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Endoplasmic reticulum stress: implications for inflammatory bowel disease pathogenesis

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Abstract

Purpose of review—To provide an overview of the emerging role of cellular stress responses in inflammatory bowel disease (IBD).

Recent findings—The unfolded protein response (UPR) is a primitive cellular pathway that is engaged when responding to endoplasmic reticulum stress and regulates autophagy. Highly secretory cells such as Paneth cells and goblet cells in the intestines are particularly susceptible to endoplasmic reticulum stress and are exceedingly dependent upon a properly functioning UPR to maintain cellular viability and homeostasis. Primary genetic abnormalities within the components of the UPR (e.g. *XBPI1*, *ARG2*, *ORMDL3*), genes that encode proteins reliant upon a robust secretory pathway (e.g. *MUC2*, *HLAB27*) and environmental factors that create disturbances in the UPR (e.g. microbial products and inflammatory cytokines) are important factors in the primary development and/or perpetuation of intestinal inflammation.

Summary—Endoplasmic reticulum stress is an important new pathway involved in the development of intestinal inflammation associated with IBD and likely other intestinal inflammatory disorders.

Keywords

autophagy; Crohn's disease; endoplasmic reticulum stress; inflammatory bowel disease; intestinal epithelial cells; ulcerative colitis; unfolded protein response

Introduction

By virtue of its specific location at the frontiers of the immune system [1], the intestinal epithelium is exposed to the largest accumulation and diversity of foreign commensal and pathogenic microbial life in mammalian hosts. At the same time, intestinal epithelial cells (IECs) are exposed to an enormous range of nutrients, factors derived from both the host and microbial metabolism and signals derived from the complex and highly dynamic immune

compartment that is immediately subjacent to the epithelium [2]. In the interactions that exist between the microbiota and immune system, the IEC is not simply a way station for signals derived from these compartments, but rather a dynamic interface that continuously interacts with them [3^{**}]. In this manner, the IEC plays a critical regulatory function by virtue of its central influence on both the microbiota and immune components of the intestine (both innate and adaptive), which, no doubt, have important systemic consequences as revealed by the influences of the microbiota and its metabolic factors on inflammatory diseases not only of the intestines but also those associated with the pancreas (diabetes mellitus), joints (immune-mediated arthritis) and skin (dermatitis) [4^{*},5]. In retrospect, this might have been anticipated by the observation that the immune composition and function of the intestine and other organs are significantly altered in germ-free mice. Recent studies in well defined model systems are beginning to elucidate the profound importance of the relationship between the intestinal epithelium and the microbiota, environmental factors and immune system, as well as the influence of the host's genetic makeup on the outcome of these interactions. Most significantly, the examination of genetic influences on host responses to the commensal microbiota that are centered on the epithelial interface has revealed a number of previously unappreciated pathways that are of considerable importance to mucosal homeostasis and inflammatory disease. One of these pathways is the one associated with the unfolded protein response (UPR) and its relationship with autophagy, which is the subject of this review [6–8,9^{**},10,11].

The unfolded protein response

The UPR has recently been identified as a pathway that is critical to the maintenance of normal epithelial function and consequently the homeostasis among the microbiota, the epithelium and the intestinal immune system [9^{**}]. The UPR is engaged upon the accumulation of unfolded or misfolded proteins within the endoplasmic reticulum. As such, cells that are highly secretory such as salivary gland acinar cells, hepatocytes, plasma cells, plasmacytoid dendritic cells, pancreatic acinar cells, neuronal cells and intestinal epithelial cells (especially Paneth cells and goblet cells) are highly susceptible to endoplasmic reticulum stress. Moreover, these highly secretory cells not only require a proper tone of the UPR to be maintained for their normal function – as revealed by the inability of plasma cells to develop from B lymphocytes in the absence of a normal UPR – but are also highly susceptible to a variety of secondary or cell-extrinsic factors that further stress the secretory requirements of the cell and/or adversely influence the environment and function of the secretory pathway of the cell [12^{**},13]. The influence of each of these secondary factors likely depends upon the specific cell type, stage of cellular differentiation and/or activation state with such secondary factors including the availability of nutrients and their metabolic by-products (e.g. fatty acids), oxygen supply, cytokines and other inflammatory mediators, metabolic products of microbes and many others [13]. Once engaged in response to endoplasmic reticulum stress, the UPR includes three major proximal effectors that regulate the size of the endoplasmic reticulum through the upregulation of lipogenesis to increase the quantity of intracellular membranes, the production of chaperones to assist in protein folding (e.g. heat shock proteins), facilitation of protein degradation machinery associated with proteasomes [e.g. endoplasmic reticulum–assisted degradation (ERAD) pathway-associated

