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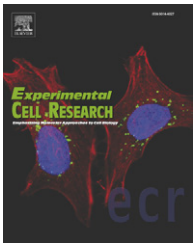

Research Article

Toxic peptides in Frazer's fraction interact with the actin cytoskeleton and affect the targeting and function of intestinal proteins☆

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ABSTRACT

Celiac disease (CD) is a multisystemic autoimmune inflammation of the intestinal tract induced by wheat gluten and related cereals in HLA-DQ2/8 positive individuals. An essential role in the pathogenesis of CD is played by a fraction of the peptic–tryptic digest of gluten, Frazer's Fraction (FF). Here, we investigate the effects of FF on the integrity of intestinal cells with particular emphasis on brush border membrane (BBM) components, their subsequent trafficking and endocytosis.

Caco-2 cells were incubated with FF at different concentrations. Thereafter, several protein and lipid components of treated and untreated cells were analysed at the molecular, functional and cellular levels. The control employed tryptic–peptic digests of ovalbumin.

Our results show that FF directly interacts with actin in an alternating manner eliciting substantial alterations in its integrity and extent in the BBM. These alterations lead to an impaired trafficking of SI to the apical membrane and reduction in its enzymatic function. ApN and DPPIV follow a transcytotic pathway and are only partly affected by FF. By contrast, the trafficking of LPH remains unaffected concomitant with its actin-independent trafficking pattern. Finally, the endocytic pathway is substantially blocked in FF-treated cells leading to an accumulation of cholesterol, and sphingolipids in the BBM.

We conclude that FF deteriorates the actin cytoskeleton in Caco-2 leading to reduced protein sorting and hampered endocytic events with subsequent alterations in the protein and lipid composition of the BBM. The reduced levels of the disaccharidase SI in the BBM suggest a potential pathomechanism of carbohydrate malabsorption in CD.

Introduction

Celiac disease (CD) is a chronic inflammatory disease of the small intestine. It is typically characterized by villus atrophy and a consecutive malabsorption syndrome in genetically predisposed individuals (HLA-DQ2/DQ8) due to gliadin toxicity [1–3]. Although it has been revealed that an altered immune response to gliadin is involved in the pathogenesis of CD [2,4] the underlying

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Abbreviations: BBM, brush border membrane; SI, sucrase-isomaltase; LPH, lactase-phlorizin hydrolase; mAb, monoclonal antibody; DPPIV, dipeptidylpeptidase IV; ApN, aminopeptidase N; FF, Frazer's Fraction; OVA, ovalbumin; PFA, paraformaldehyde; cytoD, cytochalasin D; HBB, human brush border; HLA, human leukocyte antigen; BSA, bovine serum albumin; HRP, horse radish peroxidase; WGA, wheat germ agglutinin

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mechanism by which gliadin damages the small intestine of susceptible individuals is still unclear.

The first contact of macromolecular nutrients with the intestinal epithelium occurs through the brush border membrane (BBM) that maintains the intestinal barrier function [5], whereby their internalization normally occurs by receptor-mediated endocytosis or by fluid phase endocytosis (pinocytosis) as shown for bovine serum albumin (BSA) or horse radish peroxidase (HRP) [6,7]. The mode of uptake of gliadin through the intestine has not been explored yet and it is not clear whether this uptake is receptor-mediated but Matysiak-Budnik et al. [8] were able to show that the transferrin receptor CD71 is involved in the retrotranscytosis of peptides 31–49 and that gliadin binds to chemokine receptor CXCR3 (chemokine CXC receptor 3) and leads to MyD88 (myeloid differentiation primary response gene (88))-dependent zonulin release and increased intestinal permeability [9].

Besides its barrier function between the luminal part and the interior side of the intestinal mucosa, the BBM of enterocytes consists of packed uniformly organized microvilli that provide an expanded surface enriched in a battery of glycoproteins that are essential for the digestion and absorption of nutritional components like carbohydrates and food peptides [10]. Different sorting pathways have been described for these glycoproteins in biopsy specimens and cell culture model [11,12]. Some of these proteins, e.g. sucrase-isomaltase (SI), require an intact actin cytoskeleton for an efficient sorting and trafficking to the plasma membrane. Other proteins such as lactase-phlorizin hydrolase (LPH) are transported via actin-independent pathways [13] that predominantly implicate microtubules. The actin cytoskeleton of enterocytes in biopsy samples from patients with CD [14] or of Caco-2 cells [15] has been shown to be affected upon treatment with gliadin peptides. Nevertheless, the sequence of events leading to the deterioration of the actin cytoskeleton and the mechanism by which gliadin acts on actin are entirely obscure. In the present study we utilized Caco-2 cells to assess the effects of gliadin treatment on the actin-dependent and actin-independent protein transports and to identify potential alterations in the BBM due to impaired membrane trafficking and endocytosis. Our data demonstrate that treatment with gliadin peptides does not only affect the actin cytoskeleton *per se* leading to morphological alterations, but influences the anterograde and retrograde transport of proteins and lipids concomitantly.

