Digestive Diseases

Endoplasmic Reticulum Stress and Inflammation

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Key Words

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Abstract

Endoplasmic reticulum (ER) stress due to the presence of misfolded or unfolded proteins in the ER invokes a fundamental biological response, termed the unfolded protein response (UPR). The UPR is orchestrated by three main proximal effectors, of which the IRE1/XBP1 pathway represents the evolutionarily most conserved one. Due to its high secretory burden, the intestinal epithelium is highly susceptible to perturbations in the UPR as has been revealed by functional investigations such as in mice that lack *Xbp1* expression, specifically in the intestinal epithelial cells. Genetic studies have revealed several ER stress/UPR-associated genes, including *XBP1*, *ORMDL3*, *AGR2* and *MUC19* as risk factors for IBD, and specific functional, rare variants have been described for *XBP1*. *Xbp1*$^{\Delta IEC}$ mice spontaneously develop small intestinal enteritis with crypt abscesses reminiscent of human IBD, while *Agr2*$^{-/-}$ mice develop granulomatous ileocolitis. Mechanistic studies into *Xbp1*$^{\Delta IEC}$ mice revealed a major effect on Paneth cells associated with alterations in host-microbe interactions in the intestine, and the activation of key proinflammatory pathways in the host directly associated with unresolved ER stress and hypomorphic Xbp1 function. Remarkably, the intestinal epithelium of IBD patients commonly exhibits evidence of marked ER stress, which cannot easily be attributed to these genetic risk factors alone and indicates that the paradigm of ER stress-related inflammation might be both a primary originator as well as a potent perpetuator of intestinal inflammation in IBD.

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The endoplasmic reticulum (ER) is the site of translation for secretory proteins. From an evolutionary perspective, a delicate mechanism has evolved that allows cells to adapt to different levels of protein translation which is called the unfolded protein response (UPR) [1]. The UPR is instigated by the presence of misfolded or unfolded proteins in the ER, which causes ER stress and leads to the activation of an adaptive programme. The proximal 'sensors' of ER stress are the chaperone grp78 (also known as BiP) along with PERK, ATF6 and IRE1, and their downstream transcriptional effectors ATF4, ATF6 and XBP1s, respectively [1, 2]. This adaptive programme involves a temporary halt in translation (via eIF2α), the degradation of mRNA ('RIDD', regulated IRE1-dependent mRNA decay), and the transactivation of genes involved in the expansion of the ER, the secre-

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tory machinery, protein folding and protein quality control [1]. When all these mechanisms fail, unresolved ER stress leads to the apoptotic death of stressed cells.

The intestinal epithelium, consisting of the 4 epithelial cell types differentiating from a common intestinal epithelial stem cell, can be considered a highly secretory organ [3]. Specifically, Paneth cells located at the crypt bottom secrete abundant amounts of antimicrobial peptides and play an important role in regulating the composition of the intestinal microbiome and the propensity to withstand intestinal pathogens [4, 5]. Goblet cells produce large amounts of mucins which form the basis of the intestinal mucus layer [6]. Even the absorptive epithelium per se has a considerable secretory burden. In addition to these cell-intrinsic functional aspects, the intestinal environment may pose particular challenges to the cells' capacity to fold proteins properly, especially as protein folding is an energy-dependent, active process [2]. Specifically, the anaerobic milieu (in particular in the colon), low glucose, microbial toxins and metabolites, or the presence of reactive oxygen species impose challenges to proper protein folding [7]. Based on these aspects, we hypothesized that the UPR and hence ER stress might affect intestinal epithelial cell function.

Of the 3 proximal sensors of ER stress mentioned above, the IRE1/XBP1 axis is the evolutionarily most conserved one [1]. Remarkably, only the intestinal epithelium expresses a second isoform of IRE1 (i.e. IRE1β) in addition to ubiquitously expressed IRE1α [8]. We therefore were particularly interested in studying the IRE1/XBP1 axis of the UPR in intestinal epithelial cell function. To this end, we generated a mouse model ('$Xbp1^{\Delta IEC}$') with a conditional genetic deletion of a floxed $Xbp1$ gene specifically in the epithelium of the small and large intestine by using a Villin promoter-driven Cre recombinase transgene [9].

