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Inflammation induced ER stress affects absorptive intestinal epithelial cells function and integrity

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ABSTRACT

Recent studies have linked impairment of intestinal epithelial function in inflammatory bowel disease to the disturbance of endoplasmic reticulum homeostasis (ER) in response to stress. Most studies are on goblet and Paneth cells, which are considered more susceptible to stress due to their role in the protection of intestinal epithelium against microbes and harmful substances. However, studies on the role of inflammation-induced ER stress in absorptive intestinal cells are scarce. In this study, we show, using Caco-2 cells as a model of intestinal epithelial barrier, that inducing ER stress using a cocktail mixture of pro-inflammatory mediators [TNFα (50 ng/ml), MCP1 (50 ng/ml), and IL-1β (25 ng/ml)] as observed in IBD patients induces ER stress and leads to significant changes in key proteins of the apical (sucrase-isomaltase (SI), dipeptidyl-peptidase (DPPIV), and ezrin) and basolateral (E-cadherin, zonula occludens (ZO-1), and connexin-43) membranes. Aberrant trafficking of SI, DPPIV was observed as early as 8 h post-inflammation-induced ER stress and even in the absence of loss of intestinal cell integrity. The observed effect was associated with a re-localization of ezrin, ZO-1, and connexin-43, key differentiation and junction proteins. Collectively, this study shows that disruption of the trafficking of key digestive enzymes of the intestinal epithelium occur in response to inflammation induced ER stress before the loss of monolayer integrity.

1. Introduction

Inflammatory bowel disease (IBD) is one of the most prevalent gastrointestinal disorders that encompass ulcerative colitis (UC) [1] and Crohn's disease (CD) [2,3]. While in UC only the colon is affected with occasional extension into the terminal ileum a condition designed as backwash ileitis [4], any part of the gastrointestinal tract can be affected in CD [5]. IBD pathogenesis results from a multifactorial process involving genetic, environmental, as well as immunogenic factors [6,7]. Over the last decades our understanding about the etiology of IBD has increased, yet, the exact mechanisms remain unclear. Nevertheless, it is well known that IBD is characterized by the onset of inflammation and abnormalities in the epithelial barrier [8–14]. Current knowledge shows that intestinal epithelial cells, which under physiological conditions are indispensable in maintaining selective barrier between the host and the harmful substances [15] as well as in the digestion and absorption of nutrients [16,17], have emerged as key players in the development and persistence of intestinal inflammation in IBD [18,19]. Ample evidence confirms that persistence of the disturbance of the endoplasmic reticulum (ER) homeostasis in stressed intestinal epithelial cells is the major contributing factor [20]. This is not surprising as the ER is a vital organelle responsible for the synthesis and maturation of nascent proteins [20]. Interestingly, cytokines and reactive oxygen species which are triggered by inflammation affect protein folding and lead to the accumulation of unfolded or misfolded proteins inside the ER [21,22], a state that lead to the activation of the unfolded protein response (UPR) [23]. Activation of the UPR signaling by lowering the biosynthesis of proteins, elevating the level of chaperones, increasing the degradation of unfolded proteins, and initiating apoptosis, helps the ER in regaining its homeostasis [20]. Yet, sustained ER stress results in chronic activation of the UPR, which can ultimately lead to severe inflammation and cell death [24]. Recently, ER stress and UPR have been linked to the pathogenesis of IBD [18,19,25]. While most of these reports have been investigated in Paneth and goblet cells, two secretory epithelial populations in the gut that play key roles in the defense of intestinal barrier against microbes and protection of the intestinal wall

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[18], studies on the absorptive intestinal cells are scarce. Given that diarrhea, abdominal pain, and malnutrition are prevalent in patients with IBD [26,27] and to better understand the pathophysiology of the aforementioned anomalies we investigated in this study in an in vitro model the effect of inflammation-induced ER stress on absorptive epithelial cells.

2. Materials and methods

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM) (4.5 g/l glucose) and Dithiothreitol were from Sigma (Sigma, Germany). Penicillin/streptomycin was obtained from PAA (Pasching, Austria). Fetal calf serum (FCS) and Trypsin/EDTA were from Roth (Roth, Germany). Tumor necrosis factor (TNFα), Monocyte chemo-attractant protein1 (MCP1), and Interleukin1β (IL-1β) were purchased from R&D systems biotechnology (Minneapolis, USA).

2.2. Cell culture

The human colon adenocarcinoma cell line (Caco-2 cells), purchased from the German Collection of Microorganisms and Cell Cultures (DSM2) (ACC169) (Braunschweig, Germany), were grown in DMEM (4.5 g/l glucose) supplemented with penicillin/streptomycin (100 U/ml) and 10% FCS. Cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air.

2.3. ER stress induction

Caco-2 cells seeded at equal density were maintained in culture until they reached 100% confluency. Seven days post-confluency, cells were treated for 8 and 24 h with a cocktail mixture (CK) of three pro-inflammatory mediators [TNFα (50 ng/ml), MCP1 (50 ng/ml), and IL-1β (25 ng/ml)]. The CK was used to induce ER stress in Caco-2 cells.