Materials and methods

Preparation of Frazer's Fraction

The preparation of Frazer's Fraction III (thereafter indicated as FF) was performed according to Frazer et al. [16]. This fraction contains a mixture of gliadin and glutenin peptides representing the source for toxic gliadin peptides. The control experiments employed a similarly prepared peptic–tryptic digest of ovalbumin (OVA; Sigma, Taufkirchen, Germany).

Stimulation of Caco-2 cells

Intestinal Caco-2 cells were cultured in DMEM high glucose (4500 mg/l) medium supplemented with 10% fetal calf serum and 1% penicillin/streptomycin (all from PAA Laboratories, Pasching, Austria). Cell treatment started at day 3 post-confluence (approximately 5×10^6 cells/100 mm-dish) with FF or OVA for 48 h at 37 °C. For this three different concentrations were used, 5 mg, 25 mg and 100 mg.

Cell fractionation and western blotting

Stimulated cells were fractionated using $CaCl_2$ [17] to P1 (basolateral and intracellular membranes) and P2 (BBM). Usually 20–25 μg of either P1 or P2 was subjected to western blotting. The following antibodies were used: mAb anti-SI HBB 3/705/60 (1:350), mAb anti-DPPIV (HBB 3/775/42 DPPIV; 1:3000) (both Hauri et al. [18], polyclonal anti-ApN (1:500) [19] and mAbs mLac 6 and 10 against LPH (1:250) [20]. Due to the low levels of LPH in Caco-2 cells, a hybrid clone, using LPH in a pcDNA3-Vector (Molecular Probes, Invitrogen detection technologies, Karlsruhe, Germany), was generated and immunoprecipitation of LPH with a mixture of several mAbs (mLac 1, 3, 4, 5, and 8 (1:40) [20]) preceded western blotting. Actin was detected using mAb anti-β-actin (C4) (Santa Cruz Biotechnology, Inc., Heidelberg, Germany). The mAb anti-β-tubulin (1:1000) and polyclonal anti α-gliadin antibody (1:1000) were obtained from Sigma (Taufkirchen, Germany). Anti-rabbit IgG-ECL was from Dako (1:10000) (Hamburg, Germany) and anti-mouse IgG-ECL (1:10000) (Amersham Biosciences, Freiburg, Germany) were used as secondary antibodies and the detection employed SuperSignal ELISA Femto (Perbio Science, Bonn, Germany) on x-ray (x-ray Retina XBA) film sheets (Fotochemische Werke GmbH Berlin).

Immunofluorescence

Caco-2 cells were stimulated with FF or OVA for 48 h as described. The cells were rinsed twice with phosphate buffered saline (PBS), fixed with 4% paraformaldehyde (PFA; Fluka BioChemika, Buchs, Switzerland) for 20 min and further processed according to Leitner et al. [21]. Permeabilization of the cells was performed for 30 min at room temperature with 0.5% Saponin (Sigma Aldrich Chemie, Taufkirchen) in PBS containing 1% BSA (Fluka BioChemika, Buchs, Switzerland) (indicated blocking buffer). The following antibodies were used: mAbs HSI 3 against SI (1:200) [22] and HBB 3/775/42 against DPPIV (1:2000) [18]. Phalloidin–rhodamine (1:100) for actin labeling and the anti-mouse IgG AlexaFlour488 (1:1000) as a secondary antibody were from Molecular Probes, (Karlsruhe, Germany). Confocal images were acquired using a Leica TCS SP2 microscope with an ×63 oil planapochromat lens (Leica Microsystems, Wetzlar, Germany) according to Jacob and Naim [23].

Co-immunoprecipitation of actin with anti-gliadin antibodies

Caco-2 cells were incubated for different times up to 72 h with 100 mg FF in cell culture medium at 37 °C. FF was replaced every 24 h. Cells were lysed for 2 h at 4 °C with 1% Triton X-100 in PBS and immunoprecipitation with rabbit anti-gliadin antibodies was performed. The immunoisolates were further processed by SDS-PAGE followed by western blotting and staining with the mAb anti-β-actin antibody. The control utilized lysates from cells that were not treated with FF, but treated with anti-gliadin and protein A-