

# Probiotics and inflammatory bowel diseases

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Enteric microflora profiles vary considerably between active inflammatory bowel diseases (IBD) and healthy conditions. Intestinal microflora may partake in the pathogenesis of IBD by one or some ways: specific pathogenic infection induces abnormal intestinal mucosal inflammation; aberrant microflora components trigger the onset of IBD; abnormal host immune response loses normal immune tolerance to luminal components; luminal antigens permeate through the defective mucosal barrier into mucosal lamina propria and induce abnormal inflammatory response. Preliminary studies suggest that administration of probiotics may be benefit for experimental colitis and clinical trials for IBD. Researches have been studying the function of probiotics. Introduction of probiotics can balance the aberrant enteric microflora in IBD patients, and reinforce the various lines of intestinal defence by inhibiting microbial pathogens growth, increasing intestinal epithelial tight junction and permeability, modulating immune response of intestinal epithelia and mucosal immune cells, secreting antimicrobial products, decomposing luminal pathogenic antigens.

## MICROBIAL FACTORS IN THE PATHOGENESIS OF INFLAMMATORY BOWEL DISEASES

The abnormal intestinal immunological function, resulting from an interplay between genetic susceptibility and certain environmental factors, contributes significantly to the inflammatory process of IBD.<sup>8</sup> Because the intestinal lesions in IBD occur mainly in the area with the highest bacteria concentration, it is believed that abnormal interaction between intestinal mucosal immune system and aberrant microflora in intestinal tract results in changed immunological function, and triggers the inflammatory response of IBD.<sup>9</sup> Intestinal microflora may partake in the pathogenesis of IBD by one or more ways depicted below: specific pathogenic infection induces abnormal mucosal inflammation; aberrant enteric microflora components trigger the onset of IBD; abnormal host immune response lose normal immune tolerance to luminal components; luminal antigens permeate through the defective mucosal barrier into mucosal lamina propria and induce abnormal immune response.

### Specific pathogenic infection induces abnormal intestinal mucosa inflammation

Crohn's disease and ulcerative colitis occur in the areas of the highest luminal bacterial concentration, and are similar to the defined intestinal infections. Many microbial pathogens have been suggested to cause intestinal infection and induce the disease's flare.<sup>10</sup> Many molecular techniques have been used to detect presence of the microbial pathogens in IBD patients. Sanderson *et al*<sup>11</sup> used polymerase chain reaction (PCR) to detect 5' region of IS900 DNA of *Mycobacterium paratuberculosis* genome from intestinal samples of Crohn's disease, ulcerative colitis, and control group. *M paratuberculosis* was identified in 26 of 40 (65%) Crohn's disease, 1 of 23 (4.3%) ulcerative colitis, and 5 of 40 (12.5%) control tissues. Positive samples of Crohn's disease were from both small intestine and colon, those positive of control tissues were from the colon only. The presence in two thirds of Crohn's disease tissues but in less than 5% of ulcerative colitis tissues was consistent with an aetiological role for *M paratuberculosis* in Crohn's disease. A large cluster of genetically linked *E coli* strains, according to another report,<sup>12</sup> were isolated more frequently from patients with chronic and recurrent Crohn's disease (24 of 33 patients) than from controls (9 of 21), which suggested that some genotypes of *E coli* were

The aetiology of inflammatory bowel diseases (IBD), which consist mainly of two forms as Crohn's disease and ulcerative colitis, is still unknown. However, more and more evidence shows gut microflora plays an important part in initiating and maintaining the mucosal inflammatory response in IBD.<sup>1 2</sup> Microflora in IBD patients becomes aberrant with normal microflora decreased, such as bifidobacterium and lactobacillus, pathogenic or potential harmful bacteria increased.<sup>3 4</sup> Supplement with probiotics may balance the indigenous microflora in IBD patients, and have a therapeutic effect on IBD.<sup>5 6</sup> Probiotics are live microorganisms that have a beneficial effect on health by changing the microbial environment.<sup>7</sup> Probiotic mixture often contains bifidobacteria, lactobacilli, and some non-pathogenic bacteria as *Escherichia*, enterococci. We reviewed the pathogenic effect of intestinal microflora on the pathogenesis of IBD, and the therapeutic effect of probiotics on the diseases and the possible mechanism (see box 1).

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### Box 1 Microbial factors in the pathogenesis of inflammatory bowel diseases and the therapeutic effect of probiotics on inflammatory bowel diseases

#### Microbial factors in the pathogenesis of inflammatory bowel diseases

- Specific pathogenic infection induces abnormal intestinal mucosa inflammation
- Changed enteric microflora components trigger the onset of IBD
- Aberrant mucosal immune system responds to luminal components
- Luminal antigens induce abnormal immune response through the defective mucosal barrier

#### The therapeutic effect of probiotics on inflammatory bowel diseases

- Inhibiting microbial pathogens growth
- Increasing epithelial tight junction and modifying intestinal permeability
- Modulating immune response of intestinal epithelia and mucosal immune cells
- Secreting antimicrobial products
- Decomposing luminal pathogenic antigens

associated with chronic or early recurrent Crohn's disease lesions.

Some pathogenic bacteria infections have been linked with the pathogenesis of ulcerative colitis. Ohkusa *et al*<sup>13</sup> isolated and cultured *Fusobacterium varium* from UC patients. They found the supernatants from cultures of *Fusobacterium varium* were cytotoxic. When mice were given enemas containing *Fusobacterium varium* culture supernatants, murine colitis, which was human ulcerative colitis-like lesions, was seen 24 hours later, which means *Fusobacterium varium* may be one of the elusive pathogenic factors in ulcerative colitis. Streutker *et al*<sup>14</sup> detected the ribosomal DNA of members of the *Helicobacter spp* by PCR in biopsy specimens from patients with and without ulcerative colitis. DNA from five biopsy samples of 33 ulcerative colitis patients were positive compared with 0 of 29 age matched controls. These results show that *Helicobacter spp* may be involved in some cases of ulcerative colitis.

*M paratuberculosis* and *E coli* have been investigated as possible cause of Crohn's disease, and *Fusobacterium varium* or *Helicobacter spp* may be an opportunistic pathogen in ulcerative colitis. However, no specific pathogens linked to IBD have been confirmed by blinded, controlled investigation. Treatment directed against specific putative pathogens has no obvious benefit for the diseases, but treatment with corticosteroid does have therapeutic effect on IBD, which implies IBD may be initiated and activated by commensal enteric bacteria and the products as the environmental triggers, other than a specific infectious agent.

