Endoplasmic reticulum stress and inflammatory bowel disease

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Abstract

Endoplasmic reticulum (ER) stress arises from the accumulation of misfolded or unfolded proteins in the ER and elicits the unfolded protein response (UPR), an adaptive signalling pathway which aims at resolving ER stress. Genetic loci that confer risk for both forms of inflammatory bowel disease (IBD) include genes that are centrally involved in the UPR, including X-box binding protein-1 (*XBP1*), anterior gradient protein-2 (*AGR2*) and orosomucoid-1-like 3 (*ORMDL3*). The intestinal epithelium, in particular mucin-secreting goblet and antimicrobial peptide-secreting Paneth cells appear particularly sensitive towards disturbances of the UPR. Supportive of this view are mice with a genetic deletion of *Xbp1* specifically in the intestinal epithelium, which develop spontaneous intestinal inflammation histologically remarkably similar to human IBD. Apart from such primary genetic factors that determine the threshold of tolerable ER stress within the epithelium, secondary factors emanating from the environment might intersect with the UPR as well. These secondary factors might include microbial products, inflammatory mediators *per se*, hypoxia and glucose deprivation, pharmacological agents, and many others. Interaction of such secondary factors in a genetically susceptible host might provide the basis for intestinal inflammation and might provide a framework to investigate gene – environment interactions in human IBD, whereby a normally homeostatic adaptive response (i.e. the UPR) transforms into a potent pathomechanism of intestinal inflammation in the context of unresolved (i.e. unresolvable) ER stress. (Acta gastroenterol. belg., **2011**, 74, **330-333**).

The single-layered intestinal epithelium forms the interface between the sterile host and the enormously abundant and complex microbiota of the intestines. As such, the epithelium not only forms a physical barrier, and fulfils key functions in nutrient absorption and many other physiological functions ; as more recent studies have revealed, the intestinal epithelium also fulfils key immunological functions and in fact is capable of directing the evolving innate and adaptive immune response (1-4). Such a profound immunological role of the epithelium, i.e. the cell type with immediate contact to the outside world, appears logical in view of evolution, and hence it is sensible to consider the epithelium as one of the phylogenetically oldest aspects of innate defence.

The intestinal epithelium of the small intestine is composed of four cellular subtypes, which all differentiate from a common intestinal stem cell (5). These are the absorptive epithelium ; mucin-producing goblet cells ; hormone-producing neuroendocrine cells ; and antimicrobial peptide and inflammatory mediators-secreting Paneth cells. While Paneth cells reside at the crypt base, the other four cell types migrate along the crypt-villus axis upwards and thereby exhibit a rather quick turnover (5-8).

One important feature of the intestinal epithelium, and Paneth and goblet cells in particular, is their high secretory activity (9). Secretory proteins are translated within the endoplasmic reticulum (ER), where a delicate system has evolved to control proper protein biogenesis (10,11). Specifically, misfolded or unfolded proteins within the ER induce stress in this cellular compartment and consequently elicit a fundamental biological signalling pathway, the unfolded protein response (UPR), which aims at resolving ER stress (10,11). In principle, the UPR as an adaptive response first results in a translational halt, and subsequently in transactivation and selective translation of genes that are involved in protein secretion, folding, and quality control, and only if all of these mechanisms fail, the UPR connects to apoptotic mechanisms and induces programmed cell death as a response of last resort (10,11). The UPR basically consists of three main branches, PERK/eIF2alpha, ATF6p90/ATF6p50, and IRE1/XBP1, with the latter representing the evolutionary most conserved branch (10,11).

