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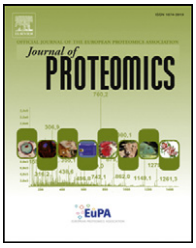

Oral administration of *Bifidobacterium longum* CECT 7347 modulates jejunal proteome in an *in vivo* gliadin-induced enteropathy animal model

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ABSTRACT

Celiac disease is an immune-mediated disorder triggered by gluten proteins of wheat (gliadins) and other cereals. Gliadin-mediated effects on weanling animals, sensitized or not with interferon (IFN)-γ, were investigated. Also, the influence of the co-administration of *Bifidobacterium longum* CECT 7347 was studied together with changes in the proteome of jejunal sections, using 2DE and MALDITOF-TOF peptide fingerprinting. Findings were compared to results for control animal groups. In the principal component analysis (PCA) of proteome pattern, two components were extracted accounting for 79.8% of variability in the expression of the identified proteins. PCA analysis clearly discriminated between the proteome of animals fed gliadins alone and those fed gliadins and *B. longum* simultaneously. However, the proteome patterns from animals sensitized with IFN-γ and fed gliadins together with *B. longum*, or alone, could not be discriminated. Gliadin feeding caused inflammatory effects as well as changes in proteins involved in intracellular ionic homeostasis, lipid turnover, cell motility and redox regulation in intestinal sections. After feeding gliadins to animals sensitized with IFN-γ, changes were also detected in proteins involved in recruitment and function of inmunocompetent cells, trophic effect on the intestine and organization of myofibers reflecting the more marked gliadin-mediated injury in jejunal sections. The administration of the bacterial strain to rats fed gliadins seemed to ameliorate the inflammation caused by gliadin feeding alone, although, in sensitized animals the co-administration of *B. longum* had less marked effects, which was probably due to the more extensive intestinal mucosal damage. The proteome patterns in animals administered *B. longum* alone did not reveal any changes reflecting impairment of jejunal functions.

1. Introduction

Celiac disease (CD) is a chronic disorder of the small intestine produced by intake of gluten proteins of wheat (gliadins) and other related cereals (rye and barley) proteins (hordeins and secalins) that cause a deregulated and abnormal immune response in genetically predisposed individuals [1]. The histological features of CD are represented by different degrees of chronic inflammation of the small intestine leading to severe villous atrophy and crypt hyperplasia [2].

It is known that innate and acquired T-cell mediated immunity play important roles in the pathogenesis of the disease [2]. In CD, the T-cell mediated adaptive response is mediated by CD4+ Th1 lymphocytes in the lamina propria that interact with

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deamidated gliadin peptides and induce the production of inflammatory cytokines. Recently, CD4+ Th17 cells have also been involved in the pathogenesis of CD [3] perpetuating inflammation by secreting several interleukins (IL) such as IL-17, IL-21, IL-22 and interferon (IFN)-γ, which act on immunocompetent cells, among others, in the tissue [4]. The inflammatory cascade induces the release of metalloproteinases and other tissue-damaging mediators that cause crypt hyperplasia and villous injury [5]. Gliadin-derived peptides in the lamina propria elicit innate immune responses, increasing the severity of mucosal lesions caused by adaptive immunity independently of the T-cell mediated response. In particular, findings indicate an increased expression of interleukin (IL)-15 by epithelial cells activates intraepithelial lymphocytes expressing the natural killer (NK) receptors CD94 and NKG2D, mediating a cytotoxic response against the enterocytes expressing the NKG2D ligand and the major-histocompatibility complex class I (MIC-A and -B) molecules [6,7]. In addition, IL-21 has been shown to trigger chemokine secretion by epithelial cells, facilitating the recruitment of immune cells in the inflamed tissue and modulating the maturation and function of CD8+ and NK cells [8].