#### Changed enteric microflora components trigger the onset of IBD

There are at least 400 bacterial species in enteric microflora, which influence mucosal immune development, structure, function, and the mucosal integrity.<sup>15</sup> Subtle change in enteric bacteria components may have profound implications for mucosal barrier function and immune response.

Enteric microflora profiles vary considerably between active IBD patients and healthy conditions, and the studies comparing enteric bacteria profiles in active and non-active IBD have shown a decrease in bifidobacterium and lactobacillus in patients with active diseases, but normal in patients with remission.<sup>16</sup> Even though no specific pathogen has been identified as the cause of IBD, pathogenic and potential harmful enteric bacteria are seen more frequently in IBD patients than in health.<sup>17, 18</sup> The pathogenic bacteria trigger intestine inflammation by secreting enterotoxins that increase epithelial cell permeability,<sup>19, 20</sup> producing immunosuppressive proteins that dysregulate the mucosal immune system, and impairing epithelial cell metabolism. Distal intestinal epithelia depend on butyrate as the primary energy source. Hydrogen sulphide and sulphate reducing bacterial counts are increased in patients with ulcerative colitis,<sup>21</sup> block of butyrate metabolism by hydrogen sulphide has detrimental effects on mucosal permeability and healing.

The hypothesis of bacterial influence on the pathogenesis of IBD is strongly supported by the IL10 knockout mouse model.<sup>22</sup> When kept in a germ free environment, IL10 deficient mice had no evidence of colitis or immune system activation. However, when contaminated with a specific pathogen free flora, IL10 knockout mice developed colitis with the time of contamination, and exhibited immune system activation as evidenced by increased expression of CD44 on CD4<sup>+</sup> T cells, increased mesenteric lymph node cell numbers, and increased production of immunoglobulin A (IgA), IgG1, and IL12 p40 from colon fragment cultures. The mice were populated with bacterial strains, including *Bacteroides vulgatus*, known to induce minimal colitis in other rodent models. Enteric bacteria were necessary for the development of spontaneous colitis and immune system activation in IL10 deficient mice.

#### Aberrant mucosal immune response to normal luminal components

IBD may be the result of abnormal host response to luminal antigens, including the resident microflora, bacterial products, toxins, or food antigens.<sup>23</sup> Hyporesponsiveness of lamina propria mononuclear cells exists to enteric flora in normal intestine, but tolerance to microflora is broken in the process of IBD.<sup>24</sup> Landers *et al*<sup>25</sup> recently analysed serum responses of patients with Crohn's disease to microbial and autoantigens, including antibodies to *E coli* outer membrane porin C, pseudomonas fluorescens associated sequence I2, antisaccharomyces cerevisiae antibody (ASCA), and perinuclear antineutrophil cytoplasmic antibodies. Eighty five per cent of patients responded to at least one antigen; only 4% responded to all four. Among microbial antigens, 78% patients responded to at least one, and 57% were double positive, but only 26% responded to three. The level of response was stable over time and with change in disease activity. That means that immune responses of Crohn's disease patients selectively lose tolerance to automicrobial and microbial antigens.

Intestinal microflora becomes aberrant in the process of IBD. There is close relation between aberrant microflora and mucosal immune function disorder, abnormal bacterial stimulation may induce mucosal inflammation, and break the delicate balance between proinflammatory molecules and activity of T lymphocytes.<sup>26</sup> Unrestrained activation of the mucosal immune cells against some commensal bacteria seems to be responsible for the characteristic relapsing course of IBD. Wide spectrum antibiotic therapy reduces bacterial load and mitigates intestinal inflammation in human IBD and in animal models. Bacterial wall products can also modify neutrophil tissue destruction mechanisms and might be pivotal for perpetuation of chronic colonic inflammation.<sup>27</sup>

### Defective mucosal barrier to luminal antigens

The normal intestinal epithelium provides a barrier comparatively impermeable to luminal constituents. Mucosal permeability is increased, and mucosal barrier is impaired during the process of intestinal inflammation, including IBD.<sup>28</sup> Impairment of mucosal barrier may result in increased uptake of antigens and proinflammatory molecules including bacterial products and endotoxins into lamina propria, followed by activation of immune cells in lamina propria, secretion of proinflammatory cytokines, products of reactive oxygen metabolites and proteases, and mucosal damage.<sup>29</sup> Madsen *et al*<sup>30</sup> have discussed the relation between the increased intestinal permeability and the onset of colitis by IL10 gene deficient mice model. At 2 weeks of age, IL10 gene deficient mice in pathogen free condition show an increment in ileal and colonic permeability in the absence of any histological injury. This primary permeability defect is associated with increased mucosal secretion of interferon gamma and tumour necrosis factor  $\alpha$  (TNF $\alpha$ ). Colonic permeability remains increased as inflammation progresses. However, IL10 gene deficient mice raised under germ free conditions do not show any evidence of intestinal inflammation, with normal intestinal permeability and cytokine levels. These data suggest that the intestinal permeability defect in IL10 gene deficient mice occurs because of a dysregulated immune response to enteric microflora, and this permeability defect exists before the development of mucosal inflammation.

Specific abnormalities in mucosal barrier function in IBD patients may be a primary event or secondary to luminal microbial or other environmental triggers, such as cigarette smoking. The investigation of increased mucosal permeability in clinically healthy relatives of patients with Crohn's disease, suggests a primary underlying defect in intestinal permeability.<sup>31</sup> The abnormal leakiness of the mucosa in IBD patients and their relatives can be amplified by oral administration of non-steroidal anti-inflammatory drugs (NSAID), such as aspirin.<sup>32</sup> As the permeability is increased in most patients with IBD, Permeability measurements in IBD patients may reflect the activity, extent, and distribution of the disease, and allow us to predict the likelihood of recurrence after surgery or medically induced remission.

