some genetic misfolding diseases only become symptomatic following viral infection.

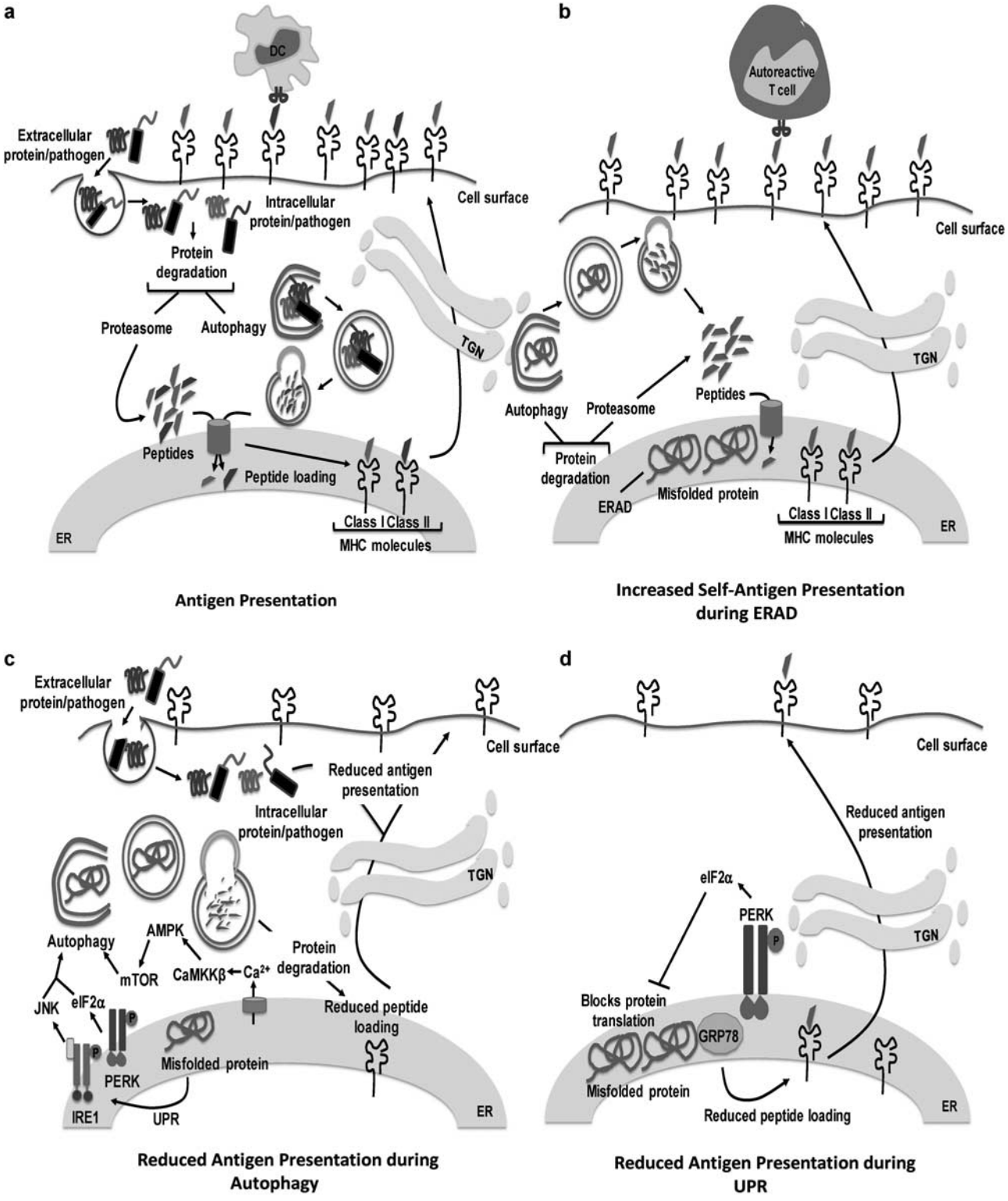
ER STRESS IN INFLAMMATORY AND AUTOIMMUNE DISEASE

The above data suggest that there are multiple levels at which inflammation and ER stress intersect and by which ER stress could either induce or modify the phenotype of inflammatory disease. Primary protein misfolding and ER stress in non-leukocytes could result in release of chemokines, cytokines and local deposition of hyaluronan resulting in recruitment, activation and retention of inflammatory leucocytes. Protein misfolding and increased autophagy could result in increased MHC Class I and Class II presentation of microbial or self-antigens. Increased cell surface calreticulin expression and ER-stress-induced apoptosis could combine to increase phagocytosis by local APCs and increased APC cytokine production and consequently T-cell activation, locally or in draining lymph nodes. Soluble factors released by ER-stressed cells could also 'transmit' ER stress to APC's and thereby, increase production of cytokines and T-cell activation. The primary ER stress could also occur in critical leucocyte populations, such as APCs, if proteins with misfolding mutations are expressed in those cells, or if the leucocytes are exposed to the local ER stressors in the affected tissues. Interestingly, environmental stressors combined with the ER load in B cells and macrophages, could affect their function as APCs as well as effector cells. ER stress in non-leukocyte and leucocyte populations are not mutually exclusive, and production of local inflammatory factors can induce or exacerbate ER stress, meaning that deciphering ER stress *in vivo* is

complex. This complexity leads to 'chicken or egg' dilemmas and many 'red-herrings' when considering the role of ER stress in inflammatory disease. Nevertheless, there is a compelling evidence that ER stress is an important facet of a broad range of inflammatory diseases, and some of this evidence is described below with an emphasis on examples from our area of research, mucosal inflammation, to illustrate the interplay between ER stress and inflammation.

Mucosal diseases

As the mucosal surfaces constitute barriers to the external world, which must also conduct other varied and complex functions, they are frequent sites of infectious disease. Consequently, these tissues have developed specialised immune surveillance to not only respond to pathogens but to maintain homeostasis usually in the presence of non-pathogenic microbial exposure.[61] Mucosal surfaces are also



