Impaired epithelial integrity in the duodenal mucosa in early stages of celiac disease

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The small-bowel mucosal damage characteristic of celiac disease (CD) develops from normal villus morphology to inflammation and finally to villus atrophy with crypt hyperplasia. Patients with early stage CD may already suffer from abdominal symptoms before the development of villus atrophy. Although epithelial junctional integrity is compromised in overt disease, the appearance of such changes in early phases of the disorder is not known. We investigated whether alterations in epithelial junction protein expression occur already in early stage CD with normal mucosal morphology, and whether this correlates with inflammation indicators and clinical symptoms. The study involved 10 patients with early stage and 10 patients with overt villus atrophy that were followed yearly according to the study protocol. As controls, 20 nonceliac subjects were included. The expression of junction proteins (occludin, claudin 3, zonula occludens 1, and E-cadherin) was studied in small-intestinal biopsies using immunohistochemistry and Western blot. The correlation between junctional proteins and mucosal morphology, autoantibodies, the number of intraepithelial lymphocytes (IELs), and gastrointestinal symptoms was assessed. The expression of all junction proteins was already decreased in early stage CD when compared with nonceliac controls ($P < 0.05$). Junction protein expression correlated positively with mucosal villus morphology and negatively with the number of IELs, the intensity of small-intestinal autoantibody deposits, and serum autoantibodies. The expression of claudin 3 showed a negative correlation with diarrheal score ($R = -0.314$, $P = 0.04$). These findings show that the mucosal epithelial integrity is disrupted already in early stage CD before the disorder progresses to full-blown enteropathy. (Translational Research 2014;164:223–231)

Abbreviations: ELISA = enzyme-linked immunosorbent assay; EmA = endomysial antibodies; GSRS = Gastrointestinal Symptom Rating Scale; HLA = human leukocyte antigen; IEL = intraepithelial lymphocyte; IgA = immunoglobulin A; TG2 = transglutaminase 2; Vh/CrD = villus height to crypt depth ratio; ZO-1 = zonula occludens 1

INTRODUCTION

In the healthy individual the epithelial layer lining the small-bowel mucosa is fairly impermeable to macromolecules, allowing only nutrients and relatively small molecules to gain access from the intestinal lumen to the *lamina propria*. However, the epithelial barrier integrity is compromised in various intestinal disorders, including celiac disease (CD), a dietary

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AT A GLANCE COMMENTARY

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Background

In celiac disease, the small-intestinal inflammation and damaged morphology are associated with increased mucosal permeability because of decreased expression of epithelial junction proteins. However, it is not known whether the junctional changes appear already in early phases of the disorder, when the small-bowel mucosal morphology is still normal.

Translational Significance

Our results suggest that mucosal epithelial integrity is disrupted already in early stage celiac disease before damaged morphology and further advances as the disease progresses to full-blown enteropathy. These findings could be clinically relevant because the compromised epithelial barrier was related to abdominal symptoms.

gluten-induced immune-mediated enteropathy occurring in genetically susceptible individuals expressing the human leukocyte antigen (HLA) subregion molecules DQ2 or DQ8.[1] The small-intestinal mucosal inflammation, crypt hyperplasia, and villus atrophy in untreated celiac patients are associated with increased mucosal permeability because of the decreased expression of epithelial junction proteins, such as occludin, claudins, E-cadherin, and β-catenin.[2-5] This makes for increased passage of gluten-derived gliadin peptides to the *lamina propria* and a subsequent downstream inflammatory immune response, including the production of disease-specific autoantibodies against endomysium and transglutaminase 2 (TG2).[6] On withdrawal of gluten from the diet, the mucosal inflammation abates and the autoantibodies disappear parallel to the normalization of mucosal epithelial barrier function.[1]

Fasano et al[7] have suggested that the mechanism leading to this epithelial barrier defect could involve a molecule called zonulin already in early phases of CD. In celiac patients, the typical gluten-induced small-intestinal mucosal damage develops gradually from normal villus structure to mild mucosal inflammation and eventually to totally flat mucosa with crypt hyperplasia.[8] Interestingly, many signs of the disease are already perceptible in the early phases of the disease when the small-bowel mucosal morphology is still normal. Such indicators include the presence of disease-specific endomysial antibodies (EmAs) and TG2-targeted antibodies in serum, small-bowel mucosal disease-specific antibody deposits, and lymphocytosis.[8-10] In addition, patients may already suffer from anemia and various gastrointestinal symptoms such as abdominal pain and diarrhea before the mucosa deteriorates.[8,11,12] This implies that the symptoms in CD are not solely because of small-bowel villus atrophy. Altogether, early developing CD offers the possibility to understand the sequence of events leading to the full-blown disease.

There are no studies addressing the expression of epithelial junction proteins in the small-bowel mucosa of celiac patients with early developing disease, when the mucosal villus morphology is still normal. With this in mind, we undertook to establish whether the expression of intestinal mucosal junction proteins is altered in this stage of CD, and whether such altered junction protein expression is gluten-dependent and correlates with mucosal inflammation and symptoms.

MATERIALS AND METHODS

Patients and study design. The study cohort comprised 20 EmA-positive adult patients who underwent upper gastrointestinal endoscopy in Tampere University Hospital because of clinical suspicion of CD. Follow-up studies were carried out according to the planned protocol at yearly intervals (Fig 1). Ten patients had normal villus structure (Marsh I) compatible with early developing CD.[12] Of these, 7 developed villus atrophy (Marsh IIII) during the follow-up when they continued 1 year on a gluten-containing diet, and later after 1 year on a gluten-free diet (GFD), the mucosal morphology reverted to normal. The remaining 3 patients with early developing CD (Marsh I) and 10 with overt small-bowel villus atrophy (Marsh III) started a GFD directly after the first biopsy (Fig 1), and after 1 year, clinical, serologic, and histologic recovery was evident in all. Twenty EmA-negative subjects, who had been investigated because of dyspepsia and had no relatives with CD, served as nonceliac controls. All had normal small-bowel mucosal morphology when on a normal gluten-containing diet.

A minimum of 6 small-bowel mucosal forceps biopsy samples were taken from the distal duodenum on upper gastrointestinal endoscopy from patients with CD representing early and overt stages of the disease and from controls. Further, at each visit a clinical examination was carried out, serum and whole blood samples were drawn and gastrointestinal symptoms assessed by structured and validated Gastrointestinal Symptom Rating Scale (GSRS) questionnaires.[13] The study protocol was approved by the Ethical Committee of Tampere University Hospital. All subjects gave their written informed consent.