### PROBIOTICS FOR THE TREATMENT OF INFLAMMATORY BOWEL DISEASE AND POSSIBLE MECHANISMS OF ACTION

Enteric microflora becomes aberrant in the process of IBD, with normal microflora decreased, such as bifidobacterium and lactobacillus.<sup>3 4</sup> Favier *et al*<sup>3</sup> cultured and counted the colonic microflora in faeces from patients with active Crohn's disease. Bifidobacteria numbers were significantly reduced in patients compared with healthy controls, whereas bacteroides and lactobacilli counts remained unchanged. Bacterial enzyme activities, especially  $\beta$ -D-galactosidase, were also decreased in faecal extracts from Crohn's disease patients, correlated with the decrease of bifidobacteria counts. Another investigation<sup>33</sup> had analysed colonic mucosa associated microflora in patients with active and inactive ulcerative colitis. Significant decrease in the number of anaerobic bacteria, anaerobic Gram negatives, and lactobacillus was shown in patients with active ulcerative colitis, whereas no changes were seen in the number of aerobic bacteria and enterobacteriaceae. However, no significant difference in colonic mucosa associated microflora could be shown in patients with inactive ulcerative colitis and healthy conditions.

The luminal microflora in IBD patients lost the anti-inflammatory function that exists in normal condition, with a reduction in the number of anaerobic bacteria and

lactobacillus. Probiotics administration can help restore microbial homeostasis in the gut, down-regulate intestinal inflammation, and ameliorate the diseases.<sup>34</sup> A lot of clinical trials have shown that probiotics have beneficial effect on IBD patients.<sup>35 36</sup> A pilot study<sup>35</sup> was conducted to investigate the possible effect of lactobacillus GG in four children with active Crohn's disease. Three patients treated with oral lactobacillus GG showed significant improvement in terms of clinical outcome, and it was possible to taper the dose of corticosteroids. That means lactobacillus GG seems to be effective in improving the clinical status of children with Crohn's disease. Gionchetti *et al*<sup>36</sup> had evaluated the efficacy of a probiotic preparation VSL#3 in maintenance of chronic pouchitis remission compared with placebo. VSL#3 contains  $5 \times 10^{11}$  per gram of viable lyophilised bacteria of four strains of lactobacilli, three strains of bifidobacteria, and one strain of streptococcus salivarius subsp thermophilus. Forty patients in clinical and endoscopic remission were randomised to receive either VSL#3, 6 g/day, or an identical placebo for nine months. Three patients (15%) in the VSL#3 group had relapses within the nine month follow up period, compared with 20 (100%) in the placebo group. Faecal concentration of lactobacilli, bifidobacteria, and *S thermophilus* increased significantly from baseline levels only in the VSL#3 treated group. These results suggested that oral administration of probiotic preparation was effective in preventing flare ups of chronic pouchitis.

Probiotics have beneficial effect on IBD, with the main related mechanisms including: inhibiting microbial pathogens growth, increasing epithelial tight junction and permeability, modulating immune response of intestinal epithelia and mucosal immune cells, secreting antimicrobial products, decomposing luminal pathogenic antigens.

### Inhibiting microbial pathogens growth

Probiotics compete with microbial pathogens for the limited number receptors present on the surface of epithelia, and inhibit epithelium attachment and invasion by enterotoxigenic and enteropathogenic bacteria.<sup>34 37</sup> If probiotic strains bound to the surface receptors or mucus of epithelia, they can inhibit successfully adhesion and cell invasion to epithelia by a large variety of diarrhoeagenic bacteria, such as *E coli*, *Yersinia pseudotuberculosis*, and *Salmonella typhimurium*. Probiotics have been shown to colonise the gut during therapy and ferment dietary fibre. Short chain fatty acids and other acids products produced by the enteric bacterial fermentation of indigestive dietary fibre, can induce specific pH and other chemical changes in the lumen,<sup>38 39</sup> these effect may directly inhibit the growth of pathogens. Short chain fatty acids are the main energy source of intestinal epithelia, and have anti-inflammatory properties.<sup>40 41</sup> Metabolic effects of microflora include nutrient, vitamin products, and detoxification of some dietary pathogens. The fermentation can be intensified by oral administration of probiotic preparation.

Probiotic agents can modulate intestinal mucins expression and inhibit adherence of attaching and effacing pathogenic organisms to intestinal epithelia. Mack *et al*<sup>42</sup> had used probiotic strains to induce MUC2 and MUC3 mucin expression of HT-29 intestinal epithelial cells. Incubation of *Lactobacillus plantarum* 299v with HT-29 cells increased MUC2 and MUC3 expression levels, and inhibited adherence of an attaching and effacing pathogenic *E coli* to HT-29 cells.

### Increasing epithelial tight junction and modifying intestinal permeability

Probiotics can stabilise the intestinal barrier and epithelial tight junction. A preliminary report has shown lactobacillus GG modulates the antigen specific immune response induced by cows' milk in suckling rats, counteracts increased

permeability disorder, and stabilises the mucosal barrier.<sup>43</sup> The benefit effect of probiotics on modifying intestinal permeability was shown by other experiments.<sup>44-45</sup> Rats were instilled in an exteriorised colonic segment with 4% acetic acid to induce colitis, the colitis in four days after acetic acid administration was featured with a threefold increase in myeloperoxidase activity, and a sixfold increase of mucosal permeability in the colonic tissue. Intracolonic administration of *L reuteri* R2LC immediately after acetic acid instillation, downregulated the morphological score, MPO activity, mucosal permeability, and prevented the development of colitis.<sup>44</sup> Madsen *et al*<sup>45</sup> have also reported administration of lactobacillus can prevent colitis of IL10 gene deficient mice. At 2 weeks of age, IL10 gene deficient mice in pathogen free conditions showed no colonic injury but did display abnormal colonic bacterial colonisation with increased colonic mucosal aerobic adherent and translocated bacteria, in conjunction with reduced *Lactobacillus spp* levels. In association with the abnormal colonic bacterial colonisation, colitis developed by 4 weeks of age. If the mice were treated by rectal delivery of *L reuteri* daily, restoring *Lactobacillus spp* to normal levels reduced the levels of colonic mucosal adherent and translocated bacteria, and attenuated the development of the colitis.