commonly involved in chronic inflammatory disease, which can occur in the presence or absence of overt pathogens. We will focus on examples of ER stress from the intestinal and respiratory tracts, but there is also considerable data implicating ER stress in the eye, kidney and other mucosal tissues.

The intestine, lined by an enormous surface area of rapidly renewing epithelium, is exposed to complex populations of microbes, and therefore has a well-controlled continuous mucosal immune response to non-pathogenic microbes while retaining capability to respond strongly to pathogens. The incidence of IBD, Crohn's disease and ulcerative colitis, is progressively increasing in the developed world, paralleling the increase in autoimmune diseases. Although the major target of immune responses in IBD appears to be the gut microbes rather than self-antigens, IBD shares many immunological and genetic features with autoimmune diseases. Genome-wide studies have identified many common alleles with typically weak contributions to IBD risk, many of which overlap with other inflammatory and autoimmune diseases. However, approximately three-quarters of the genetic risk for IBD remains unexplained by common alleles.[62]

A steadily growing body of evidence in human IBD and animal models of intestinal inflammation implicates ER stress in IBD.[6] Secretory epithelial cells that produce anti-microbial molecules and the mucus barrier, which separate the epithelium from the luminal microbes, are vulnerable to ER stress. Intestinal goblet cells continuously secrete complex mucin glycoproteins that homo-oligomerise into very large molecular complexes, which give mucus its viscous barrier properties and aid retention of antibodies and anti-microbial molecules at the apical surface of all mucosal epithelia.[61] These mucins are classic candidates for misfolding in the ER, because of their size (>5000 amino acids) and their N- and C-terminal cysteine-rich domains (the MUC2 intestinal mucin has 215 cysteines) folded into complex structures, which oligomerise via inter-molecular disulphide bonds. Small intestinal Paneth cells secrete high concentrations of anti-microbial proteins into crypt base mucus overlying intestinal stem cells to ensure sterility of this region. Paneth cells produce a variety of molecules, including defensins, lectins and enzymes etc, many of which are cysteine-rich and likely to present a challenge for correct folding. ER stress in intestinal secretory cells is likely to have two pro-inflammatory consequences that are not mutually exclusive and almost certainly synergistic: (a) reducing the efficacy of the mucus barrier, thus increasing exposure of epithelial cells and underlying immune cells to luminal microbes, and (b) pro-inflammatory signalling by the ER-stressed secretory cells.

