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Alimentary Tract

Endoplasmic reticulum stress and unfolded protein response are involved in paediatric inflammatory bowel disease

Anna Negroni[a,*], Enrica Prete[a], Roberta Vitali[a], Vincenzo Cesi[a], Marina Aloi[b], Fortunata Civitelli[b], Salvatore Cucchiara[b], Laura Stronati[a]

[a] Department of Radiobiology and Human Health, ENEA, Rome, Italy
[b] Department of Pediatrics and Infantile Neuropsychiatry, Paediatric Gastroenterology and Liver Unit, Sapienza University of Rome, Rome, Italy

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ABSTRACT

Background: Endoplasmic reticulum stress and unfolded protein response have been recently associated with the development of inflammatory bowel diseases in adults. We aimed at assessing the involvement of these pathways also in paediatric inflammatory bowel disease by analysing the expression of the main genes involved in endoplasmic reticulum stress and correlating them with the degree of intestinal inflammation.
Methods: Real-time PCR and Western blot analysis of the expression of the endoplasmic reticulum stress marker HSPA5 and of selected genes representing the three pathways of unfolded protein response (IRE-XBP1, PERK-ATF4, ATF6p90-p50) in inflamed and uninflamed biopsies from 28 inflammatory bowel disease paediatric patients and 10 controls.
Results: HSPA5, PDIA4, as well as unspliced and spliced XBP1 mRNAs were significantly increased in patients' inflamed colonic mucosa compared to uninflamed mucosa and controls. HSPA5, PDIA4, ATF6, and phospho-IRE proteins were also upregulated, indicating the activation of the IRE-XBP1 and ATF6p90-p50 branches of unfolded protein response. A positive significant correlation between interleukin-8 levels, as a marker of inflammation, and upregulated genes was found in the inflamed colonic mucosa.
Conclusion: A deregulation of the genes involved in the endoplasmic reticulum stress and unfolded protein response pathways may be a key component of the inflammatory response in paediatric patients with inflammatory bowel disease.

1. Introduction

Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are chronic, debilitating, and unremitting conditions that affect the gastrointestinal tract. Although their aetiology is still unknown, recent studies have suggested an association of endoplasmic reticulum (ER) stress to the development of these conditions [1–4]. ER stress is a phenomenon that occurs when excessive protein misfolding accumulates during biosynthesis: it triggers a series of signalling and transcriptional events, known as the unfolded protein response (UPR), that attempt to restore cell homeostasis [5]. The UPR is mediated by three major pathways and their associated transcription factors: PERK-ATF4 (pancreatic ER kinase (PKR)-like ER kinase-activating transcription factor 4), ATF6p90-ATF6p50 (activating transcription factor 6) and IRE1-XBP1 (inositol requiring enzyme 1-X-box binding protein-1) [6–8]. In the absence of misfolded proteins, the three ER transmembrane sensors, IRE1, PERK, and ATF6, are maintained in an inactive state through the association with heat shock protein 5 (HSPA5) [9]. Upon ER stress, HSPA5 binds to misfolded proteins and therefore separates from the ER sensors, resulting in their activation. Activated sensors induce distinctive signalling pathways that lead to the block of translation and the activation of UPR target genes. Prolonged or unresolved ER stress can trigger apoptosis in the stressed cells as well as local inflammation [10].

ER stress has been linked to an increasing number and variety of inherited and sporadic human diseases including neurodegenerative diseases, developmental disorders, cancer, diabetes, cystic fibrosis, as well as infectious and inflammatory diseases [8,11–16]. Recently, some studies related ER stress and UPR signalling to the pathogenesis of IBD by showing that patients with active CD and UC exhibit signs of ER stress in their ileal and colonic

* Corresponding author at: Department of Radiobiology and Human Health, ENEA CR Casaccia, Via Anguillarese 301, 00123 Rome, Italy. Tel.: +39 0630483623; fax: +39 0630486559.
E-mail address: anna.negroni@enea.it (A. Negroni).

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epithelium [17–20]. The XBP1 pathway has been the most studied in IBD: in mice, deletion of XBP1 in intestinal epithelial cells leads to spontaneous enteritis, sensitivity to colitis induction by dextran sodium sulfate (DSS), increased ER stress in the intestinal epithelial cells' (IEC) compartment, apoptosis of Paneth cells, reduction in the number of goblet cells and, finally, impaired defence to Listeria monocytogenes [4,17,21,22]. Furthermore, analysis of single nucleotide polymorphisms (SNPs) in the XBP1 locus has demonstrated an association between XBP1 and both UC and CD [4].

The aims of this study were: (1) to assess the involvement of ER stress in paediatric IBD by analysing the expression levels of several genes representing the three UPR pathways; (2) to compare UPR activation in the ileal and colonic districts and in inflamed and uninflamed mucosa of both patients and controls; (3) to correlate the modulation of the UPR genes to intestinal inflammation.