Apoptosis of intestinal epithelia can be induced by a lot of proinflammatory cytokines such as TNF $\alpha$ , IL1 $\beta$ , and interferon gamma during the process of intestinal inflammation.<sup>46</sup> Probiotics can counteract the apoptosis disorder of intestinal epithelia. Yan *et al*<sup>47</sup> studied the effect of *Lactobacillus rhamnosus* GG on preventing cytokine induced apoptosis in two different intestinal epithelial cell models. Culture of LGG with either mouse or human colon cells activated the antiapoptotic Akt/protein kinase B. This model probiotics also inhibited activation of the pro-apoptotic p38/mitogen activated protein kinase induced by TNF $\alpha$ , IL1 $\beta$ , and interferon gamma. Furthermore, products from LGG culture supernatant showed concentration dependent activation of Akt and inhibition of cytokine induced apoptosis. These findings suggested probiotic microorganisms could increase survival of intestinal epithelia in an environment of proapoptotic cytokines and keep intestinal cell homeostasis.

### Modulating immune response of intestinal epithelia and mucosal immune cells

Probiotic treatment can downregulate the expression of proinflammatory cytokines as TNF $\alpha$ , IL1 $\beta$ , and interferon gamma, inducible nitric oxide synthase, and matrix metalloproteinase activity in inflamed mucosa of active IBD or

experimental colitis.<sup>48-52</sup> and the relevant mechanisms are well studied (see box 2).

In inflammatory conditions of IBD, immune cells secrete excessive inflammatory products, such as cytokines chemokines and a lot of active oxides. Overproduction of cytokines affect the biological action of epithelial cells, for instance, TNF $\alpha$  induces epithelial cells secreting IL8, expressing membrane TLR4 excessively.<sup>53</sup> TLR4 provides intestinal epithelia hyperreaction in response to LPS, the product of bacteria wells. IL8 has leucocytes chemotactic and stimulatory properties. The pathogens also lead intestinal epithelia to secrete increased products as IL8 and TNF $\alpha$ .<sup>54</sup> Probiotic strains interact with intestinal epithelia, and attenuate synthesis of inflammatory effector molecules elicited by diverse proinflammatory stimuli.<sup>55-57</sup> This immunosuppressive effect involves inhibition of the inhibitor  $\kappa$ B/nuclear factor  $\kappa$ B (I $\kappa$ B/NF- $\kappa$ B) pathway by block of I $\kappa$ B- $\alpha$  degradation, which prevents nuclear translocation of active NF- $\kappa$ B dimer and subsequent relevant gene expression.<sup>58-59</sup> That means probiotics can be responsible for the unique tolerance of the gastrointestinal mucosa to proinflammatory stimuli.

The intestinal apical di-/tripeptide transporter PepT1, responsible for the uptake of a broad array of small peptides transports bacterial proteoglycan derived muramyl dipeptide (MDP). PepT1 expression in chronic colonic inflammation is increased.<sup>60</sup> In colonic epithelial cells, MDP taken up by hPepT1 activates NF- $\kappa$ B and chemokine production. In this way, PepT1 may play an important part in promoting colonocyte participation in host defence and pathogen clearance through increased uptake of MDP. Probiotic bacteria such as *Lactobacillus casei* can change the function of intestinal PepT1, as a recent study has shown.<sup>61</sup> Incubation of Caco-2 cells with *L casei* for 48 hours leads to significant increase in hPEPT1 mediated [<sup>3</sup>H] Gly-Sar uptake, which means direct contact of probiotics with the Caco-2 apical membrane can increase PepT1 activity. As the data are poor in this area, more studies should be carried out to explore this bacterial/intestinal transporter interaction, and to find out how probiotics participate in host defence and pathogen clearance.

Probiotic strains may modify immune response of the intestinal immune cells.<sup>62</sup> Mucosal specimens from Crohn's disease patients and controls were cultured for 24 hours alone or with probiotic strains as non-pathogenic *E coli*, *L casei* DN-114001, *L bulgaricus* LB10, or *L crispatus*. Release of TNF $\alpha$  by inflamed Crohn's disease mucosa was significantly reduced by coculture with *L casei* or *L bulgaricus*, whereas changes induced by *L crispatus* or *E coli* were not significant. Coculture with *L casei* and *L bulgaricus* reduced the number of CD4 cells as well as TNF $\alpha$  expression among intraepithelial lymphocytes from Crohn's disease mucosa. None of the bacteria induced changes in non-inflamed mucosa. Probiotics interact with immunocompetent cells using the mucosal interface and modulate locally the production of proinflammatory cytokines.

Toll-like receptors (TLRs) are mediators of pathogen recognition in innate immune system.<sup>63</sup> Such recognition results in the initiation of an inflammatory immune response and subsequent instruction of the adaptive immune system, both of which are designed to rid the host of the invading pathogen. Probiotics can modify mucosal immune function via TLRs signalling. Preliminary data from experimental colitis show that TLR9 signalling is essential in mediating the anti-inflammatory effect of probiotics.<sup>64</sup> Intragastric or subcutaneous administration of probiotics and *E coli* DNA ameliorates the severity of DSS induced colitis in TLR2 and TLR4 deficient mice, whereas methylated probiotic DNA, calf thymus DNA, and DNase treated probiotics have no effect on TLR9 deficient mice. The protective effect of probiotics is

### Box 2 Possible mechanisms of probiotics modulating mucosal immune response in IBD

#### Downregulating proinflammatory cytokines secretion

- Inhibiting NF- $\kappa$ B activation
- Modulating PepT1 activity
- Reducing the number of CD4 intraepithelial lymphocytes
- Regulating anti-inflammatory effect via TLR9 signalling pathway
- Modulating apoptosis and proliferation of immune cell by TLR2 signalling
- Modulating peroxisome proliferator activated receptor (PPAR)  $\gamma$  pathway

mediated by their own DNA via TLR9 signalling pathway, rather than by their metabolites or ability to colonise the colon. Sturm *et al*<sup>65</sup> recently had demonstrated one probiotic strain, *E coli* Nissle 1917, could affect cell cycling and apoptosis of peripheral blood T cells (PBT). When anti-CD3 stimulated PBT are treated with *E coli* Nissle 1917 conditioned medium, *E coli* Nissle 1917 medium inhibit cell cycling and expansion of peripheral blood T cells, decrease cyclin D2, B1, and retinoblastoma protein expression contributing to the reduction of T cell proliferation, and regulate the decreased expression of IL2, TNF $\alpha$ , and interferon gamma but increased IL10 production in PBT. The inhibition of PBT proliferation by *E coli* Nissle 1917 conditioned medium is TLR2 dependent, shown by using toll-like receptor 2 (TLR-2) knockout mice. *E coli* Nissle 1917 may downregulate the expansion of newly recruited T cells into the mucosa and limit intestinal inflammation by TLR2 signalling.

