

## Butyric acid-producing anaerobic bacteria as a novel probiotic treatment approach for inflammatory bowel disease

Inflammatory bowel disease (IBD), of which Crohn's disease (CD) and ulcerative colitis (UC) are the most common manifestations, is characterized by chronic inflammation of the lining of the gastrointestinal tract, which causes severe watery and bloody diarrhoea, and abdominal pain. IBD is often debilitating and is characterized by onset at a young age and extra-intestinal manifestations. Whereas CD can affect any part of the gastrointestinal tract, UC is usually confined to the colon and rectum. IBD is an emerging disease and the incidence amounts to 20/100 000 in Europe and North America. There is a link with the social and economic development of the countries: the increase in IBD was first seen in northern Europe and North America, followed by the rest of Europe, Japan, South America and certain parts of Asia (Cohen, 2000; Ouyang *et al.*, 2005). Although the exact aetiopathogenesis of IBD are not clear, it is widely accepted that the disease derives from an inappropriate immune response in genetically susceptible individuals as the result of a complex interaction between environmental factors, the microbiota and the intestinal immune system (Danese & Fiocchi, 2006).

In IBD, the response to conventional drug therapy is suboptimal. This therapy is associated with a number of disadvantages, including a considerable burden of side effects, and surgery is still required in a subgroup of patients. Because IBD represents a distortion of the relationship between the gut microbiota and the host response, probiotics and prebiotics have been proposed as possible therapeutic products. The probiotics that are being tested for treatment of IBD are mainly lactic acid bacteria, i.e. lactobacilli and bifidobacteria. Although a decrease in the number of lactic acid bacteria has been shown in IBD, administration of lactic acid bacteria has yielded inconsistent results with respect to the treatment of IBD

(Gueimonde *et al.*, 2007; Hedin *et al.*, 2007). The only well-documented indication for the use of probiotics in IBD is in the treatment of pouchitis, particularly using a mixed lactobacilli–bifidobacteria product (Mimura *et al.*, 2004). Many trials using probiotics either as maintenance therapy in UC, to treat active UC, to maintain remission in CD, or to treat active CD, have yielded mixed results, concluding that their use cannot be recommended based on the available evidence (Hedin *et al.*, 2007).

Butyric acid, produced within the intestinal lumen by bacterial fermentation of dietary carbohydrates, exerts a wide variety of effects on intestinal function (Hamer *et al.*, 2008). First of all, butyric acid is the preferred source of energy for colonocytes. It affects cellular proliferation, differentiation and apoptosis. Moreover, butyric acid has well documented anti-inflammatory effects. Inhibition of histone deacetylase activity, resulting in hyperacetylation of histones, and as a consequence suppression of nuclear factor- $\kappa$ B activation, is a likely explanation. Secondly, it has been proposed that butyric acid reinforces the colonic defence barrier by increasing production of mucins and antimicrobial peptides. Thirdly, it has been shown that butyric acid decreases intestinal epithelial permeability by increasing the expression of tight junction proteins. Anti-inflammatory activities, combined with a strengthening of the mucosal barrier integrity, are ideal properties for therapeutic compounds against IBD-like syndromes. Indeed it has been shown that butyrate enemas can yield positive results in the treatment of active UC (Breuer *et al.*, 1997).

Butyric acid thus has a potential therapeutic value against IBD, but the actual delivery into the gut is still problematic. Several mechanisms are possible.

- (a) Fermentation of dietary fibre leads to butyrate production. An

increase in butyrate production may result from a direct stimulation of butyrate producers or indirect effects such as metabolic cross-feeding of fermentation products from other bacterial groups (Flint *et al.*, 2007).

- (b) The use of butyric acid-coated tablets is a possible delivery method, but the tablets may not always release their content at the intended location because of inter-individual differences in gut lumen pH and transit time. Moreover the taste is very unpleasant. Alternative approaches are the consumption of butyrylated starch and the use of butyryl-L-carnitine as a potential prodrug (Srinivas *et al.*, 2007; Bajka *et al.*, 2008).
- (c) Rectal butyric acid enemas hold promise but the use of enemas is often hampered by a low compliance rate, and a short and discontinuous exposure of the colon mucosa to the butyrate (Breuer *et al.*, 1997).
- (d) A final possibility is the administration of colonizing butyric acid-producing bacteria, enabling *in situ* production of butyric acid.