proteins] and the induction of apoptosis in the context of unabated endoplasmic reticulum stress. The UPR is initiated by the binding of misfolded proteins to glucose-regulated protein 78 (grp78), which normally associates itself with the endoplasmic reticulum luminal domains of the three arms of the UPR: pancreatic endoplasmic reticulum kinase (PERK), activation factor 6 (ATF6) and inositol-requiring enzyme 1 (IRE1). IRE1 is the most evolutionarily conserved with a homologue in yeast [13]. The UPR has been extensively reviewed elsewhere and will, therefore, only briefly be summarized here [12[•],13,14].

Once engaged through the release of grp78 from the endoplasmic reticulum luminal domain in the context of misfolded proteins, the three main arms of the UPR are engaged and regulated in a characteristic fashion. Dimerization and autophosphorylation of PERK result in phosphorylation of elongation initiation factor 2 α (eIF2 α) and consequently inhibition of protein translation except for a select group of proteins in which translation evades the inhibition by phosphorylated eIF2 α , such as ATF4, a transcription factor that is downstream of PERK. Similarly, dimerization of ATF6p90 results in its migration to the Golgi apparatus, where its cytoplasmic tail is proteolytically released as a transcriptionally active factor (ATF6p50) under the influence of site 1-specific and site 2-specific proteases (S1P and S2P). Finally, in the context of endoplasmic reticulum stress, IRE1, which has two isoforms, the ubiquitously expressed IRE1 α and IRE1 β , which is restricted in its expression to the intestinal epithelium, undergoes dimerization and autophosphorylation. Activated IRE1 possesses both endoribonuclease activity for X box binding protein 1 (XBP1) and kinase activity that results in the activation of both Jun-related kinase (JNK) and NF κ B through separate pathways. Splicing of XBP1 as a consequence of the removal of a 26-bp fragment within the intron between exons two and three creates an alternative transcript which when translated is stable and highly active as a transcription factor relative to unspliced XBP1.

Primary alterations of the IRE1/XBP1 axis and intestinal inflammation

Initial evidence that endoplasmic reticulum stress may be related to intestinal inflammation came from the observation that genetic deletion of the IEC-specific isoform of IRE1 (Ire1 β , *Ern2*) resulted in increased susceptibility of *Ire1 β ^{-/-}* mice to dextran sodium sulfate (DSS)-induced colitis [10] (Table 1 [9[•],10,11,15[•],16[•],17–20]). Although the mechanism was undefined, at the very least, it provided the first functional evidence that IRE1 β played an important role in the ability of the IEC to manage injury derived from environmental challenges.

To specifically interrogate the contribution of the IRE1/XBP1 pathway in normal IEC physiology, mice with a conditional genetic deletion of *Xbp1* were generated, wherein a Villin-promoter-driven *Cre* recombinase transgene directed deletion of a 'floxed' *Xbp1* allele specifically within the small and large intestinal epithelium [9[•]]. Together with evidence for increased endoplasmic reticulum stress in the absence of functional XBP1, these mice were observed to develop spontaneous small intestinal inflammation that strikingly resembled multiple features observed in human inflammatory bowel disease (IBD) such as crypt abscesses, leukocyte infiltration and ulcerations [9[•]]. The most highly secretory cell type in the IEC compartment is the Paneth cell, which delivers abundant