Genetically manipulated mice demonstrate that defects in protein folding or in the UPR pathway lead to spontaneous intestinal inflammation. We have characterised mice with ENU-derived misfolding mutations in Muc2, the major intestinal gel-forming mucin, which lead to ER stress in goblet cells and Paneth cells, activation of the UPR and complex inflammation involving both innate and adaptive immune responses, resembling the sort of inflammation seen in IBD.[63–65] As an instructive example of how ER stress causes inflammation and inflammation also exacerbates ER stress, recent studies in our laboratory show that, even though the primary defect in these mice is the single amino-acid substitution in MUC2, the amount of ER stress and consequent decrease in Muc2 biosynthesis is dependent on the development of inflammation. Treatment of these mice with anti-inflammatory drugs suppresses ER stress and restores mucin production even though some misfolding still occurs (see Figure 3). Interestingly, knock out of the Agr2 ER-resident protein disulphide isomerase that is tightly co-expressed with mucins also results in inflammation in the colon and small intestine.[66,67]

Coping with ER stress via the UPR is likely to be an essential element of maintaining secretory function in goblet cells and Paneth cells. Consistent with this, in the absence of any increase in primary misfolding, defects in multiple individual arms of the UPR are sufficient to induce either spontaneous or more easily inducible

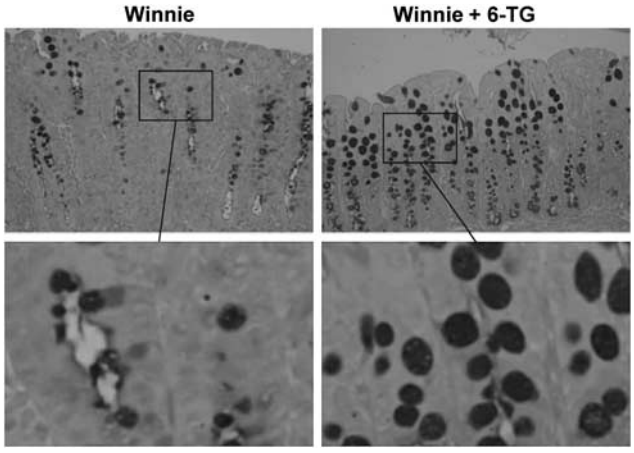


Figure 3 Demonstration of the importance of inflammation on the phenotype of misfolding disease. Production of goblet cell mucins stained with Alcian blue and PAS in mice with a Muc2 mucin misfolding mutation (Winnie) and in Winnie mice treated with the anti-inflammatory drug 6-thioguanine (6-TG). Goblet cells in Winnie mice have small blue-staining thecae (a small reservoir of granules of Muc2 for secretion) and large accumulations of pink-staining misfolded protein. After treatment with 6-TG, mucin production is restored (large blue thecae of stored mucin granules), although a small amount of misfolded protein is still seen. A full colour version of this figure is available at the *Immunology and Cell Biology* journal online.

Figure 2 ER stress and its possible effects on antigen presentation. (**a**) Intracellular self and pathogen proteins are degraded by the proteasome to form peptides that are transported to the ER and coupled to the major histocompatibility complex (MHC) Class I molecules, before being transported to the cell surface where they are recognised by immune cells, for example, dendritic cells (DC). Professional APCs can also present antigens on MHC Class II. (**b**) During ER-associated protein degradation (ERAD), misfolded proteins accumulated within the ER are translocated to the proteasome for degradation into peptides. Misfolding can thereby result in greater presentation on MHC Class I at the cell surface resulting in increased likelihood of activation of autoreactive T cells. (**c**) Misfolded proteins unable to be cleared by ERAD activate PERK and IRE1 and their downstream factors eIF2α and JNK, respectively, which can activate autophagy; a process by which misfolded protein aggregates from the ER are engulfed and degraded by lysosomal proteins. Autophagy in APCs can result in reduced protein processing by the proteasome from the ER, hence reduced peptide loading and reduced MHC Class I presentation at the cell surface, although there is some evidence for derivation of Class I peptides from autophagolysosomes. Autophagy could also result in increased presentation of self-proteins on MHC Class II. (**d**) During the UPR, GRP78 dissociates from PERK, allowing the phosphorylation of eIF2α, which can inhibit protein translation, potentially resulting in reduced synthesis of MHC molecules and an overall reduction in protein degradation and peptide loading onto MHC, and therefore reduced antigen presentation. A full colour version of this figure is available at the *Immunology and Cell Biology* journal online.