$Xbp1^{\Delta IEC}$ mice indeed exhibited evidence of increased ER stress as detected by increased expression of the chaperone grp78 [9]. Remarkably, these mice spontaneously developed intestinal inflammation in the small intestine that included histological features typically seen in human IBD; these included crypt abscesses, leukocyte infiltration and ulcerations [9].

Prompted by these results and earlier genetic linkage studies published by several groups that pointed to a locus on chromosome 22 close to the $XBP1$ gene as a potential risk locus for both forms of IBD [10–12], Crohn's disease (CD) and ulcerative colitis (UC), we performed a candidate gene study covering the $XBP1$ locus and its vicinity by 20 HapMap-selected tagging single nucleotide polymorphisms (SNPs) [9]. These studies identified a locus signal in the index cohort, which was also found in two replication cohorts [9]. Of note was the complex genetic architecture of the $XBP1$ locus, which, for example, exhibited very little linkage disequilibrium as measured by R^2, and with the $XBP1$ gene flanked by recombination hot spots [9]. We hypothesized that the locus association signal might be due to rare/private functional variants and therefore embarked on a deep sequencing effort of the $XBP1$ gene and its promoter involving a total of approximately 1,000 IBD patients and healthy controls [9]. This revealed 3- to 4-fold more rare SNPs in the IBD patients than in the controls, and identified several nonsynonymous SNPs (nsSNPs) that were only found in the patients [9]. These IBD-associated nsSNPs, when engineered into expression vectors of $XBP1$, exhibited evidence of hypomorphic induction of the UPR [9]. Altogether, these genetic studies revealed an association of the $XBP1$ locus with IBD and identified rare (private) coding variants of $XBP1$ that may represent the functional-genetic basis of this signal [9]. Moreover, this genetic insight along with the fact that $Xbp1$ conditional knock-out mice spontaneously develop intestinal inflammation that is reminiscent of human IBD posited a unique opportunity to investigate the in vivo mechanism of how this genetic risk factor (and unresolved ER stress in general) may lead to pathology [9].

In this context, an intriguing observation was that $Xbp1^{\Delta IEC}$ mice lack Paneth cells; this was associated with a virtual absence of transcripts for antimicrobial peptides that are commonly secreted by these cells [9]. Using a model pathogen, $Listeria monocytogenes$, it was established that $Xbp1^{\Delta IEC}$ mice exhibit an approximately 2-log increase in the number of $L. monocytogenes$ colonies that can be recovered from feces 10 h after oral infection [9]. Along the same lines, $Xbp1$ deficiency in the intestinal epithelium also resulted in the increased translocation of $L. monocytogenes$ to the liver, implying that absence of $Xbp1$ in the epithelium leads to an impairment of the intestinal barrier towards invading pathogens [9]. Of note is that a similar phenotype of Paneth cell dysfunction with increased $L. monocytogenes$ translocation had previously been reported for $Nod2^{-/-}$ mice [13], and decreased expression of the human α-defensins HD4 and HD5 has been found in ileal Crohn's disease, with lowest HD5 levels in patients harbouring the major $NOD2^{3020insC}$ risk allele [14]. This is important as $NOD2$ represents the genetic risk factor for CD with the largest effect size [15–17]. Further evidence for impairment of Paneth cell function as a common theme in IBD arose from a recent discovery

that hypomorphic *Atg16l1* function, another major genetic risk factor for Crohn's disease, results in a major impairment in Paneth cell granule morphology [18–20]. Remarkably, a similar phenotype of Paneth cells has been observed in CD patients homozygous for the *ATG16L1^T300A* variant [19]. The discovery of *ATG16L1* as a genetic risk factor of CD by Hampe et al. [18] has provided the first hint toward autophagy as a pathomechanism of CD, and more recent studies have reported that NOD2 is a major regulator of autophagy as well [21, 22]. This supports that the observed phenotype in Paneth cells in *Nod2^−/−* and *Atg16l1^HM* mice might possibly have a common underlying mechanism.

Paneth cell dysfunction has been reported to lead to alterations in the structural composition of the intestinal microbiome ('dysbiosis') [5]. Dysbiosis induced by host genetic alterations may also affect the susceptibility to dextran sodium sulphate (DSS)-induced colitis [23], a barrier-breaching model of intestinal inflammation. Accordingly, conditional knock-out mice that lack either one or both *Xbp1* alleles exhibit a gene-dosage-dependent increase in colitis severity in the DSS model [9]. Reduction of the microbial flora via broad-spectrum antibiotics substantially reduced the severity of colitis in this model and abrogated the genotype-related differences [9]; indeed, implying a prominent role of microbial alterations in the phenotype of *Xbp1^ΔIEC* mice.

