

LETTER

Is it true that coeliacs do not digest gliadin? Degradation pattern of gliadin in coeliac disease small intestinal mucosa

Prolyl-endopeptidase supplementation has been proposed to favour gliadin degradation as an alternative treatment for coeliac disease (CD), although the real usefulness of this therapy *in vivo* is still under discussion.¹ However, our data point to alternative treatments aiming to modify the intestinal microbiota in patients with CD by the use of probiotics and/or prebiotics. We propose that the induction of gliadin proteolysis in the human gut might not be the solution but the origin of CD.

We have carried out gliadin zymograms using a complete protein solution from duodenal mucosa of patients with CD, both untreated patients with positive serology, genetics and duodenal inflammation (n = 20), and treated patients on a gluten-free diet (GFD) with negative serology and recovering mucosa (n = 9). We have also analysed 18 non-CD controls, without mucosal inflammation and negative serology.

Figure 1 reveals for the first time to our knowledge, and in contrast to what would be expected, the existence of a specific gliadinase pattern in duodenal samples from patients with CD. This pattern was observed regardless of the phase of the disease, either active or treated with a

GFD, and therefore dismisses a secondary effect due to the effect of matrix metalloproteinases (MMPs) given that gliadinases were also found in non-altered serology-negative samples from GFD-treated patients who do not express MMPs.^{2,3} Neither gender- nor age-related effects (children or adults) were found. The existence of gliadinases in all the assayed samples (patients with CD and non-CD controls) suggests that the absence of gliadinases in non-CD healthy controls was not due to protease degradation in the latter, and confirms that gliadinases are CD specific.

Gliadin zymograms were also performed after intraepithelial and lamina propria leucocyte isolation from duodenal samples. The results obtained dismiss the leucocyte origin of gliadinases, since they were not detected in any case, as compared with non-treated explants from the same patients.

After MALDI-TOF (matrix-assisted laser desorption ionisation-time of flight) and ion-trap mass spectrometry analyses of the 26 kDa gliadinase, a biological cleavage point Pro-Pro in γ gliadin was identified. According to the MEROPS peptidase database (v 8.0),⁴ this protease point can only be cleaved by three known human proteases: MMP9 (M10.004, 92.0 kDa), prolyl oligopeptidase (POP, S09.001, 80.7 kDa) and dipeptidyl-peptidase II (DPP7; S28.002, 54.3 kDa); or by the metalloprotease proline-specific peptidyl-dipeptidase from *Streptomyces* (M9D.002, mass not specified). None of the human proteases was revealed as responsible for the gliadinase activities, since no inhibition was obtained even after

100 K_i specific inhibitory concentrations (all from Calbiochem) in the incubation buffer of zymograms. On the contrary, all gliadinases were MMPs since these were inhibited after 20 mM EDTA supplementation to the incubation buffer. That led us to think that gliadinases might have a bacterial origin within the duodenum of patients with CD. The latter was demonstrated after completely inhibiting all gliadinases after 24 h culture with antibiotics (penicillin, streptomycin and gentamicin) of duodenal explants, as compared with non-treated explants from the same patients. Moreover, gliadinase activities were detected in five out of five duodenal biopsy-associated microbiota extracts from untreated patients with CD recovered from biopsy culture in two bacteriological culture media (YCFA and BH). These gliadin-metabolising bacteria may represent one of the environmental missing links in the development of CD. These bacteria could be absent, or present to a much lower degree, in the duodenum of all non-predisposed individuals, as compared with those patients who develop CD. Interestingly, the possible implication of duodenal bacteria in the pathogenesis of CD has recently been described.⁵⁻⁷ Therefore, identifying duodenal bacterial strains responsible for the gliadinase activities may help to confirm this point.

In conclusion, our data point to a possible contribution of specific CD mucosal-associated bacteria to the development of the disease, due to metallo-gliadinase activities. This provides new insight into the pathogenesis of CD and helps to lay the foundation for the proposal of new prophylactic and therapeutic alternatives to restore the composition of the gut microbiota and its metabolic activity

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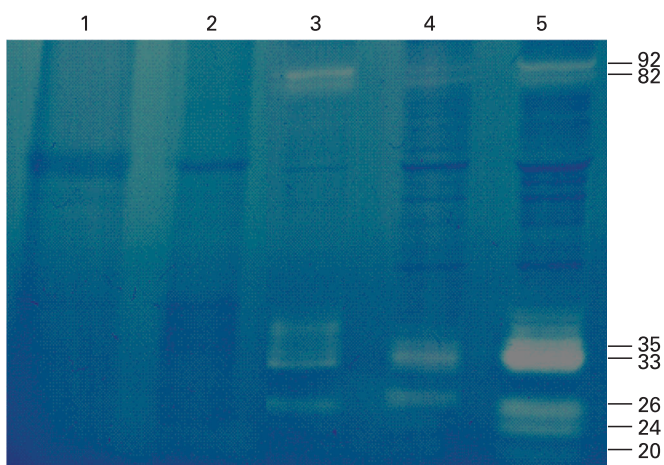


Figure 1 Gliadin zymogram representative of several whole protein duodenal biopsy explants from non-coeliac disease (CD) healthy controls (lanes 1 and 2), and patients with CD, both untreated (lanes 3 and 4) and treated by a gluten-free diet (GFD) with no duodenal histological changes (lane 5). The identified gliadin-degrading protease (gliadinase) pattern is characterised by seven CD-specific gliadinases with approximative masses of 92, 82, 35, 33, 26, 24 and 20 kDa. It was found in almost all samples from patients with CD regardless of GFD treatment (17 out of 20 untreated patients, 8 out of 9 GFD-treated patients) and remained nearly absent in non-CD samples (1 out of 18). Although there were interindividual differences in band intensity among patients with CD, it was not possible to discern an intensity pattern that could differentiate treated or untreated patients with CD. On the right-hand side, approximative molecular masses in kDa of the identified proteases are shown.

Letter

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