amounts of antimicrobial peptides into the lumen and is capable of secreting various inflammatory mediators including tumor necrosis factor (TNF) and adipocytokines [21,22**]. Notably, Paneth cells, which normally reside within the small intestine and in the colon during inflammation, were completely depleted in the small intestines of *Xbp1*^{Vil-*fl/fl*} mice [9**]. Moreover, mucin-secreting goblet cells were numerically reduced in *Xbp1*^{Vil-*fl/fl*} mice, and both *Xbp1*-deficient Paneth and goblet cells exhibited ultrastructural evidence of a condensed endoplasmic reticulum and only few granule remnants, in stark contrast to their wild-type counterparts. Depletion of Paneth cells was observed to be secondary to induction of apoptosis and not owing to a role for XBP1 as a lineage-committing transcription factor [9**]. As a consequence of Paneth cell depletion, like *Nod2*^{-/-} mice [23], *Xbp1*^{Vil-*fl/fl*} mice exhibited increased susceptibility to infection with *Listeria monocytogenes* as observed by increased *L. monocytogenes* in the feces upon oral infection together with increased *L. monocytogenes* translocation into the liver [9**]. Importantly, *Xbp1*^{Vil-*fl/+*} mice, which were heterozygous for *Xbp1* within the epithelium, displayed diminished crypt bactericidal activity that was intermediate to that observed in *Xbp1*^{+/+} and *Xbp1*^{Vil-*fl/+*} mice despite morphologic evidence of intact quantities of Paneth cells. This was physiologically meaningful because a substantial proportion of the *Xbp1*^{Vil-*fl/+*} mice developed spontaneous intestinal inflammation that was histologically indistinguishable from that of mice that lacked both *Xbp1* alleles [9**]. Perhaps most strikingly, within days of the conditional deletion of *Xbp1* specifically within intestinal epithelial cells during adult life, evidence of superficial intestinal inflammation and Paneth cell loss by programmed cell death can be detected [9**]. This demonstrates the primary importance of XBP1 within the epithelium in the maintenance of tissue homeostasis and conditional deletion of *Xbp1* in the intestinal epithelium generates an animal model of intestinal inflammation that is unique among those described to date [24].

However, *Xbp1* deletion affects not only Paneth cells but also the overall dynamics of epithelial cell renewal as evidenced by accelerated migration of IECs along the crypt/villus axis and, to a lesser numerical extent, mucin-producing goblet cells, which are also decreased in number within the small intestine. It is interesting that *Xbp1* deletion within IECs of the colon is not associated with spontaneous colitis but, as observed with *Irelβ*^{-/-} mice, increased susceptibility to DSS colitis. There are many possible explanations for this finding, which must await additional investigation. Nonetheless, these studies show that IEC function is generally dependent upon XBP1 function with a hierarchy of susceptibilities to XBP1 hypofunction that varies according to the secretory status of the cell types (Paneth cells > goblet cells > absorptive epithelial cells).

Insights into the mechanisms by which loss of XBP1 function results in intestinal inflammation are beginning to emerge. In introducing these mechanisms, it is important to first note that elimination of Paneth cells and loss of normal Paneth cell function do not lead to spontaneous intestinal inflammation. Specifically, neither selective depletion of Paneth cells [25] nor an inability to convert procryptdins to cryptdins via loss of MMP7 function [26] leads to spontaneous intestinal inflammation despite recent observations that Paneth cell dysfunction associated with such animal models is associated with alterations in the composition of the commensal microbiota [27]. These observations suggest that alterations

in intestinal microbiota composition (or dysbiosis) or its physical relationship with the epithelium *per se* [27] are not necessarily associated with intestinal inflammation. Recent observations that IECs with hypomorphic XBP1 function exhibit increased evidence of IRE1 activity together with hypersensitivity to what is likely to be IRE1-mediated, JNK-associated signaling in response to either cytokines (e.g. TNF) or microbial-derived factors (e.g. flagellin, a TLR5 ligand and an important antigen in Crohn's disease) [9**] suggest that dysbiosis owing to Paneth cell function may lead to intestinal inflammation when the innate and/or adaptive immune systems are sensitized to hyperrespond to a disordered microbial community. This so-called 'two-hit model' [6] would appear to be the case in the context of XBP1-deficiency wherein Paneth cell dysfunction and heightened inflammatory signaling of the epithelium in response to the local intestinal milieu of the lumen and lamina propria coincide.