intestinal inflammation, including: (a) knockout of the intestinal specific isoform of Ire1 (Ire1β),[68] (b) intestinal epithelium-specific knockout of Xbp1,[69] and (c) hypomorphicity for the Golgi proteases that cleave Atf6.[70] Thus, either an increase in misfolding, which could be environmental or genetic, or an inappropriate UPR to the normal level of misfolding, which would be likely to be genetic, could predispose to IBD. In case–control studies, polymorphisms in genes encoding the mucin secretory protein, *MUC2*,[71] the protein disulphide isomerase, *AGR2*[72] and the UPR transcription factor, *XBP1*,[69] have all been shown to be associated with IBD, although none of these were replicated in the genome-wide studies of common alleles.

Morphological changes in goblet cells and Paneth cells are well-accepted features of IBD. These usually involve decreased intracellular secretory granules of mucin/anti-microbial proteins and vacuolation of the ER,[46,63,73,74] which are both consistent with ER stress. The usually accepted interpretation that this phenotype is because of increased secretion, ignores the capacity of intestinal goblet cells to secrete large amounts of mucins under the influence of appropriate stimuli such as classical T_H2 cytokines.[75] Most studies of ER stress in IBD, including our own, have been small and have not examined factors, which may compound such as treatment (the influence of anti-inflammatory drugs on ER stress is discussed later).[22,63,69] However, a recent more comprehensive study has shown clear evidence of ER stress in ulcerative colitis, apparently independent of treatment, and implicated regulation of translation of secretory proteins in the disease aetiology.[76] Whether ER stress proves to be primary or secondary in IBD is yet to be determined, but we would argue that it is a viable therapeutic target, regardless of the situation.

ER stress has been implicated in multiple forms of respiratory inflammatory disease. The respiratory mucosa also contains epithelial cells that synthesise complex cell surface proteins, and secrete mucins and anti-microbial proteins. Idiopathic pulmonary fibrosis (IPF) is a form of interstitial pneumonia that has a strong linkage to ER stress. Families with familial IPF that carry misfolding mutations in the surfactant protein-C (*SFTPC*) gene show evidence of ER stress and UPR activation in epithelial cells that is enhanced following viral infection, which is often the trigger for the onset of clinically evident disease in these individuals,[77,78] and is known to induce ER stress.[79] This suggests that an infection mediated boost in translation of SFTPC, perhaps coupled with secreted inflammatory ER stressors, exacerbates ER stress and leads to a cycle of chronic inflammation and fibrosis after the pathogen is cleared. In addition to this linkage with familial IPF, there is also evidence of ER stress in sporadic IPF.[80] A relatively common promoter polymorphism in the *MUC5B* mucin ($\sim$10% allele frequency in healthy individuals) increases the risk of familial interstitial pneumonia 7-fold in heterozygotes and 21-fold in homozygotes, and sporadic IPF 9-fold in heterozygotes and 22-fold in homozygotes.[81] This was an unexpected finding given that IPF is generated in the small airways not usually characterised by mucin production, and one possible mechanism postulated was ER stress.[81,82] At the same time an independent study showed atypical expression of MUC5B in surface epithelial cells in peripheral bronchioles in the majority of IPF patients in the absence of the normal goblet cell transcription factor SPDEF.[83] SPDEF drives not only mucin expression but AGR2 and other molecules required for mucin biosynthesis.[84] Taken together, these studies suggest that the MUC5B polymorphism results in inappropriate expression of MUC5B in cells lacking protein disulphide isomerases and other molecules required for correct folding, resulting in misfolding of MUC5B, ER stress, UPR and NF-κB activation, chronic inflammatory signalling and eventually the clinical presentation as fibrotic disease.

