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Imbalance in *Bacteroides* species composition associated with coeliac disease

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The gut microbiota and the human host have developed a mutualistic relationship, contributing to the host's ability to avoid pathogen infection, suppress chronic inflammation and preserve intestinal homeostasis. The gut immune system has to maintain a balance between the need to respond to pathogens and tolerate the presence of commensal bacteria, whose breakdown leads to disease. Coeliac disease (CD) is a chronic inflammatory disorder of the small intestine that presents in genetically predisposed individuals, following gluten consumption. Imbalances in the intestinal microbiota of CD patients, characterised by increases in *Bacteroides* proportions, have been reported[(1,2)]. In addition, the unbalanced gut microbiota activated *in vitro* the production of pro-inflammatory cytokines and, therefore, its contribution to CD pathogenesis cannot be disregarded. The objective of this study was to characterise the *Bacteroides* species composition of duodenal biopsies of CD patients by PCR and denaturing gradient gel electrophoresis (DGGE))in order to progress in the knowledge of the possible contribution of this genus to the CD pathogenesis.

The diversity of *Bacteroides*-related species was studied in biopsy specimens of 20 active CD patients, 4 non-active CD patients and 8 control children. DNA from biopsies and from pure cultures of the bacterial strains used as ladder was extracted by using the QIAamp DNA stool Mini kit. Partial 16S rRNA gene amplification was performed using the primer pair Bfra 531-f and Bfra 766r-GC, and the obtained PCR amplicons (293 bp) were separated by DGGE. Identity of the amplicons was determined by their comparison with those of the reference strains and DNA sequencing. The presence or absence of DNA bands was registered and these data were analysed by using the Shannon–Wiener diversity index and the Dice/UPGMA cluster analysis.

Clustering analysis of the DGGE profiles showed two differentiated clusters: the first, grouping most children with active CD; and, the second, subdivided into two clusters, one grouping control subjects and the other grouping non-active CD children. The individual DGGE profiles showed similarity values ranging from 35 to 100%. The biological diversity of *Bacteroides* species was significantly greater in control children ($H' = 1.67$) than in non-active CD ($H' = 1.1$, $P = 0.006$) and active CD patients ($H' = 0.99$, $P < 0.001$).

Bacteroides distasonis, Bacteroides fragilis, Bacteroides thetaiotaomicron, *Bacteroides uniformis* and *Bacteroides vulgatus* were significantly more abundant in DGGE profiles from control children than in those from active and non-active CD patients ($P < 0.01$). By contrast, *Bacteroides ovatus* was significantly more abundant in controls and non-active CD children than in active CD patients ($P < 0.01$). *Bacteroides coprocola* was more frequently detected in non-active CD patients than in controls and active CD children ($P < 0.01$). *Bacteroides dorei* was more abundant in active CD patients than in non-active CD patients and control children ($P < 0.01$).

In summary, the duodenal *Bacteroides* population of CD patients shows differences in biological diversity and species composition in comparison with that of control subjects. These changes do not seem to be completely dependent on the inflammatory status of the mucosa and, therefore, might be additional factors contributing to the risk and/or pathogenesis of the disease.

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