The meaningfulness of these pathways to human IBD has recently been revealed through an examination of the *XBPI* gene. Consistent with three earlier studies that reported linkage between IBD and a locus on chromosome 22 within the immediate vicinity of *XBPI* [28–30], an association between polymorphisms within the *XBPI* gene and both forms of IBD, Crohn's disease and ulcerative colitis was recently discovered with replication in two additional cohorts [9**]. In these studies, the observation that the *XBPI* gene is characterized by a high degree of conservation throughout evolution and a lack of significant linkage disequilibrium despite being flanked by recombination hotspots along with several additional features indicates that the *XBPI* locus is highly complex. Given this, deep sequencing of the promoter and coding region was undertaken in more than 1000 Crohn's disease, ulcerative colitis patients and healthy controls in an effort to discover rare functional variants. These endeavors revealed approximately four-fold more rare variants in both Crohn's disease and ulcerative colitis patients as compared to healthy controls [9**]. Among these were five potentially functional nonsynonymous single-nucleotide polymorphisms (nsSNPs). An assessment of their actual frequencies revealed that four of these variants could only be detected in Crohn's disease and ulcerative colitis patients, but not in controls. When these variants were engineered into expression vectors and transfected into either a small intestinal epithelial cell line (MODE-K) or *Xbp1*^{-/-} murine embryonic fibroblasts (MEFs), it was shown that the nsSNPs observed only in IBD were hypomorphic inducers of the UPR in contrast to the one *XBPI* variant that occurred in equal frequency in IBD patients and controls and was functionally indistinguishable from wild-type *XBPI* [9**]. Obviously, given their frequencies, it is not possible to show whether any of the IBD-associated rare variants with hypomorphic function are in linkage disequilibrium with any of the multiple common variants of *XBPI* that are significantly associated with IBD. It is notable, however, that simulations of similar rare, potentially causal variants that presumably possess large biological effect sizes have suggested that the common SNPs with modest effect sizes that are detectable by genome-wide association studies may in fact be 'synthetic associations' that may be landmarks for much rarer variants with pathophysiologic relevance [31]. It remains to be determined whether this is the case for the hypomorphic rare variants that have been detected for *XBPI* in Crohn's disease and ulcerative colitis. However, taken together with the observations from XBP1-deficient mice,

these studies in humans support the possibility that IBD may emanate directly from hypomorphic XBP1 function within the intestinal epithelium.

Primary genetic alterations within the unfolded protein response leading to endoplasmic reticulum stress and intestinal inflammation

In addition to primary defects within the IRE1/XBP1 axis, evidence is emerging that other primary genetic alterations in components contained within the UPR may also associate themselves with intestinal inflammation (Table 1) [17,32*]. One example is anterior gradient 2 (*Agr2*). AGR2 is a member of the endoplasmic reticulum-resident protein disulfide isomerase (PDI) family of proteins that are important for bringing their client proteins into their proper conformation [32*]. A failure of PDI substrates to isomerize results in the accumulation of misfolded proteins within the endoplasmic reticulum and consequently endoplasmic reticulum stress. Indeed, *Agr2*^{-/-} mice exhibit increased endoplasmic reticulum stress in the intestinal epithelium as revealed by increased *grp78* expression and increased XBP1 splicing [32*]. Notably, AGR2 appears to physically interact with MUC2, an important secretory protein that requires an appropriate chaperone machinery within the endoplasmic reticulum, such that *Agr2*^{-/-} mice exhibit disrupted MUC2 protein stability and consequently decreased mucus production [32*]. In other studies, *Muc2*^{-/-} mice develop spontaneous colitis showing the importance of mucin production in intestinal homeostasis. Consistent with this, germline deletion or conditional deletion of *Agr2* results in the development of severe spontaneous ileocolitis together with decreased goblet cell MUC2 expression and an abnormal localization and expansion of Paneth cells [32*]. In contrast to *Xbp1*-deficient mice, *Agr2*^{-/-} mice do not exhibit Paneth cell depletion and display decreased, rather than increased, replenishment of the absorptive epithelium as revealed by BrdU staining [32*]. The histological infiltrate in the terminal ileum and colon of *Agr2*^{-/-} mice is characterized by dense neutrophilic infiltration in the lamina propria and submucosa, loss of goblet cells, crypt elongation and multinucleated giant cells suggestive of a granulomatous inflammation [32*]. The phenotype of these *Agr2*^{-/-} mice is particularly interesting because an earlier candidate-gene study revealed that AGR2 variants that decrease AGR2 mRNA expression are associated with Crohn's disease as well as ulcerative colitis [17].

A mouse model that originated from a forward-genetic approach, termed woodrat, further corroborates the link between unresolved endoplasmic reticulum stress and intestinal inflammation [18]. Specifically, woodrat mice, which harbor a hypomorphic variant of *Mbtps1*, encoding membrane-bound transcription factor peptidase site 1 (S1P), exhibit increased susceptibility to DSS colitis. S1P activates via cleavage several cAMP response element-binding protein/ATF transcription factors, the sterol regulatory element-binding proteins (SREBPs) and others [18]. S1P has a nonredundant role in the activation of ATF6, one of the major branches of the UPR [13]. Woodrat mice exhibit decreased colonic expression of *grp78* during DSS colitis, which may be indicative of inefficient induction of an endoplasmic reticulum stress response because *grp78* is an important transcriptional target of the UPR. S1P's role in this model could be linked to nonhematopoietic cells through experiments involving bone marrow chimeric mice [18]. These studies, thus,

indicate that the ability of non-hematopoietic cells such as intestinal epithelial cells to manage endoplasmic reticulum stress by an appropriate UPR in response to endoplasmic reticulum stress is a potential primary risk factor for the development of intestinal inflammation.