Hypomorphic (or absent) function of a key mediator of the UPR, XBP1 (X-box binding protein 1, a UPR transcription factor) specifically in the intestinal epithelium results in unresolved stress in the ER as measured by the increase in grp78, a molecular chaperone within the ER (12). Remarkably, partial or complete deficiency of *Xbp1* in the intestinal epithelium results in spontaneous intestinal inflammation in the small intestine (12). The inflammatory changes are patchy and exhibit hallmarks of human inflammatory bowel disease (IBD), namely crypt abscesses, neutrophil infiltration, and ulcerations (12). *Xbp1* deletion in the intestinal epithelium has also profound implications for the composition of the epithelium, as deletion of both *Xbp1* alleles results in loss of Paneth cells and a reduction in goblet cells in the small intestine ; however, in epithelia with only one allele deleted, the cellular composition of the epithelium appears largely normal, as does the large intestine with one or two *Xbp1* alleles deleted (12). The absence of Paneth cells in $Xbp1^{-/-}$ mice results from their depletion through apoptosis due to unresolved ER stress. As a

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consequence, epithelial *Xbp1* deletion results in impaired handling of an oral infection with a model pathogen, *Listeria monocytogenes*, and in vitro bactericidal activity assays of isolated crypts revealed a functional impairment even upon deletion of only one *Xbp1* allele (12), which may imply that *Xbp1* deficiency might have substantial implications for the composition of the intestinal microbial flora as well. Hypomorphic XBP1 function also results in an increased inflammatory responsiveness of the epithelium towards signals emanating from the microbial flora (e.g. the TLR5 ligand flagellin) or the mucosal immune compartment (e.g. TNF-alpha) (12). This is highlighted be increased phosphorylation of c-Jun N terminal kinase (JNK) in vitro and in vivo (12). Mechanistically, it is remarkable that hypomorphic XBP1 function yields to massive overactivation of the kinase and endoribonuclease upstream of this molecule, namely IRE1 (12). IRE1 senses the presence of misfolded proteins in the ER and upon activation not only activates XBP1 by excising a 26nt stretch resulting in XBP1s mRNA ('s' for spliced ; only this species gives rise to the active transcription factor), but IRE1 also recruits the adapter molecule TRAF2 and as a consequence thereof results in JNK activation (13).

While the large intestine did not show evidence of spontaneous colitis, *Xbp1*$^{-/-}$ and *Xbp1*$^{+/-}$ mice exhibited increased sensitivity toward dextran sodium sulphate (DSS) (12), a well-established model of experimental colitis (14). Depletion of the microbial flora with broad-spectrum antibiotics established that the intestinal microbiota is required for the more profound manifestation of DSS colitis in *Xbp1*-deleted mice (12).

A candidate gene survey established the *XBP1* locus on chr 22 as a genetic risk locus for both forms of IBD, Crohn's disease (CD) and ulcerative colitis (UC) (12). In an effort to identify functional variants, a deep sequencing effort of the *XBP1* gene revealed a three-fold higher prevalence of rare variants in IBD patients compared to those found in healthy control subjects (12). Indeed, rare variants only found in IBD patients exhibited hypomorphic UPR induction compared to those rare variants that were present in equal frequencies in IBD patients and healthy controls, suggesting that the mechanisms established through the conditional *Xbp1* mouse model could provide a framework to understand the contribution of these IBD-associated *XBP1* variants to IBD (12).

Further mechanistic evidence that links alterations in the UPR and unresolved ER stress to intestinal inflammation comes from forward-genetics mouse models with point mutations in *Muc2* (15). These mice exhibit a severe phenotype and spontaneously develop colitis (15). *Muc2* encodes the primary constituent of mucus secreted from goblet cells, which is an important component of the intestinal mucosal barrier (16). The colitis-inducing *Muc2* mutations induce substantial ER stress, both biochemically and ultrastructurally, which has been speculated to arise from altered oligomerization of these MUC2 variants, and which has been suggested as the mechanistic basis for induction of colitis in this model (15). Importantly, intestinal epithelium from IBD patients also exhibits evidence of increased ER stress (12,15,17), and evidence of an altered glycosylation pattern has been reported for UC (15).