However, it is also of note that Paneth cell depletion [24] or the inability to convert the proform of α-defensins to their active form [25], both of which lead to microbial dysbiosis [5], do not per se result in spontaneous intestinal inflammation. This is in line with the lack of spontaneous intestinal inflammation in *Nod2^−/−* and *Atg16l1^HM* mice [13, 19], and argues for additional mechanisms that drive intestinal inflammation in the context of Paneth cell dysfunction and dysbiosis. In this context, specific aspects of the IRE1/XBP1 branch of the UPR deserve further consideration.

IRE1, an ER transmembrane protein that is activated and phosphorylated upon the presence of misfolded proteins in the ER, activates XBP1 via an unconventional mechanism that involves IRE1's endoribonuclease activity to excise a 26-nt fragment from *Xbp1* mRNA (termed 'XBP1u' for unspliced) [26–29]. This excision leads to a frame shift in the translated protein (termed 'XBP1s' for spliced), and only this protein encodes the transactivation domain at its C terminus in addition to the N-terminal DNA-binding domain that is encoded by both *Xbp1u* and *Xbp1s*. In its activated state and in specific contexts of ER stress, active IRE1α may also recruit TRAF2 and

may thereby directly activate downstream inflammatory pathways [26], like JNK and NFκB. Of note is that even a biologically typically minor (i.e. 50%) reduction in *Xbp1* transcript levels by genetic deletion of one *Xbp1* allele leads to massive overactivation of IRE1 in the small intestinal epithelium, as evidenced by the predominant presence of *Xbp1s* over *Xbp1u* mRNA [9]. Using an in vitro model system (the murine small intestinal MODE-K cell line) where *Xbp1* expression is silenced via a retroviral shRNA expression vector, we noted a marked overactivation of the NFκB pathway upon stimulation with tumor necrosis factor (TNF). This was evident at several levels, including increased phosphorylation of IKK, IκBα, and NFκB p65 proteins, NFκB p65 DNA binding activity and transcription of prototypical NFκB target genes. Of note, cosilencing of *Ern1* transcription, the gene encoding IRE1α, in *Xbp1*-silenced MODE-K cells resulted in the abrogation of NFκB hyperactivation upon stimulation with TNF [Niederreiter and Adolph, manuscript in preparation]. These data suggest that NFκB signaling could be an important consequence of unresolved ER stress due to Xbp1 hypofunction. To test this, we treated *Villin-Cre-ER^T2;Xbp1^ΔIEC* mice with a selective and irreversible inhibitor of IκBα phosphorylation, BAY11-7082 [30]. *VillinCre-ER^T2;Xbp1^ΔIEC* mice express Cre recombinase under the epithelium-specific *Villin* promoter as a fusion protein with a mutated estrogen receptor, which allows controlling the time point of *Xbp1* deletion [9, 31]. Indeed, BAY11-7082 significantly ameliorated small intestinal inflammation as measured by a histological enteritis score; it also prevented Paneth cell depletion in *Xbp1*-deficient mice [Niederreiter and Adolph, manuscript in preparation]. These data might indicate that NFκB signaling may play an important role in driving enteritis under condition of unresolved ER stress in *Xbp1*-deficient mice.

Using the *VillinCre-ER^T2;Xbp1^ΔIEC* mouse model system, we also noted that TNF expression is increased in the intestinal epithelium upon genetic deletion of *Xbp1* [9]. TNF is a prototypical inflammatory mediator that is induced by several inflammatory signal transduction pathways (including NFκB), and activates a wide variety of downstream inflammatory effector mechanisms [32]. TNF signaling has been identified as a quintessential common mechanism of both forms of IBD based on the substantial efficacy of neutralizing monoclonal antibodies in CD and UC [33, 34]. Remarkably, we barely understand at the moment how the genetic underpinning of IBD connects with TNF signaling [7, 17, 35–37]. TNF signals through two distinct receptors, TNFR1 and TNFR2, with the proinflammatory signals being almost exclu-