Probiotics can modulate the inflammatory response in inflamed mucosa of IBD patients through peroxisome proliferator activated receptor (PPAR)  $\gamma$  pathway. PPAR $\gamma$  has been proposed as a key inhibitor of colitis through attenuation of NF- $\kappa$ B activity.<sup>66</sup> However, PPAR $\gamma$  expression is impaired in patients with ulcerative colitis,<sup>67</sup> compared with normal controls. *Bacteroides thetaiotaomicron*, as recently investigated, increase NF- $\kappa$ B subunit RelA exporting from nuclear through PPAR $\gamma$  dependent anti-inflammatory mechanism.<sup>68</sup> PPAR $\gamma$  in complex with nuclear RelA undergoes nucleocytoplasmic redistribution in response to *B thetaiotaomicron*, which shows a clue for therapeutic drug design and treatment of IBD.

### Secreting antimicrobial products

The normal intestinal microflora participates in the barrier effect by developing antimicrobial activity against enterovirulent bacteria, and the antimicrobial activity is related to bacteriocin produced by normal microflora. Vero cytotoxin produced by *E coli* O157:H7 is associated with haemorrhagic colitis and haemolytic uraemic syndrome in humans. One recent study<sup>69</sup> had shown some substance in the culture supernatant of *Bifidobacterium longum* HY8001 could neutralise the virulence of Vero cytotoxin successfully. Control mice were inoculated intragastrically with *E coli* O157:H7, *B longum* HY8001 culture supernatant were inoculated intragastrically to mice simultaneously for challenge with *E coli* O157:H7. TNF $\alpha$  and IL1 levels in sera were decreased in mice treated with *B longum* culture supernatant compared with those control mice. The concomitant experiment in vitro showed the culture supernatant of *B longum* primarily bound vero cytotoxin to interfere attachment and invasion of *E coli* to epithelia. These results suggest that soluble substance in *B longum* HY8001 culture supernatant may have inhibitory activity on the virulence of *E coli*.

Same reports had discussed the antimicrobial activity of bacteriocin produced by probiotics.<sup>70 71</sup> Inhibition of *Salmonella typhimurium* SL1334 for cell association and cell invasion was investigated using Caco-2 cells in a study in vitro.<sup>70</sup> Two strains of bifidobacterium (CA1 and F9) expressed antagonistic activity against pathogens in vitro, inhibited cell entry, and killed intracellular *S typhimurium* SL1334 in Caco-2 cells. The antibacterial component produced by CA1 and F9 was found to be a lipophilic molecule with a molecular weight less than 3500 Da. Other data<sup>71</sup> showed a small heat stable bacteriocin, ABP-118, which was a Class IIB two peptide bacteriocin composed of Abp118 $\alpha$ , and produced by *L salivarius* UCC118. Heterologous expression of ABP-118 was achieved, as the study showed, in *Lactobacillus plantarum*, *L lactis*, and *Bacillus cereus*.

Probiotics can also stimulate the intestinal innate defence through the upregulation of defensins. Crohn's disease is

characterised by an impaired induction of human  $\beta$  defensins 2 and 3, a decrease or lack of mucosal peptide antibiotics may play a central part in the aetiopathogenesis of Crohn's disease.<sup>72</sup> *E coli* strain Nissle 1917 and a variety of other probiotics can induce the expression of the antimicrobial peptide human  $\beta$ -defensin-2 (hBD-2) in Caco-2 intestinal epithelial cells in a time and dose dependent manner, which mechanism is related to NF- $\kappa$ B and AP-1 activation in Caco-2 for induction of hBD-2.<sup>73</sup> The induction of hBD-2 may contribute to an increased mucosal barrier to the luminal bacteria.

### Decomposing luminal pathogenic antigens

Intestinal inflammation resulting in disruption of mucosal barrier function has been proposed as a cause of increased incidence of allergic diseases.<sup>74 75</sup> Dietary antigens may trigger aberrant immune response in mucosa and flare the onset of colitis.<sup>76</sup> Some studies have shown that probiotics can decompose luminal pathogenic antigens, and induce hyporesponsiveness of mucosal immune system to dietary antigens. Peripheral blood mononuclear cells from young atopic patients secrete high level of the IL4 and interferon gamma production in response to the stimulation of anti-CD3 antibody and bovine casein simultaneously. If peripheral blood mononuclear cells are stimulated by bovine casein degraded by enzymes of *Lactobacillus GG*, *Lactobacillus GG*-degraded casein down regulated the IL4 production, with no effect on interferon gamma. The result shows that intestinal bacteria may modify immunomodulatory properties of native food proteins and provide protection from potentially harmful dietary antigens at a young age.<sup>77</sup> Another report shows that administration of yoghurt supplemented with probiotics including different species and strains of lactic acid bacteria, can increase mucosal and systemic IgA responses in mice to the cholera toxin immunogen.<sup>78</sup>

Probiotics can alleviate milk hypersensitivity in hypersensitive subjects, different from that in healthy subjects.<sup>79</sup> In the double blind, crossover study, challenges with milk in milk hypersensitive and healthy adults with or without *Lactobacillus GG* were performed, and milk challenge induced immunoinflammatory response was measured by the expression of phagocytosis receptors before and after the challenge. In milk hypersensitive subjects, milk challenge increased significantly the expression of phagocytosis receptors in monocytes. Milk with *Lactobacillus GG* prevented the increase of the receptor expression. In healthy subjects, milk challenge did not influence receptor expression while milk with *Lactobacillus GG* increased significantly the expression of phagocytosis receptors in neutrophils. That means probiotic bacteria can modulate the non-specific immune response differently in healthy and hypersensitive subjects, as an immunostimulatory effect in healthy subjects, and a down-regulation of immunoinflammatory response in milk hypersensitive subjects.

### CONCLUSIONS

Enteric microflora become aberrant in IBD patients, with a decrease in bifidobacterium and lactobacillus, and a significant increase in pathogenic and potential harmful enterobacteria. The aberrant microflora may partake in the pathogenesis of IBD. Preliminary studies suggest that oral administration of probiotics may be effective in treating intestinal inflammation in animal colitis and clinical trials. Probiotic mixture often contains bifidobacteria, lactobacilli, and some non-pathogenic bacteria as escherichia and enterococci. The effect of probiotic administration is often different in each clinical trials as probiotic use differs in those respects: the dose used, the frequency and duration of use, and the use of concomitant treatment with other drugs as

corticosteroids and antibiotics. More controlled trials are needed to search the best efficient use of probiotics for treating the diseases.