2.4. Cell proliferation and metabolic activity

The effect of inflammatory cytokines on the proliferation and the metabolic activity of Caco-2 cells was evaluated up to 72 h post-treatment, using trypan blue dye exclusion method and the WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzenedisulfonate) assay (Roche Diagnostics GmbH, Mannheim, Germany). This latter assay measures the ability of the metabolically active cells to cleave by mitochondrial dehydrogenases the WST-1 tetrazolium salt to the red colored formazan. The absorbance was measured at 440 nm using a microplate spectrophotometer Epoch (Bio Tek Instruments, USA).

2.5. Cytotoxicity assay

Caco-2 cells were plated at equal density in 96-well plates. At day 7 post-confluency, cells were treated with the CK for 8 and 24 h and the cytotoxicity was monitored by measuring, using CytoTox-ONE™ Promega kit (Promega Corporation, USA), the level of lactate dehydrogenase (LDH), a stable cytosolic enzyme that is released upon cell lysis. Released LDH in culture supernatants is measured with a coupled enzymatic assay which results in the conversion of a tetrazolium salt into a red formazan product. The absorbance was recorded at 544 nm using a microplate reader FLUO star OPTIMA (BMG LABTECH, Germany).

2.6. Transepithelial electrical resistance (TEER)

The effect of the CK on Caco-2 cells monolayer integrity was assessed by measuring the transepithelial electrical resistance (TEER). Cells were seeded on Millipore Milicell® cell culture inserts (0.4 μm pore size) (BD, Transduction Laboratories, USA) in 6 well plates. 21 days post-seeding, cells were treated with the CK for 0, 8, and 24 h. TEER was recorded, at each time point, using a Millicell-ERS device (Merck-Millipore, Germany).

2.7. Western blot analysis

Total cell lysates were denatured in Laemmli buffer and 50 mM Dithiothreitol for 5 min at 95 °C. Equal amount of protein samples (50 μg) was then subjected to gel electrophoresis on sodium dodecyl sulfate (SDS) polyacrylamide gels (80 V for around 10 min followed by 120 V for 120 min). Proteins were then transferred (240 mA for 100 min) onto a PVDF transfer membrane (Roth, Germany). Consequently, PVDF membranes were immunoblotted with the following primary monoclonal antibodies: BIP, CHOP, caspase-3, E-cadherin, connexin-43 (Cell Signalling, Germany), ZO-1 (Thermo Fisher Scientific, USA), ezrin (Santa Cruz Biotechnology, CA, USA), SI-705 (Kindly provided by Dr. Hans-Peter Hauri, University of Basel, Switzerland), DPPIV (kindly provided by Dr. Ewin Sterchi, University of Bern, Switzerland), and β-actin (Santa Cruz Biotechnology, CA, USA). After incubation of the membranes with secondary antibodies and with the chemiluminescence reagents (Thermo Fisher Scientific, USA), bands were visualized by a ChemiDoc XRS System (Bio-Rad, Munich, Germany). The Bands were quantified using Quantity One 1-D Analysis Software (Bio-Rad Laboratories GmbH).

2.8. Brush border membrane isolation

Caco-2 cells, grown as previously described, were treated 7 days post-confluency with the CK for 8 and 24 h. Brush border membranes of Caco-2 cells were isolated by the modified divalent cation precipitation method [28,29]. Briefly, Caco-2 cells were solubilized and homogenized using a Potter-Elvehjem homogenizer in the hypertonic homogenization buffer [Tris-HCl (2 mM), mannitol (50 mM pH 7.1)] supplemented with protease inhibitor mixtures (Sigma, Germany). The homogenates were passed 20 times through a 21 Gauge needle. After the addition of $CaCl_2$ (at a final concentration of 10 mM), and following a gentle rotation at 4 °C, the homogenates were centrifuged at 2000*g* for 30 min. The obtained pellet contains basolateral and intracellular membranes. The supernatant was then centrifuged at 25,000*g* for 30 min. The obtained pellet which contains the brush border membranes was re-suspended in brush border membrane buffer [Tris-HCl (10 mM), NaCl (150 mM pH 7.4)] and further used for the biochemical analysis.

2.9. Enzyme activity measurements

The enzymatic activity of sucrase-isomaltase (SI) was measured in total homogenates (H) by the Glucose oxidase (GOD)-PAP mono-reagent method (Axiom mbH, Germany). Briefly, GOD converts the glucose, obtained due to the action of active SI, into gluconate. This process is accompanied by the generation of hydrogen peroxide that is degraded by peroxidase (POD) into a colored product the absorbance of which was measured at 492 nm using a microplate spectrophotometer Epoch (Bio Tek Instruments, USA). Protein concentration was quantified by the Bradford method (Bio-Rad) using bovine serum albumin as a standard. In each sample the enzymatic activity was normalized to the protein amount detected by western blot.

2.10. Statistical analysis

Results, shown as mean ± standard error of mean (SEM), are representative of at least three independent experiments. Comparison between the different groups was performed using either One way ANOVA followed by Tukey's multiple test or paired Student's *t*-test. The