sively relayed via the TNFR1 [38, 39]. Interestingly, TNFR1 can be recruited to active IRE1 and has been implied to mediate ER stress-induced activation of the MAP kinase JNK [40], which is activated in the $Xbp1$-deficient intestinal epithelium [9]. We were therefore interested whether TNFR1 might have a role in mediating intesti-nal inflammation in $Xbp1^{\Delta IEC}$ mice. Indeed, $Tnfr1^{-/-};Xbp1^{\Delta IEC}$ double-knockout mice were almost completely protected from spontaneous small intestinal enteritis; remarkably, this did not involve the prevention of Paneth cell depletion [Niederreiter and Adolph, manuscript in preparation]. These experiments establish that TNFR1 signaling is required for the development of intestinal inflammation in the context of unresolved ER stress due to $Xbp1$ deletion in the epithelium.

Altogether, these published and unpublished studies have established that the intestinal epithelium appears particularly susceptible to perturbations in ER stress mechanisms, which may emanate from genetic variants or environmental triggers [3, 41, 42]. Consequently, a hypomorphic ER stress response in the intestinal epithelium can lead to organ-specific inflammation as in IBD.

However, $XBP1$ is no longer the only IBD risk gene that maps to ER stress/UPR pathways. Genome-wide association studies have identified $ORMDL3$ as a genetic risk factor of CD [43] and UC (and asthma [44], another mucosal barrier disease). ORMDL3 (which localises to the ER [45, 46]) appears to be involved in protein folding, regulating the UPR [45, 46] and playing a role in sphingolipid metabolism [47]. Another particularly interesting IBD risk gene identified through a candidate gene study is $AGR2$ [48]. The protein product of this gene is an ER-resident protein disulphide isomerase and, as such, is involved in protein folding. $Agr2^{-/-}$ mice have recently been reported to spontaneously develop severe granulomatous ileocolitis, closely resembling human CD [49]. Remarkably, these mice exhibit a marked disruption of Paneth and goblet cell homeostasis [49]. A further potential ER stress-related risk gene is $MUC19$ [43], encoding a mucin gene (see below).

Irrespective of specific IBD risk genes that are related to the UPR (and which might be infrequent such as $XBP1$ [9]), it is noteworthy that several studies have reported unresolved ER stress in the intestinal epithelium as a very common phenomenon in IBD patients [9, 50–52]. Shkoda et al. [50] reported the first observation of increased grp78 expression in the epithelium in inflamed mucosa, and a more recent paper by Treton et al. [52] showed several-fold increased levels of $XBP1s$ in essentially all UC patients they had studied. In this context, it is interesting to note that Heazlewood et al. [51] reported two mouse models (called Eeyore and Winnie) generated through forward-genetic approaches that spontaneously developed colitis resembling human UC, and which were genetically mapped to distinct functional mutations of the $Muc2$ gene, which encodes the predominant mucin. These point mutations cause aberrant MUC2 biosynthesis which leads to ER stress in goblet cells, which has been suggested to be the underlying mechanism for the development of colitis [51]. Remarkably, goblet cells of UC patients exhibit evidence of MUC2 glycosylation defects [51]. Based on results from $Xbp1^{\Delta IEC}$ mice, goblet cells are susceptible to unresolved ER stress, which leads to their numeric reduction and ultrastructural changes with smaller granule size [9].

A further notable ER stress-related mechanism in IBD recently reported by Rolhion et al. [53] is related to the ER stress chaperone Gp96. Gp96 is overexpressed on the intestinal epithelium of CD patients and regulates the invasion of adherent-invasive *Escherichia coli*. Increased numbers of mucosa-adherent adherent-invasive *E. coli* are observed in patients with CD, involving the ileal mucosa in particular [54, 55].

In summary, the UPR has emerged as an important regulator of intestinal epithelial cell function and, as such, affects the host-microbial mutual relationship. Unresolved ER stress, either due to genetic variants such as $XBP1$ or environmental factors that impede proper resolution of ER stress, can be an originator of intestinal inflammation in human IBD. By means of in vivo mouse models, it has been possible to establish a mechanistic framework that connects these genetic risk factors with the evolution of intestinal inflammation. The paradigm of ER stress-related inflammation not only furthers our understanding of the mechanistic basis of IBD, but might also allow for the development of novel therapeutic approaches.

Disclosure Statement

No conflicts of interest are declared.

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