The orosomucoid1-like 3 (*ORMDL3*) gene has been shown to be associated with asthma [33], primary biliary cirrhosis [34], insulin-dependent diabetes mellitus [35] as well as Crohn's disease [16**] and ulcerative colitis [15**]. The *ORMDL3* protein is located mainly in the endoplasmic reticulum [36], and a recent study has shown that it can regulate the levels of cytosolic Ca^{2+} levels and endoplasmic reticulum-mediated Ca^{2+} signaling. Moreover, overexpression of *ORMDL3* facilitates the activation of the PERK/eIF2 α -dependent UPR signaling pathway [37]. Thus, *ORMDL3* represents another genetic risk factor that lies directly within the UPR that is associated with endoplasmic reticulum stress.

Primary genetic disturbances that secondarily impact upon the unfolded protein response and intestinal inflammation

Although not yet directly linked to the genetic basis of IBD, it is increasingly evident that abnormalities in proteins that require a robust secretory machinery may precipitate endoplasmic reticulum stress and intestinal inflammation. Whether the intestinal inflammation that is observed in these circumstances is due to the absence of the secreted protein and hence endoplasmic reticulum stress occurring secondary to inflammation, or whether the inflammation is primarily due to endoplasmic reticulum stress arising from the presence of misfolded proteins, remains to be defined. Nonetheless, the following are excellent examples of a potential cause of endoplasmic reticulum stress-induced inflammation that is secondary to primary defects in secretory proteins.

The first to be discussed is that associated with two mouse models, termed Eeyore and Winnie, which were developed through a forward-genetic approach. Both mouse models are characterized by spontaneous colitis that histologically resembles human ulcerative colitis [11]. In these models, independent missense mutations in the *Muc2* gene could be identified as the causal variants for this phenotype. *Muc2* encodes the major constituent of mucin that covers the intestinal epithelial lining, and MUC2 is secreted from goblet cells. Although inflammation in these animal models is located in the colon, the histological features observed are reminiscent of the histological changes observed in the small intestine of *Xbp1* hypomorphic mice [11]. IECs from these mouse models exhibit evidence of increased endoplasmic reticulum stress, including increased grp78 expression and XBP1 splicing, which might occur secondary to an oligomerization defect of MUC2 [11]. Of note, IECs from ulcerative colitis patients also exhibit evidence of an improperly activated UPR (as further discussed below) and appear to exhibit a glycosylation defect in MUC2. Interestingly, a locus on chromosome 12q12, which is associated with Crohn's disease, contains *MUC19*. Although other genes at this locus appear equally likely candidates that could explain the genome-wide association signal, this observation does raise the possibility that primary genetic abnormalities that are associated with alterations in the structure of mucin proteins may be a primary risk factor for the development of IBD [16**].

Another example of an abnormally folded protein that could lead to endoplasmic reticulum stress and intestinal inflammation is HLA-B27. The association between HLA-B27 and ankylosing spondylitis [38] can be explained by the capacity of HLA-B27 to induce endoplasmic reticulum stress. The heavy chain of HLA-B27 has an intrinsic tendency to misfold during assembly with β 2-microglobulin (β 2m) and peptides in the endoplasmic reticulum [39,40]. Transgenic rats expressing HLA-B27 and human β 2m develop inflammatory disease resembling HLA-B27-associated diseases, including ankylosing spondylitis and IBD [19]. In this animal model, the misfolding of HLA-B27 is associated with endoplasmic reticulum stress and subsequent activation of the UPR [41]. Moreover, there is an increase in IL-23 production by macrophages and also activation of Th17 cells suggesting that endoplasmic reticulum stress emanating from hematopoietic cells may also be a source of inflammation in IBD [42].