Several studies have shown that, numerically, the majority of butyrate-producing bacteria found in human faeces are highly oxygen-sensitive anaerobes belonging to the clostridial clusters IV and XIVa (Louis & Flint, 2009). Decreases in members of the butyrate-producing clostridial clusters IV and XIVa have been reproducibly reported in the gut of IBD patients (Sokol *et al.*, 2008; Takaishi *et al.*, 2008). These species are thus probiotic candidates for the treatment of IBD. Recently, considerable progress has been made in the isolation of these strictly anaerobic butyric acid-producing bacteria from the human gut. It has been shown

that lactic acid, produced *in vitro* by lactic acid bacteria, is used by some strictly anaerobic butyrate-producing bacteria of clostridial cluster XIVa for the production of high concentrations of butyric acid (Louis & Flint, 2009). This mechanism is called cross-feeding and could explain why administration of lactic acid bacteria to IBD patients can in certain cases be an advantage, i.e. through stimulation of butyric acid production. However, other possible mechanisms of action, including immunoregulatory effects, also have been suggested (Geier *et al.*, 2007). An ideal probiotic would thus be a colonizing bacterium that combines systemic anti-inflammatory and immunoregulatory effects with delivery of high butyrate levels at the site of action and that can be ingested in a stable form, such as spores. One potential problem is that bowel conditions in IBD patients may not be optimal to support growth of these bacterial species. However, butyrate producers present in the colon are phylogenetically diverse and it has not been investigated in detail yet which species are decreased in IBD. Therefore, it is likely that strains can be identified that are good colonizers. Furthermore, a synbiotic approach, using a prebiotic in combination with a butyrate producer, may help to re-establish such bacteria in the individual in the diseased state. First attempts have been made in animal models of IBD to apply butyrate-producing bacteria (Sokol *et al.*, 2008; Eckhau *et al.*, 2009). Strains of clostridium cluster IV and XIVa were found to be highly successful in decreasing inflammation and necrosis in rodent IBD models. Indeed, *Faecalibacterium prausnitzii*, a species belonging to cluster IV, has been shown to be much less prevalent in the gut microbiota of IBD patients. This species showed great promise in counterbalancing dysbiosis in a mouse IBD model (Sokol *et al.*, 2008, 2009). Interestingly, components of the culture supernatant of this bacterium had the capacity to decrease production of the inflammatory cytokines interleukin 12 and gamma interferon, and to increase production of the anti-inflammatory cytokine interleukin 10, in peripheral blood mononuclear cells in a way independent of butyrate (Sokol *et al.*, 2008). Oral and intraperitoneal administration of the culture supernatant in a mouse IBD model reduced colitis and mortality, respectively

(Sokol *et al.*, 2008). This clearly suggests that *F. prausnitzii* secretes certain molecules that have the potential to be used as therapeutics. In fact it has not been resolved whether butyrate is the key molecule in the anti-colitis effects of *F. prausnitzii* or whether these depend on a combination of secreted compounds yet-to-be identified that might act independently or together with butyrate. Notably, however, *F. prausnitzii* administration led to a shift in the microbiota composition that could also have complex effects on colitis (Sokol *et al.*, 2008). It has been shown that the inflamed intestinal mucosa of IBD patients has a defective butyrate metabolism, leading to reduced efficiency of butyrate, again suggesting that other compounds in addition to butyrate may be involved in the beneficial effects of the butyrate-producing microbiota (Thibault *et al.*, 2010). Nevertheless, it is clear that the pool of butyrate-producing bacteria from clostridial cluster IV and XIVa harbours very promising preventive and therapeutic probiotic strains. In a comparative study using four strains, it was shown that a particular strain from clostridium cluster IV, *Butyricoccus pullicaecorum*, was superior in decreasing lesions in a rat IBD model compared to *F. prausnitzii* (Eckhau *et al.*, 2009). It is therefore suggested that research efforts should be increased to test the therapeutic value of these next generation probiotic strains, and to identify further strains that are able to colonize the gut of IBD patients without being outcompeted.

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