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Original Article

Gluten-degrading bacteria are present in the human small intestine of healthy volunteers and celiac patients

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Abstract

Gluten is the only known environmental factor that triggers celiac disease. Several studies have described an imbalance between the intestinal microbiota of different individuals based on diagnoses. Moreover, recent studies have suggested that human bacteria may play an important role in gluten hydrolysis. However, there has been no research focusing on the small intestine.

This study aimed to characterize the adult small intestine microbiota possibly implicated in gluten hydrolysis. Duodenal biopsies from different diagnosed individuals were cultured in a gluten-containing medium, and the grown microbiota was analyzed by culture dependent/independent methods. Results showed that gluten-degrading bacteria can be found in the human small intestine. Indeed, 114 bacterial strains belonging to 32 species were isolated; 85 strains were able to grow in a medium containing gluten as the sole nitrogen source, 31 strains showed extracellular proteolytic activity against gluten protein and 27 strains showed peptidolytic activity towards the 33 mer peptide, an immunogenic peptide for celiac disease patients. We found that there are no differences based on the diagnosis, but each individual has its own population of gluten-hydrolyzing bacteria. These bacteria or their gluten-degrading enzymes could help to improve the quality of life of celiac disease patients'.
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Keywords: Gluten; Celiac disease; Hydrolysis; Protease

1. Introduction

Celiac disease (CD) is a common inflammatory enteropathy of the small intestine that may also have systemic manifestations [1]. It can develop at any age and affects around 1% of the general population, with the only accepted treatment being a life-long gluten-free diet [2]. Despite the fact that it is a well-studied disease due to its high prevalence, the exact mechanisms of the disease remain unknown.

CD is a complex disorder, with multiple factors contributing to its etiology. To date, gluten is the only known environmental factor implicated in the development of CD. Gluten protein has a high proline content, and human gastrointestinal enzymes lack prolylendopeptidasic activity. This results in incomplete digestion of gluten and the generation of immunogenic peptides such as the 33-mer [3], which are deaminated by tissue transglutaminase (TGt), the molecule considered to be the autoantigen of CD. This process increases peptide binding affinity to antigen-presenting cells (APCs), initiating a characteristic CD-4

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T-cell response in CD patients. The presence of anti-tTG antibodies plays an important role in the serological work-up for CD [4,5]. In addition, genetic factors are involved. The main genetic factors affecting CD are histocompatibility complex II class HLA-DQ2 and HLA-DQ8, which are present in 90% and 10% of patients, respectively [1]. It is important to consider that 30–40% of the overall population carries either HLA-DQ2 or DQ8, and only a small proportion of them develop CD [1]. Despite the fact that HLA molecules DQ2/DQ8 are high risk factors, their presence is not sufficient for development of the disease; important aspects regarding the development of CD remain unclear. Why do some individuals who eat gluten and carry the HLA DQ2/DQ8 complex develop CD and others do not? What makes the difference?

Throughout a number of years, several additional risk factors have been postulated. Dietary factors appear to be particularly relevant during the early stages, when the immature neonate gut is acquiring and shaping its own microbiota. Many authors propose that introduction of gluten to the diet at an early age, as well as breastfeeding instead of formula, could have a protective role in CD [6,7]. In contrast to these results, a recent study concluded that breastfeeding and the time of gluten introduction are not implicated in CD development [8]. The relationship between the high frequency of rotavirus infections and an increase in the risk of developing CD in genetically predisposed children was described by Stene et al. [9]. However, it was after Forsberg et al. described a rod-shaped bacterium attached to the small intestine epithelium of some untreated CD patients, but not to the epithelium of healthy controls [10], that the possible implications of the intestinal microbiota in the development of CD were hypothesized.