Recent researches are enlarging the insight into the composition and function of probiotics. Oral introduction of probiotics can normalise of the properties of aberrant indigenous microflora and reinforce the various lines of intestinal defence. However, different probiotic bacteria may be distinct in immunological effects, and have special properties when normalising in the inflamed mucosal of IBD patients or in the healthy. In the respect, the properties of different probiotics strains might be intensively explored to determine and screen the optimal probiotics strains and proper ingredients for therapeutic intervention of IBD in the future.

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#### REFERENCES

- Darfeuille-Michaud A, Boudeau J, Bulois P, et al. High prevalence of adherent-invasive *Escherichia coli* associated with ileal mucosa in Crohn's disease. *Gastroenterology* 2004;**127**:412–21.
- Bell AJ, Nicholls RJ, Forbes A, et al. Human lymphocyte stimulation with pouchitis flora is greater than with flora from a healthy pouch but is suppressed by metronidazole. *Gut* 2004;**53**:1801–5.
- Favier C, Neut C, Mizon C, et al. Fecal beta-D-galactosidase production and Bifidobacteria are decreased in Crohn's disease. *Dig Dis Sci* 1997;**42**:817–22.
- Cummings JH, Macfarlane GT, Macfarlane S. Intestinal bacteria and ulcerative colitis. *Curr Issues Intest Microbiol* 2003;**4**:9–20.
- Fooks LJ, Gibson GR. Probiotics as modulators of the gut flora. *Br J Nutr* 2002;**88**(suppl 1):S39–49.
- Sartor RB. Targeting enteric bacteria in treatment of inflammatory bowel diseases: why, how, and when. *Curr Opin Gastroenterol* 2003;**19**:358–65.
- Schrezenmeir J, de Vrese M. Probiotics, prebiotics, and synbiotics—approaching a definition. *Am J Clin Nutr* 2001;**73**(suppl 2):361–4S.
- Oliva-Hemker M, Fiocchi C. Etiopathogenesis of inflammatory bowel disease: the importance of the pediatric perspective. *Inflamm Bowel Dis* 2002;**8**:112–28.
- Feillet H, Bach JF. Increased incidence of inflammatory bowel disease: the price of the decline of infectious burden? *Curr Opin Gastroenterol* 2004;**20**:560–4.
- Farrell RJ, LaMont JT. Microbial factors in inflammatory bowel disease. *Gastroenterol Clin North Am* 2002;**31**:41–62.
- Sanderson JD, Moss MT, Tizard ML, et al. Mycobacterium paratuberculosis DNA in Crohn's disease tissue. *Gut* 1992;**33**:890–6.
- Masseret E, Boudeau J, Colombel JF, et al. Genetically related *Escherichia coli* strains associated with Crohn's disease. *Gut* 2001;**48**:320–5.
- Ohkusa T, Okayasu I, Ogihara T, et al. Induction of experimental ulcerative colitis by *Fusobacterium varium* isolated from colonic mucosa of patients with ulcerative colitis. *Gut* 2003;**52**:79–83.
- Streutker CJ, Bernstein CN, Chan VL, et al. Detection of species-specific helicobacter ribosomal DNA in intestinal biopsy samples from a population-based cohort of patients with ulcerative colitis. *J Clin Microbiol* 2004;**42**:660–4.
- Hooper LV, Wong MH, Thelin A, et al. Molecular analysis of commensal host-microbial relationships in the intestine. *Science* 2001;**291**:881–4.
- Giaffer MH, Holdsworth CD, Duerden BI. The assessment of faecal flora in patients with inflammatory bowel disease by a simplified bacteriological technique. *J Med Microbiol* 1991;**35**:238–43.
- Seksik P, Rigottier-Gois L, Gramet G, et al. Alterations of the dominant faecal bacterial groups in patients with Crohn's disease of the colon. *Gut* 2003;**52**:237–42.
- Cummings JH, Macfarlane GT, Macfarlane S. Intestinal bacteria and ulcerative colitis. *Curr Issues Intest Microbiol* 2003;**4**:9–20.
- Wells CL, van de Wosterlo EM, Jechorek RP, et al. *Bacteroides fragilis* enterotoxin modulates epithelial permeability and bacterial internalization by HT-29 enterocytes. *Gastroenterology* 1996;**110**:1429–37.
- Robertson DJ, Sandler RS. Measles virus and Crohn's disease: a critical appraisal of the current literature. *Inflamm Bowel Dis* 2001;**7**:51–7.
- Pitcher MC, Cummings JH. Hydrogen sulphide: a bacterial toxin in ulcerative colitis? *Gut* 1996;**39**:1–4.
- Sellon RK, Tonkonogy S, Schultz M, et al. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. *Infect Immun* 1998;**66**:5224–31.
- Podolsky DK. Inflammatory bowel disease. *N Engl J Med* 2002;**347**:417–29.
- Duchmann R, Kaiser I, Hermann E, et al. Tolerance exists towards resident intestinal flora but is broken in active inflammatory bowel disease (IBD). *Clin Exp Immunol* 1995;**102**:448–55.
- Landers CJ, Cohavy O, Misra R, et al. Selected loss of tolerance evidenced by Crohn's disease-associated immune responses to auto- and microbial antigens. *Gastroenterology* 2002;**123**:689–99.
- Guarner F, Casellas F, Borruel N, et al. Role of microecology in chronic inflammatory bowel diseases. *Eur J Clin Nutr* 2002;**56**(suppl 4):S34–8.
- Nielsen SE, Vainer B, Nielsen OH. Comparative studies of superoxide production by microbial wall product-primed neutrophils in ulcerative colitis. *Dig Dis Sci* 2004;**49**:878–82.
- Patel B, Schutte R, Sporns P, et al. Potato glycoalkaloids adversely affect intestinal permeability and aggravate inflammatory bowel disease. *Inflamm Bowel Dis* 2002;**8**:340–6.
- Kennedy RJ, Kirk SJ, Gardiner KR. Mucosal barrier function and the commensal flora. *Gut* 2002;**50**:441–2.
- Madsen KL, Malfair D, Gray D, et al. Interleukin-10 gene-deficient mice develop a primary intestinal permeability defect in response to enteric microflora. *Inflamm Bowel Dis* 1999;**5**:262–70.
- Gordon JI, Hooper LV, McNevin MS, et al. Epithelial cell growth and differentiation. III. Promoting diversity in the intestine: conversations between the microflora, epithelium, and diffuse GALT. *Am J Physiol* 1997;**273**:G565–70.
- Hollander D. Intestinal permeability, leaky gut, and intestinal disorders. *Curr Gastroenterol Rep* 1999;**1**:410–16.
- Fabia R, Ar'Rajab A, Johansson ML, et al. Impairment of bacterial flora in human ulcerative colitis and experimental colitis in the rat. *Digestion* 1993;**54**:248–55.
- Schultz M, Scholmerich J, Rath HC. Rationale for probiotic and antibiotic treatment strategies in inflammatory bowel diseases. *Dig Dis* 2003;**21**:105–28.
- Guandalini S. Use of Lactobacillus-GG in paediatric Crohn's disease. *Dig Liver Dis* 2002;**34**(suppl 2):S63–5.
- Gionchetti P, Rizzello F, Venturi A, et al. Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial. *Gastroenterology* 2000;**119**:305–9.
- Bernet MF, Brassart D, Neeser JR, et al. Lactobacillus acidophilus LA 1 binds to cultured human intestinal cell lines and inhibits cell attachment and cell invasion by enterovirulent bacteria. *Gut* 1994;**35**:483–9.
- Le Blay G, Michel C, Blottiere HM, et al. Prolonged intake of fructo-oligosaccharides induces a short-term elevation of lactic acid-producing bacteria and a persistent increase in cecal butyrate in rats. *J Nutr* 1999;**129**:2231–5.
- Babakissa C, Colomb V, Andrieux C, et al. Luminal fermentation and colonocyte metabolism in a rat model of enteral nutrition. *Dig Dis Sci* 2003;**48**:1339–45.
- Wilson AJ, Gibson PR. Short-chain fatty acids promote the migration of colonic epithelial cells in vitro. *Gastroenterology* 1997;**113**:487–96.
- Liu Q, Shimoyama T, Suzuki K, et al. Effect of sodium butyrate on reactive oxygen species generation by human neutrophils. *Scand J Gastroenterol* 2001;**36**:744–50.
- Mack DR, Michail S, Wei S, et al. Probiotics inhibit enteropathogenic *E coli* adherence in vitro by inducing intestinal mucin gene expression. *Am J Physiol* 1999;**276**:G941–50.
- Isolauri E, Majamaa H, Arvola T, et al. Lactobacillus casei strain GG reverses increased intestinal permeability induced by cow milk in suckling rats. *Gastroenterology* 1993;**105**:1643–50.
- Fabia R, Ar'Rajab A, Johansson ML, et al. The effect of exogenous administration of Lactobacillus reuteri R2LC and oat fiber on acetic acid-induced colitis in the rat. *Scand J Gastroenterol* 1993;**28**:155–62.
- Madsen KL, Doyle JS, Jewell LD, et al. Lactobacillus species prevents colitis in interleukin 10 gene-deficient mice. *Gastroenterology* 1999;**116**:1107–14.
- Bruewer M, Luegering A, Kucharzik T, et al. Proinflammatory cytokines disrupt epithelial barrier function by apoptosis-independent mechanisms. *J Immunol* 2003;**171**:6164–72.
- Yan F, Polk DB. Probiotic bacterium prevents cytokine-induced apoptosis in intestinal epithelial cells. *J Biol Chem* 2002;**277**:50959–65.
- Ulisse S, Gionchetti P, D'Alo S, et al. Expression of cytokines, inducible nitric oxide synthase, and matrix metalloproteinases in pouchitis: effects of probiotic treatment. *Am J Gastroenterol* 2001;**96**:2691–9.
- Furie E, Macfarlane S, Kennedy A, et al. Synbiotic therapy (Bifidobacterium longum/Synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial. *Gut* 2005;**54**:242–9.
- Schultz M, Linde HJ, Lehn N, et al. Immunomodulatory consequences of oral administration of Lactobacillus rhamnosus strain GG in healthy volunteers. *J Dairy Res* 2003;**70**:165–73.
- Schultz M, Veltkamp C, Dieleman LA, et al. Lactobacillus plantarum 299V in the treatment and prevention of spontaneous colitis in interleukin-10-deficient mice. *Inflamm Bowel Dis* 2002;**8**:71–80.
- Dieleman LA, Goerres MS, Arends A, et al. Lactobacillus GG prevents recurrence of colitis in HLA-B27 transgenic rats after antibiotic treatment. *Gut* 2003;**52**:370–6.
- Hausmann M, Kiessling S, Mstermann S, et al. Toll-like receptors 2 and 4 are up-regulated during intestinal inflammation. *Gastroenterology* 2002;**122**:1987–2000.
- Eckmann L, Jung HC, Schurer-Maly C, et al. Differential cytokine expression by human intestinal epithelial cell lines: regulated expression of interleukin 8. *Gastroenterology* 1993;**105**:1689–97.
- Otte JM, Podolsky DK. Functional modulation of enterocytes by gram-positive and gram-negative microorganisms. *Am J Physiol Gastrointest Liver Physiol* 2004;**286**:G613–26.