Secondary (environmental) factors that promote endoplasmic reticulum stress and their implications for intestinal inflammation

Substantial evidence has accumulated that unresolved endoplasmic reticulum stress owing to a variety of environmental factors might also be a prevalent secondary cause of intestinal inflammation or important factors in the perpetuation of intestinal inflammation once induced by other causes (Table 2) [7,43–46,47*,48–50]. Of note, three independent studies in various patient populations have reported evidence of increased markers indicative of endoplasmic reticulum stress in ileal and colonic tissues from human Crohn's disease and ulcerative colitis and, in some instances, have localized the endoplasmic reticulum stress to the IEC compartment [9**,11,43]. It seems likely that the endoplasmic reticulum stress is in large part owing to the effects of inflammation and potentially other environmental factors. However, these studies show that endoplasmic reticulum stress is quite common during the course of IBD and support the notion that a host's genetically endowed ability to manage endoplasmic reticulum stress is an important determinant of tissue homeostasis versus inflammation (Fig. 1). The remainder of this discussion will focus on the secondary (environmental) factors that are known to affect endoplasmic reticulum stress pathways.

A variety of inflammatory mediators have been shown to either enhance or diminish endoplasmic reticulum stress. As examples, whereas IL-10 has been reported to ameliorate endoplasmic reticulum stress [43], TNF has been recognized to be an important exacerbating factor [44]. Similarly, conditions frequently encountered in inflammatory states, such as redox balance or hypoxia, are important inducers of endoplasmic reticulum stress [12**]. Hence, an imbalance between pro-inflammatory and anti-inflammatory mediators might be an important contributor to induction of the UPR beyond levels that are necessary for the maintenance of homeostasis (Fig. 1).

In view of the substantial environmental contribution to ulcerative colitis and Crohn's disease, it is also noteworthy that specific metabolic products of the intestinal microbiota might directly affect the UPR. A macrocyclic lactam termed trierixin produced by *Streptomyces* sp. [45], or the AB₅ subtilase cytotoxin, produced by Shiga toxigenic strains of *Escherichia coli* [51], may serve as examples of interactions between the (metabolic) products of specific microbes and the UPR. Specifically, trierixin inhibits XBP1 splicing

[45], and hence could possibly result in consequences similar to mice observed with a hypomorphic *Xbp1* allele. In contrast, the A subunit of AB₅ subtilase specifically cleaves and hence inactivates grp78, resulting in broad activation of the branches of the UPR [51]. Notably, oral infection with *E. coli* engineered to express this toxin results in weight loss in mice along with a lethargic and ill phenotype, suggesting the induction of a severe and pathologic UPR [46]. Of further note is that proteins with significant sequence homology to the A and B subunits of this cytotoxin have been reported in various bacterial strains [46], raising the possibility that the normal or diseased gut microbiota might also contain variants of such a cytotoxin that directly intersects with the UPR in a detrimental manner.

Another potentially relevant ‘environmental’ factor that may be of importance to IBD is the ability of viral infections to intersect with the UPR. It is possible that this might have a role in disease induction in a genetically susceptible host as observed by the frequent occurrence of IBD relapse secondary to a variety of viral infections [52,53]. As an example, the picornavirus enterovirus 71 (EV71) induces endoplasmic reticulum stress upon infection and replication as evidenced by grp78 induction, eIF2 α phosphorylation and inhibition of translation together with activation of ATF6 [54].

From a more general perspective, it is important to also consider the recent evidence that Toll-like receptor (TLR) signaling may suppress a specific UPR target gene, *Ddit3* (Chop), which is important to induction of apoptosis during a UPR. Recent studies suggest that the decrease in Chop expression is owing to impaired eIF2 α -dependent translation of ATF4 resulting in decreased apoptosis of macrophages, renal tubule cells and hepatocytes [47*]. During states of systemic endoplasmic reticulum stress, ligation of TLR4 by its ligand was observed to prevent renal dysfunction and hepatic steatosis [47*]. This suppressive effect of TLR signaling on Chop expression was dependent on Toll/IL-1-receptor domain-containing adaptor inducing IFN- α (TRIF) [47*]. It was speculated that this mechanism might have evolved to allow for the survival of TLR-expressing cells that experience prolonged physiological UPR activation in response to microbial invasion of the host [47*]. This mechanism needs further specific consideration in the context of IECs, which experience a physiologic state of continuous exposure to pathogen-associated membrane proteins and may as such be an important protective pathway [2]. A further aspect that might be relevant in this context is that TRIF in myeloid cells has been identified as a key mediator that is associated with the pro-inflammatory state that has been observed in ATG16L1-deficient cells and hence might represent an important additional link between endoplasmic reticulum stress and autophagy pathways (see below) [55].