One of the major drawbacks hampering progress in our understanding of the mechanisms underlying CD pathogenesis is the lack of appropriate models. Such models should reproduce the main features of the disease, including the immune response(s), the mucosal lesions and symptoms [2,9,10]. Although recent significant advances have attempted to refine animal models, they have only managed to partially reproduce the main characteristics of the disease [11,12]. It has been demonstrated that feeding gluten to human leukocyte antigen (HLA)-DQ8/CD4, or to single HLA-DQ8 transgenic mice, triggers abnormal immune response(s) of both the adaptive and innate systems [13,14]. This leads to changes in gut neuromuscular and epithelial secretory function [14] but enteropathy does not develop. Repeated oral administration of gliadin-derived peptides to rats, which had been sensitized with interferon gamma (IFN-γ), immediately after birth, was found to cause high cellular infiltration and maturation of T-cell populations, reproducing an intermediate state between the proliferative and destructive phases of the disease [12] similar to that described in CD patients. This model has previously been used to explore the immunomodulatory properties of orally administered *Bifidobacterium longum* CECT 7347 [12]. This study has revealed the positive effects of *B. longum* CECT 7347 in attenuating the CD4+ T-cell-mediated immune response(s) in weanling animals.

Both the innate and acquired immune mechanism(s) must be involved for intestinal tissue damage to develop, which depends on genetic determinants and environmental factors (gluten) [15]. Although the pathogenic mechanisms underlying CD have been thoroughly investigated, little is known about the early interactions and effects of gliadins on the intestinal epithelium, prior to the appearance of severe mucosal damage. In this respect, proteomic approaches have been applied to investigate the molecular changes in response to gluten of intestinal epithelial cell cultures [16], intestinal tissue of animals [17] and duodenal biopsies of CD patients [18]. These studies have provided important information, shedding light on the underlying mechanisms of CD and the way epithelial cells or intestinal tissue sense and respond to gliadins. In *in vivo* animal studies, the chemokine CXCR3 receptor, a 7-transmembrane G-protein, present in the brush border membrane, has been identified as responsible for initial gliadin interaction with intestinal cells triggering inflammatory signals [17], which could be similarly identified in human intestinal epithelial (Caco-2) cells [16,19]. Moreover, proteomic analyses of biopsies obtained from CD patients, with different Marsh classification, also revealed both up-regulated expression of calcium-activated channels and down-regulated expression of proteins involved in the peroxisome proliferator-activated receptors (PPAR) pathway [18], which participates in the regulation of immune activation mediated by the NFκB pathway [20,21].

In view of the limited knowledge of the role played by components of the intestinal microbiota on gliadin-mediated CD pathogenesis, this study aims to compare changes in the proteome of jejunal sections of weanling animals, sensitized or not with interferon (IFN)-γ, and fed gliadin. Moreover, the study determines the effect of the co-administration of *B. longum* CECT 7347, and compares the results obtained with those for control animals.

2. Material and methods

2.1. Bacterial strain and culture conditions

Cell cultures of *B. longum* CECT 7347, isolated from healthy infants, were grown in Man-Rogosa-Sharpe agar and broth (Scharlau, Barcelona, Spain) supplemented with 0.05% (w/v) cysteine (MRS-C; Sigma-Aldrich, St. Louis, USA), and kept at 37 °C in anaerobic conditions (AnaeroGen, Oxoid, Basingstoke, UK) for 24 h [12]. For animal studies, the strain was grown overnight and the culture used to inoculate fresh MRS-C broth incubated for 22 h. Cells were harvested by centrifugation (6000 ×*g* for 15 min), washed twice in phosphate buffered saline (PBS, 130 mM sodium chloride and 10 mM sodium phosphate, pH 7.4), and re-suspended in a hypoallergenic milk-based formula (Nutramigen©, Mead Johnson B.V., Nijmegen, Netherlands) [12]. Aliquots of these cell suspensions were frozen in liquid nitrogen and stored at −80 °C until use. The number of live cells after freezing and thawing was determined by plate counting and no significant differences were found during storage time. One fresh aliquot was thawed for every new dosage to avoid variability in bacterial cell viability between the different treatment groups.

2.2. Animals and experimental design

All experiments were carried out according to the guidelines for the Care and Use of Laboratory Animals of the University of Valencia (SCSIE, University of Valencia, Spain) and the protocol was approved by its Ethic Committee. The adult females were date-mated, and fed *ad libitum* with a standard diet (Harlan Bioproducts, Indianapolis, USA). Experimental animals were female, weanling Wistar rats, provided by the SCSIE. Shortly after spontaneous birth, animals were randomly distributed into six different groups (n=6 per group), as previously described [12]: 1) artificially reared (AR) with the