- 56 **Bai AP**, Ouyang Q, Zhang W, *et al.* Probiotics inhibit TNF-alpha-induced interleukin-8 secretion of HT29 cells. *World J Gastroenterol* 2004;**10**:455-7.
- 57 **Lammers KM**, Helwig U, Swennen E, *et al.* Effect of probiotic strains on interleukin 8 production by HT29/19A cells. *Am J Gastroenterol* 2002;**97**:1182-6.
- 58 **Neish AS**, Gewirtz AT, Zeng H, *et al.* Prokaryotic regulation of epithelial responses by inhibition of IkappaB-alpha ubiquitination. *Science* 2000;**289**:1560-3.
- 59 **Neish AS**. Bacterial inhibition of eukaryotic pro-inflammatory pathways. *Immunol Res* 2004;**29**:175-86.
- 60 **Vavricka SR**, Musch MW, Chang JE, *et al.* hPepT1 transports muramyl dipeptide, activating NF-kappaB and stimulating IL-8 secretion in human colonic Caco2/bbe cells. *Gastroenterology* 2004;**127**:1401-9.
- 61 **Neudeck BL**, Loeb JM, Faith NG. Lactobacillus casei alters hPEPT1-mediated glycylsarcosine uptake in Caco-2 cells. *J Nutr* 2004;**134**:1120-3.
- 62 **Borrueil N**, Carol M, Casellas F, *et al.* Increased mucosal tumour necrosis factor alpha production in Crohn's disease can be downregulated ex vivo by probiotic bacteria. *Gut* 2002;**51**:659-64.
- 63 **Andreaskos E**, Foxwell B, Feldmann M. Is targeting Toll-like receptors and their signaling pathway a useful therapeutic approach to modulating cytokine-driven inflammation? *Immunol Rev* 2004;**202**:250-65.
- 64 **Rachmilewitz D**, Katakura K, Karmeli F, *et al.* Toll-like receptor 9 signaling mediates the anti-inflammatory effects of probiotics in murine experimental colitis. *Gastroenterology* 2004;**126**:520-8.
- 65 **Sturm A**, Rilling K, Baumgart DC, *et al.* Escherichia coli Nissle 1917 distinctively modulates T-cell cycling and expansion via toll-like receptor 2 signaling. *Infect Immun* 2005;**73**:1452-65.
- 66 **Nencioni A**, Wesselborg S, Brossart P. Role of peroxisome proliferator-activated receptor gamma and its ligands in the control of immune responses. *Crit Rev Immunol* 2003;**23**:1-13.
- 67 **Dubuquoy L**, Jansson EA, Deeb S, *et al.* Impaired expression of peroxisome proliferator-activated receptor gamma in ulcerative colitis. *Gastroenterology* 2003;**124**:1265-76.
- 68 **Kelly D**, Campbell JI, King TP, *et al.* Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. *Nat Immunol* 2004;**5**:104-12.
- 69 **Kim SH**, Yang SJ, Koo HC, *et al.* Inhibitory activity of Bifidobacterium longum HY8001 against Vero cytotoxin of Escherichia coli O157:H7. *J Food Prot* 2001;**64**:1667-73.
- 70 **Lievins V**, Peiffer I, Hudault S, *et al.* Bifidobacterium strains from resident infant human gastrointestinal microflora exert antimicrobial activity. *Gut* 2000;**47**:646-52.
- 71 **Flynn S**, van Sinderen D, Thornton GM, *et al.* Characterization of the genetic locus responsible for the production of ABP-118, a novel bacteriocin produced by the probiotic bacterium Lactobacillus salivarius subsp. salivarius UCC118. *Microbiology* 2002;**148**:973-84.
- 72 **Wehkamp J**, Fellermann K, Stange EF. Human defensins in Crohn's disease. *Chem Immunol Allergy* 2005;**86**:42-54.
- 73 **Wehkamp J**, Harder J, Wehkamp K, *et al.* NF-kappaB- and AP-1-mediated induction of human beta defensin-2 in intestinal epithelial cells by Escherichia coli Nissle 1917: a novel effect of a probiotic bacterium. *Infect Immun* 2004;**72**:5750-8.
- 74 **Fargeas MJ**, Theodorou V, More J, *et al.* Boosted systemic immune and local responsiveness after intestinal inflammation in orally sensitized guinea pigs. *Gastroenterology* 1995;**109**:53-62.
- 75 **Isolauri E**, Kirjavainen PV, Salminen S. Probiotics: a role in the treatment of intestinal infection and inflammation? *Gut* 2002;**50**(suppl 3):III54-9.
- 76 **Björsten M**, Hultgren Hornquist E. Dietary antigen-specific T-cell responses: switch from an interleukin-10-dominated response in normal mice to a T-helper 1 cytokine profile in Galp1a2-deficient mice prior to colitis. *Scand J Immunol* 2005;**61**:29-35.
- 77 **Sutas Y**, Hurme M, Isolauri E. Down-regulation of anti-CD3 antibody-induced IL-4 production by bovine caseins hydrolysed with Lactobacillus GG-derived enzymes. *Scand J Immunol* 1996;**43**:687-9.
- 78 **Tejada-Simon MV**, Lee JH, Ustunol Z, *et al.* Ingestion of yogurt containing Lactobacillus acidophilus and Bifidobacterium to potentiate immunoglobulin A responses to cholera toxin in mice. *J Dairy Sci* 1999;**82**:649-60.
- 79 **Pelto L**, Isolauri E, Lilius EM, *et al.* Probiotic bacteria down-regulate the milk-induced inflammatory response in milk-hypersensitive subjects but have an immunostimulatory effect in healthy subjects. *Clin Exp Allergy* 1998;**28**:1474-9.