Environmental stressors of the UPR might also be present in food, toxins (as discussed above) as well as pharmacological agents that are commonly ingested. It is well known, for example, that free fatty acids or glucose deprivation can induce the UPR. Whether these metabolic factors are associated with pathophysiological alterations of the UPR within the intestines remains to be established. However, it is notable that the HIV protease inhibitors lopinavir and ritonavir have recently been reported to induce endoplasmic reticulum stress resulting in partial impairment of IEC barrier integrity through the induction of apoptosis via a Chop-dependent mechanism [48]. This appears particularly relevant as HIV protease

inhibitor-induced adverse events in the intestines, notably diarrhea, are common in patients on highly active antiretroviral therapy [48].

Endoplasmic reticulum stress and autophagy

Based on the discussion above, it is clear that endoplasmic reticulum stress and its management through the UPR represents a major pathway that is involved in cellular homeostasis in response to the challenges associated with metabolism, cellular differentiation and secretory function as well as the unique microbial milieu that normally resides within the complex ecosystem of the intestines. As noted, this is particularly relevant to the IEC. Another related pathway is autophagy with which the endoplasmic reticulum stress response interacts.

Autophagy represents another fundamental cellular pathway that is involved in the maintenance of homeostasis. In the classical case, autophagy is typically induced in response to starvation, whereupon it is involved in protein and organelle catabolism via a lysosomal-dependent pathway that homeostatically assists in the recycling of critical metabolic substrates [56]. As detailed elsewhere in this issue, the discovery that polymorphisms in *ATG16L1* [57], which was at that time a presumptive autophagy gene based on sequence homology with other autophagy-related genes, is specifically associated with Crohn's disease introduced this pathway to the pathophysiology of IBD. In addition to *ATG16L1*, *IRGM* and *LRRK2* have also been shown to be genetic risk factors for the development of Crohn's disease [58], lending further strong support for the importance of autophagy as a pathway that is involved in Crohn's disease, but interestingly not ulcerative colitis. *ATG16L1* hypomorphic mice, as well as Crohn's disease patients carrying a Crohn's disease-associated causal *ATG16L1* variant, exhibit a structural defect in Paneth cell granules together with an altered transcriptional profile that is consistent with a pro-inflammatory state [22^{**}]. Moreover, myeloid-specific genetic deficiency of *ATG16L1* leads to lipopolysaccharide-induced hyperactivation of the inflammasome, resulting in increased IL-1 α and IL-18 expression via a TRIF-dependent pathway [55]. However, it is interesting that neither *Atg16l1*-hypomorphic nor myeloid-specific *Atg16l1*-deficient mice develop spontaneous intestinal inflammation [22^{**},55]. Unexpectedly, activation of NOD2 with muramyl dipeptide (MDP) has recently been reported to induce autophagy via an *ATG16L1*-dependent pathway, and Crohn's disease-associated *NOD2* variants fail to induce autophagy in response to MDP and bacterial infection [59^{**},60^{**}]. Moreover, consistent with these observations that NOD2 regulates autophagy, the Crohn's disease-associated T300A *ATG16L1* variant, like the Crohn's disease-associated *NOD2* variants [59^{**},60^{**}], exhibits impaired capture and internalization of *Salmonella typhimurium* [61]. Although *Nod2*^{-/-} mice do not develop intestinal inflammation [23], they share with *Atg16l1*-deficient and *Xbp1*-deficient mice a phenotypic impairment of Paneth cell function [9^{**},22^{**},55]. It might be speculated that the mechanistic basis for this phenotypic presentation might lie in the remarkable triangulation between intracellular bacterial sensing via NOD2, endoplasmic reticulum stress and autophagy pathways. It is, therefore, intriguing that a mouse model of amyotrophic lateral sclerosis, based on the expression of mutant *Sod1* gene, recently identified an important physiologic relationship between endoplasmic reticulum stress and autophagy [62^{*}]. Specifically, in this model, unresolved endoplasmic reticulum stress owing