Another interesting example of the nexus between ER stress and inflammation in the lung is α-1 anti-trypsin (AAT) deficiency that arises owing to misfolding mutations in AAT, a serine protease inhibitor synthesised mainly in the liver, but which is present in serum and has an important role in the respiratory tract.[85] AATD patients present with liver disease in childhood and emphysema in adulthood. The major contributor to lung disease has always been thought to be simply due to deficiency of the protease inhibitor leading to tissue damage from granulocyte proteases such as neutrophil elastase. However, a recent study has shown that resting monocytes from individuals homozygous for the misfolding mutation show AAT ER accumulation and UPR activation, and following LPS activation these monocytes secrete more inflammatory cytokines.[86] The conclusion is that the lung disease arises, at least in part, because of hypersensitivity to inflammation perhaps following an infectious trigger, which will be compounded by the loss of the protease inhibitor. Interestingly, the authors speculate the reason why this misfolding mutation may be common is that heterozygous individuals may have some priming of their innate immunity because of misfolding while still producing AAT from their wild-type allele, giving a selective advantage under some infectious challenges. Another respiratory disease involving misfolding is cystic fibrosis (CF). The CFTR chloride ion transporter has a high propensity to misfold and the most common *CFTR* mutation (Δ508) in CF is a misfolding mutation resulting in ER retention. Although loss of CFTR function is the critical cause of CF, the Δ508 mutation results in UPR and NF-κB activation and there is substantial interest in how ER stress augments the CF phenotype in the lung and the gut.[5,87,88] Bronchial epithelial cells with the Δ508 mutation are more susceptible to respiratory viral infection/replication that appears to relate to altered ER conditions induced by misfolding of CFTR.[89] ER stress has also been reported in human chronic obstructive pulmonary disease, a largely a smoking-induced condition that responds partially to anti-inflammatory therapy, and in cigarette smoke exposed mouse lungs.[90] Asthma is characterised by a T_H2 immune response and goblet cell hyperplasia and mucus secretion, and there are no studies addressing ER stress in asthma. However, the *ORMDL3* gene, which has been linked with asthma in genome-wide studies modulates ER Ca^{2+} and the UPR.[91]

Metabolic diseases

There is growing interest in the contributions of both inflammation and ER stress to metabolic diseases including obesity and type 1 and type 2 diabetes, and readers are referred to current comprehensive reviews in this area.[1] There is evidence of ER stress in multiple tissues in metabolic diseases including the pancreas, liver, adipose tissue and the hypothalamus. In metabolic disease the initiation of ER stress is thought to mainly involve altered fatty acids consequent to high nutrient diets. In the hypothalamus,[92] pancreatic β-cells,[93] adipocytes[94] and hepatocytes[95] ER stress appears to trigger inflammation. Given the complexity of these diseases, their progressive nature and the involvement of multiple tissues, the interplay between ER stress and inflammation is particularly hard to dissect and requires much further research.

Bone and joint diseases

The main interest around ER stress in bone and joint inflammation centres around misfolding of MHC protein encoded by the HLA-B27 allele, which is tightly linked to the development of ankylosing spondylitis.[4] Whilst originally this association pointed to the development of autoimmunity against HLA-B27 presented peptides, attention is now turning to the role of ER stress in the development of

inflammation, which in this disease often also involves the intestine. Misfolding of HLA-B27 in macrophages activates the UPR, resulting in increased production of IFN-β, IL-23, and, if ER stress is more severe, IL-12.[4,96–98] Thus, it appears that misfolding of HLA-B27 may be central to the development of this autoimmune disease with other environmental and genetic factors determining whether a HLA-B27 individual will develop disease. Given the predisposition to misfolding, and the links between inflammation and ER stress, it is interesting to speculate whether infections, perhaps in the gut, could be important environmental triggers for spondyloarthropathies.