## CORRECTION

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An authors' error occurred in this paper by Dr Alagiakrishnan and others (2006;**82**:101-5). The competing interests statements were not included in the paper. The statements are printed below.

Peter N McCracken: in the past three years I have received grant research support from Pfizer, Eisai, Sanofi-aventis, Lundbeck Canada, Servier Canada, Janssen-Ortho, and Neurochem Inc, Syreon Corporation, and

Wyeth. I have served as a consultant for Pfizer, Eisai, Novartis, Lundbeck Canada, and Janssen Ortho. I have been on the Speaker's Bureau or served in CME programmes with Pfizer, Eisai, Novartis, Lundbeck, and Janssen Ortho. I am not a shareholder in these firms and receive no other financial or material support.

Howard Feldman: in the past three years, I have/have had a financial interest/arrangement or affiliation with the following organisations: grant/research support (external): Pfizer, Eisai, Janssen, Lilly, Astra Zeneca, Sanofi Synthelabo, Glaxo Smith Kline. Consultant: Pfizer, Eisai, Novartis, Janssen,

Servier, Sanofi Synthelabo, Glaxo Smith Kline, Myriad, Targacept, Lundbeck, Forest, Axonyx. Speakers' Bureau, or CME programmes: Pfizer, Eisai, Janssen, Novartis, Forest. For this specific article I have had financial arrangements with Pfizer, Eisai, Novartis, and Janssen as listed above. They are the manufacturers of the acetylcholinesterase inhibitors. Pfizer is also the manufacturer of atorvastatin, a statin that is being investigated in Alzheimer's disease and for which I have been a consultant.

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