A similar forward-genetics approach revealed that mice with mutant *Mbtps1*, which encodes membrane-bound transcription factor peptidase site 1 (S1P), present with increased sensitivity toward DSS colitis (18). S1P is localized in the Golgi membrane and plays a central role in activating several cAMP response element binding protein / ATF transcription factors (18). Further direct human genetic evidence that unresolved ER stress may link to IBD comes from genome-wide association studies, which revealed the *ORMDL3* locus as associated with both forms of IBD (19,20). ORMDL3 is thought to be involved in protein folding and localizes to the ER (19,21). While overexpression of ORMDL3 decreased levels of ER stress, its knock-down resulted in a more vigorous UPR upon pharmacological stimulation of ER stress (19). Further genetic evidence on a role of ER stress in IBD comes from another candidate-gene study on *AGR2*, which also revealed an association with both CD and UC (22). AGR2 (anterior gradient 2) is an ER-localized protein disulfide isomerase expressed in mucus-secreting cells and Paneth cells (23). Its germline deletion results in disruption of Paneth and goblet cell homeostasis and increased ER stress (24). At a phenotypic level, *Agr2*$^{-/-}$ mice develop terminal ileitis and colitis, with histological evidence of multinucleated giant cells suggestive of granulomatous inflammation in interfollicular zones (24).

In addition to primary genetic factors, various secondary factors might also induce ER stress and thereby contribute to intestinal inflammation (1,25-27). These secondary, environmental factors might be particularly relevant in hosts with a primary genetic impairment of the UPR, which might therefore be particularly sensitive to such insults. Such secondary insults might arise from microbial products, which have been described to directly intersect with grp78 (e.g. AB_5 cytotoxin subtilase, produced by Shiga toxigenic strains of *E. coli* (28,29)) or XBP1 splicing (e.g. trierixin from *Streptomyces sp.* (30,31)). However, also broader microbial functions apart from specific toxins could play a role in inducing ER stress in the epithelium, e.g. TLR signalling has recently been shown to intersect with XBP1 signaling in macrophages (32). It can also be envisioned that intestinal infections or specific properties of commensals or pathobionts might elicit an enhanced secretory response of Paneth cells (or other epithelial cell types) (33,34), and as has been shown in *C. elegans*, a proper UPR and IRE1/XBP1 signaling may be required for cellular 'survival' in the context of the increased secretory demand imposed by microbial infections (34,35). Protein folding is also profoundly affected by the availability of oxygen and glucose (36-39), both of which can be limiting at the epithelial surface adjacent to the complex aerobic/

anaerobic milieu of the intestinal lumen (40,41). Similarly, dietary iron might affect the UPR (41), and pharmacological compounds have also been described to directly intersect with the UPR (e.g. specific HIV protease inhibitors (42)).

In addition to the aforementioned secondary factors that may induce ER stress, inflammatory mediators *per se*, like TNFalpha, can induce the UPR, while anti-inflammatory mediators like IL-10 have been shown to alleviate ER stress (17). This is relevant to consider as the mechanisms described above that link unresolved ER stress with intestinal inflammation might thereby amplify inflammatory circuits instigated through primarily ER stress-unrelated pathways.

In summary, the UPR represents a homeostatic response aimed to resolve ER stress (27). However, when this pathway is impaired, be it through germline genetic variants or through secondary insults that either increase the burden of ER stress or impair the mechanisms that have developed to resolve it, the primary outcome of the UPR might no longer be re-establishment of protein folding homeostasis but rather instigation of inflammatory signalling pathways, tissue inflammation and also autophagic cell death (10,26,43). Several lines of genetic evidence point toward the ER stress response as one of the mechanisms that can lead to IBD (12,19, 20,22). In this context it is particularly remarkable to extrapolate from the murine conditional *Xbp1*$^{-/-}$ model of enteritis that the intestinal epithelium can be a cellular originator of IBD (12).

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