Neuromuscular diseases

ER stress has been linked with neurodegenerative diseases such as amyotrophic lateral sclerosis, Parkinson's disease, Huntington's disease and Alzheimer's disease, autoimmune neuromuscular inflammation, such as autoimmune myositis and multiple sclerosis, and with acute brain injury following ischaemia/hypoxia.[2,3] Inflammation is involved in many of these conditions and the interactions between ER stress and inflammation also appear, as in other diseases, to be a two-way street. For example, in myelin producing oligodendrocytes the response to IFNγ, which is produced in the CNS by autoreactive T cells in multiple sclerosis, is modulated by the UPR, specifically the PERK pathway.[99] Therefore, appropriate strategies to treat these complex, and often progressively degenerative conditions requires a deeper understanding of the relationship between ER stress and inflammation.

POTENTIAL THERAPEUTIC OPPORTUNITIES

The translational significance of developing knowledge of ER stress in inflammatory disease hinges mainly on the development and application of therapeutic strategies to alleviate symptoms of disease. There are multiple extracellular and intracellular pathways in a variety of cell types, targeting of which with drugs could potentially be used to reduce ER stress associated inflammation. The most appropriate strategies will vary depending on the nature of the ER stress and other features of the disease. However, drugs targeting these pathways are in development and are being trialled in pre-clinical models and human disease.

One therapeutic goal is to reduce ER stress by promoting correct folding of proteins, enhancing efficiency of ERAD or reducing the threshold of sensing of misfolded proteins to promote exit of misfolded proteins from the ER. The hydrophilic bile acid tauroursodeoxycholic acid and 4-phenylbutyric acid belong to a class of drugs, which act as ER chaperones and promote successful folding of proteins, and have been used successfully in pre-clinical models of diabetes and obesity, liver transplant related steatosis, glaucoma, cerebral ischaemic injury and Alzheimer's disease.[100–105] Tauroursodeoxycholic acid is currently in clinical trials in obesity and appears to reduce insulin resistance.[106] Although there are inhibitors of ERAD, which may be useful to exacerbate ER stress in cancer, currently there are no ERAD-promoting drugs to test the validity of this approach. However, drugs which enhance autophagy such as rapamycin and everolimus have been tested in inflammation models, although attributing their action to reducing ER stress is complicated by the other inflammation-related roles of autophagy and mammalian target of rapamycin.[107] Progressive trimming of N-glycans of misfolded ER proteins by mannosidases is the mechanism by which misfolded proteins are firstly retained in the calnexin/calreticulin folding cycle and then targeted to ERAD. The drug kifunensine is a mannosidase inhibitor, which can promote exit of misfolded proteins from the ER into the secretory pathway. In a mouse model of limb girdle muscular dystrophy type 2D caused by misfolding mutations in the alpha-sarcoglycan gene, kifunensine promoted exit of the protein from the ER and reduced ER stress.[108] Using fibroblasts derived from patients with lysosomal storage disorders, it has been shown that prolonging ER retention of misfolded proteins by using ERAD inhibitors like kifunensine in combination with proteostasis regulators that enhance the cellular protein folding capacity can synergistically rescue protein misfolding.[109]

Another therapeutic approach is to block specific elements of UPR signalling, which promote inflammation. Although there are prototype enzyme inhibitors of PERK and IRE1, one of the problems with this approach is attaining specificity for the inflammatory consequences of the UPR. Total blockage of individual UPR pathways is likely to cause severe problems in the ER due to the lack of adaptive responses to misfolding, as occurs when these enzymes are knocked out in mice. Salubrinal is an inhibitor of eIF2-α dephosphorylation by protein phosphatase 1,[110] which has been used successfully to reduce pathology in a rat model of ischaemic brain injury, mouse models of inflammation from sepsis and acute lung injury, and mouse models of emphysema and chronic obstructive pulmonary disease.[111–113] Guanabenz is another protein phosphatase 1 inhibitor, which blocks the regulatory domain and reduces ER stress by inhibiting translation without inhibiting the related PPP1R15B-phosphatase complex and constitutive protein synthesis, but is yet to be tested in pre-clinical models of misfolding.[114] Further progress in this area is dependent on the development of new more specific drugs for the UPR pathways including for specific transcription factors such as activating transcription factor 4, ATF6 and XBP1.