2. Materials and methods

2.1. Patients

Overall, 13 patients with CD (median age: 14 years; range: 10–17 years), 15 with UC (median age: 13.2 years; range: 10–17 years), and 10 controls (median age: 11.0 years; range: 5–17 years), were referred to the Paediatric Gastroenterology and Liver Unit at the Sapienza University of Rome. All selected patients had an established diagnosis of IBD and were in the active phase of the disease despite being on therapy. Children with incapacitating functional gastrointestinal disorders requiring extensive investigation, having normal endoscopy and histology, were enrolled as controls. Diagnosis of CD was based on widely agreed endoscopic and histological criteria as well as on the exclusion of infectious and systemic diseases, food allergies, and malabsorption syndromes [23]. Disease activity was measured by the PCDAI (Paediatric Crohn's Disease Activity Index) score, a multi-item measure of clinical and laboratory parameters [24]. Disease was defined as inactive if the score was <10, mild between 10 and 30, and moderate-to-severe if the score was >30. UC activity was graded according to the PUCAI (Paediatric Ulcerative Colitis Activity Index) score [25], i.e. a recently validated, non-invasive, multi-item score of disease activity with established cutoff values for remission (<10), mild disease (10–34), moderate (35–64), and severe disease (65–85). All patients' data are reported in Table 1.

Table 1
Characteristics of inflammatory bowel disease patients and healthy controls.

	CD	UC	HC
N	13	15	10
Male gender	8 (61.5%)	7 (46.7%)	5 (50%)
Mean age (years ± SD)	14.1 ± 2.4	13.2 ± 2.2	11.3 ± 3.8
Mean disease duration (years ± SD)	3.1 ± 2.4	2.1 ± 2.2	
Disease location			
L1/L2/L3	3/3/7		
E1/E2/E3		3/8/4	
Treatment			
None	0	3	
5-ASA	1	7	
AZA	5	0	
IFX, ADA	2	0	
5-ASA, CS	0	4	
AZA, CS	3	0	
IFX, CS	1	0	
AZA, nutrition	1	0	
MTX, 5-ASA	0	1	
Disease activity			
PCDAI (10–30)	6		
PCDAI (>30)	7		
PUCAI (10–34)		8	
PUCAI (>34)		7	

CD, Crohn's disease; UC, ulcerative colitis; HC, healthy control.
L1, ileal; L2, colonic; L3, ileocolonic; E1, ulcerative proctitis; E2, left-sided UC (distal UC); E3, extensive UC (pancolitis) according to the Montreal classification of IBD (Can J Gastroenterol 2005;19:5A–36A).
5-ASA, aminosalicylates; AZA: azathioprine; IFX: infliximab; ADA: adalimubab; CS, steroids; MTX, metotrexate.
PCDAI, Paediatric Crohn's Disease Activity Index; PUCAI, Paediatric Ulcerative Colitis Activity Index.

Patients were treated with immunomodulators (azathioprine or methotrexate), mesalamine, or oral corticosteroids at low doses; two patients underwent biological therapy. Endoscopy was always performed by the same endoscopist with a paediatric video-colonoscope (Olympus PCF 140L, Tokyo, Japan) after conscious

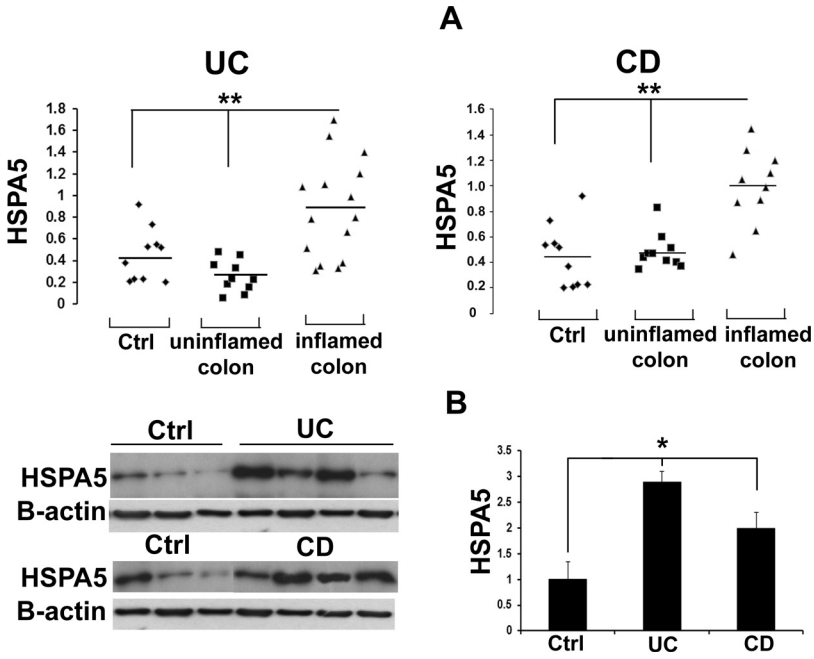

Fig. 1. (A) Colonic HSPA5 mRNA expression, evaluated by real-time PCR, in the uninflamed and inflamed colon of ulcerative colitis and Crohn's disease patients. (B) A representative Western blot showing HSPA5 protein expression in the colon of ulcerative colitis and Crohn's disease patients. The histogram represents mean ± SD of the densitometrical analysis. Ctrl: control group; UC: ulcerative colitis patient group; CD: Crohn's disease patient group. $^{**}p < 0.01$; $^{*}p < 0.05$.