Several researchers have since attempted to find a dysbiosis pattern in the intestinal microbiota of different individuals based on diagnosis, with contradictory conclusions; although most studies describe an imbalance in the bacteria composition between CD and healthy individuals [11–14], some studies conclude that there are no differences between these groups [15,16]. It is also important to consider that the majority of the studies analyzed the colonic microbiota, but only a few focused on the small intestine due to the difficulty of sample acquisition [13,15–25]. However, the underlying mechanisms through which bacteria are implicated in CD are unknown. Small intestinal bacteria could play a role in triggering of CD in genetically susceptible persons in different ways, such as modulating an adaptive immune response through microbe–gluten interactions [26] or an innate immune response through microbe–host interactions [27]. Recent studies have suggested that human associated bacteria could play an important role in gluten hydrolysis [28,29], but no studies focused on the small intestine.

The presence of several microorganisms that produce proteases able to hydrolyze peptides rich in proline and glutamine residues in the oral cavity has been described [29–31]. In other studies, bacterial strains from feces capable of digesting gliadin-derived peptides have been isolated [19]. Caminero et al. showed that the human large intestine exhibits a wide variety of bacteria capable of utilizing gluten proteins and peptides as nutrients [32]. Although there exist no studies performed in the small intestine, Bernardo et al. [22] described a specific gliadinase pattern in duodenal samples from patients with CD, and these authors suggested that specific bacteria are responsible for such proteolytic activities [33].

This study aims to characterize the adult small intestine microbiota with gluten-degrading ability and analyze whether such bacteria are present indistinctly in all individuals or differ among the different diagnosed individuals.

2. Materials and methods

2.1. Study subjects and sampling

Duodenal biopsies were collected at the Department of Gastroenterology of the Hospital de León, Spain. In order to be able to analyze whether there were differences in the microbiota based on diagnosis or not, samples were collected from different diagnosed individuals.

For cultivable microbiome analysis, 42 adults were included in the study: 16 first-degree relatives of CD patients (R) (mean age 41.7 years, range 28–65 years; 10 men and 6 women); 7 healthy volunteers (H) (mean age 42.7 years, range 23–63 years; 6 men and 1 woman); 14 treated CD (TCD) patients on a gluten-free diet (GFD) (mean age 43.6 years, range 18–69 years; 5 men and 9 women); and 5 untreated CD patients (UCD) (mean age 35 years, range 25–44 years; 1 man and 4 women).

For PCR-DGGE analysis, 52 adults were included in the study: 26 CD relatives (R) (mean age 42.3 years, range 28–65 years; 14 men and 12 women); 7 healthy volunteers (H) (mean age 42.7 years, range 23–63 years; 6 men and 1 woman); 14 treated CD (TCD) patients on a GFD (mean age 44.6 years, range 18–69 years; 5 men and 9 women); 5 untreated CD patients (UCD) (mean age 35 years, range 25–44 years; 1 man and 4 women).

Inclusion criteria of the volunteers were: (H) First-degree relatives of CD patients in whom CD was ruled out, had no genetic susceptibility for CD and did not show signs of any other gastrointestinal disease; (UCD) CD patients who were diagnosed by positive tTG antibodies, HLA-DQ2 or DQ8 phenotype and a positive duodenal biopsy (>Marsh IIIa: partial villous atrophy, increased intra-epithelial lymphocytes (≥40/≥25/100 enterocytes) and a villous:crypt ratio, 1:1 [34]); (TCD) Treated CD patients who were instructed by the gastroenterology department to comply with a strict GFD and had recovered the normal architecture of the duodenal biopsy and normal tTG antibody levels after a GFD of at least one year. (R) First-degree relatives of CD patients in whom CD was ruled out, but who were positive for HLA-DQ2 or HLA-DQ8. None of the individuals included in the study had been treated with antibiotics for at least 1 month prior to the sampling time. Duodenal biopsy specimens were collected by upper endoscopy and were cultured for a maximum of 3 h after being taken. H, R and UCD individuals declared having a normal diet. TCD declared being under a GFD, and this was proven by different analyses as indicated above.

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