to *Xbp1* deficiency in the nervous system resulted in amelioration of disease owing to expression of the mutant SOD1 protein [62*]. Moreover, protection from disease was associated with increased levels of autophagy in neurons and reduced accumulation of mutant SOD1 aggregates in the spinal cord, showing that XBP1 is a regulator of autophagy [62*]. Experiments using the NSC34 motoneuron cell line indicated that silencing of *Xbp1* and *Irel1* (*Ern1*) expression similarly resulted in decreased SOD1 aggregation, suggesting that endoplasmic reticulum stress and the consequent UPR owing to XBP1-deficiency lead to induction of autophagy as a protective compensatory mechanism in a pathway that does not require IRE1 activation but does require the absence of XBP1 [62*]. This is in contrast to previous studies that have also shown that autophagy is induced by endoplasmic reticulum stress but through a pathway that was dependent upon IRE1 α [63] through activation of TRAF2 and ultimately JNK signaling [63–65]. Another arm of the UPR, which is related to the PERK/eIF2 α pathway, has also been demonstrated to be capable of inducing autophagy [66,67]. Thus, multiple components of the UPR secondary to endoplasmic reticulum stress are linked to the activation of autophagy. Given observations discussed above, it might be speculated that the ability to activate autophagy in the context of endoplasmic reticulum stress plays an important role in the development of intestinal inflammation.

Conclusion

In summary, unresolved endoplasmic reticulum stress is currently emerging as an important mechanism that links cell-intrinsic stress with organ-specific inflammation. Apart from primary genetic causes that lie directly within pathways associated with the UPR (e.g. *XPB1*, *ORMDL3*, *ARG2*) or those that are outside the UPR pathway but that are able to influence its activity (e.g. *HLAB27*, *MUC2*), a multitude of environmental, including microbial agents as well as inflammation *per se*, might intersect with this pathway and contribute to intestinal inflammation. This might be particularly relevant in conditions where a primary, genetically encoded ‘hit’ combines with an environmentally derived second ‘hit’ to initiate the cascades of inflammation. Moreover, the close intersection between endoplasmic reticulum stress and autophagy shows that this pathway is one of the major converging pathways in the pathogenesis of IBD.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 404–405).

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Figure 1.

The unfolded protein response may be considered a continuum ranging from homeostatic resolution of endoplasmic reticulum stress to an unabated endoplasmic reticulum stress response that results in tissue inflammation

Table 1

Genetic factors leading to endoplasmic reticulum stress and intestinal inflammation

Primary UPR genetic alterations	Disease association	Intestinal phenotype/animal model	References
<i>XBP1</i>	UC/CD	Spontaneous enteritis (<i>Xbp1</i> ^{-/-}); increased susceptibility to DSS colitis (<i>Xbp1</i> ^{-/-})	[9**]
<i>ORMDL3</i>	UC/CD	–	[15**,16**]
<i>AGR2</i>	UC/CD	Spontaneous ileitis and colitis (<i>Agr2</i> ^{-/-})	[17]
<i>Mbtps1</i> (S1P)	–	Increased susceptibility to DSS colitis (<i>woodrat</i>)	[18]
<i>Ern2</i> (IRE1β)	–	Increased susceptibility to DSS colitis (<i>Ire1β</i> ^{-/-})	[10]
Genetic factors secondarily leading to ER stress			
HLA-B27	AS	Spontaneous colitis (HLA-B27 Tg)	[19]
<i>MUC2</i>	–	Spontaneous colitis (<i>Eeyore</i> and <i>Winnie</i>)	[11]
<i>MUC2</i>	CD	–	[20]
<i>MUC4, MUC13</i>	UC	–	[20]
<i>MUC19</i>	CD	–	[16**]

CD, Crohn's disease; AS, ankylosing spondylitis; UC, ulcerative colitis; UPR, unfolded protein response.

Table 2

Secondary factors that promote endoplasmic reticulum stress

Secondary (environmental) factors related to ER stress	Effect on ER stress	Reference
IL-10	Reduction	[43]
TNF- α	Promotion	[44]
Hypoxia	Promotion	[12**]
Oxidative stress	Promotion	[12**]
Trierixin (<i>Streptomyces</i> sp.)	Promotion	[45]
AB ₅ subtilase cytotoxin (<i>E. coli</i>)	Promotion	[46]
TLR4 ligand	Reduction	[47*]
Lopinavir (HIV protease inhibitor)	Promotion	[48]
Thiazolidinedione	Reduction	[49]
Dopaminergic signaling	Promotion	[50]

ER, endoplasmic reticulum; IL, interleukin; TNF, tumor necrosis factor.