Given that inflammation generally appears to not only arise in misfolding diseases but to contribute to the severity of ER stress, many of these diseases should be considered candidates for specific or generalised immunosuppressive therapy. In fact, while many of these diseases are already treated with anti-inflammatory drugs, what is not clear is the contribution of these treatments to reducing ER stress. Appropriate anti-inflammatory therapy needs to be designed knowing the nature of the ER stress, the affected cells, and the inflammatory factors contributing to ER stress, and after consideration of the possible adverse effects of immunosuppression. Drugs or biologicals with specificity for the inflammatory pathways involved in exacerbating ER stress are likely to be the best way to reduce protein misfolding while not compromising immunity to infection.

PRIORITIES FOR FUTURE RESEARCH

The overall thesis of this review is that the ER stress and UPR pathways are often tightly entwined with inflammation in human disease, as illustrated in Figure 4. However, there are many significant knowledge gaps impacting on our ability to propose appropriate therapeutic approaches. Recommended areas for future research to address include:

- Identifying primary misfolding mutations underlying human inflammatory and autoimmune disease, and the cell types, including immune cells, in which these misfolded proteins are expressed.
- Defining the nature of the UPR in different cell and tissue types, particularly with respect to induction or suppression of inflammatory signals.
- Exploration of the effects of environmental ER stressors on APCs and effector cells of the immune system.
- More comprehensive analysis of the effects of different forms of ER stress and ER stress-induced autophagy on antigen presentation by both immune cells and autoimmune target cells.

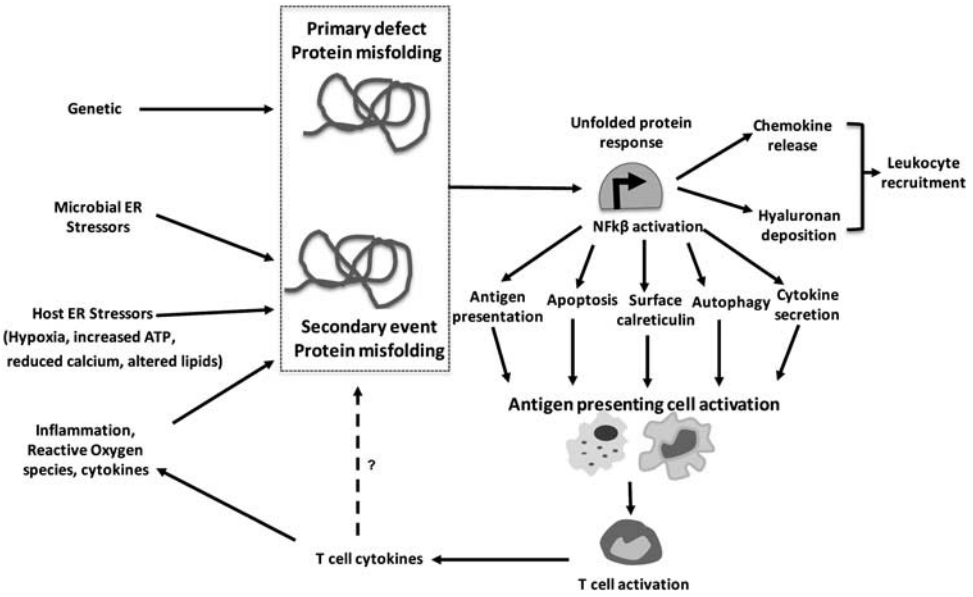


Figure 4 Schematic illustration of the interactions between protein misfolding, the UPR and inflammation. Protein misfolding can be caused by a primary defect, such as a genetic mutation, or can occur as a secondary event as a consequence of microbial ER stressors (for example, bacterial toxins), host ER stressors (for example, hypoxia and increased ATP levels) and/or of inflammation (for example, ROS, cytokines). Misfolding triggers the UPR and NF-κB activation (Figure 1) activating a network of signalling and transcriptional events that result in increased leukocyte recruitment and T-cell activation leading to increased inflammation. Inflammation once it develops exacerbates ER stress potentially leading to unresolved cycles of inflammation in susceptible individuals. A full colour version of this figure is available at the *Immunology and Cell Biology* journal online.

- Identification of which inflammatory factors modulate ER stress and elucidation of their mechanisms of action.
- Development of new drugs to alleviate ER stress, specifically block UPR-initiated inflammatory signalling, and block inflammatory factors known to trigger ER stress.

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