

## Review

## Gut flora in health and disease

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**The human gut is the natural habitat for a large and dynamic bacterial community, but a substantial part of these bacterial populations are still to be described. However, the relevance and effect of resident bacteria on a host's physiology and pathology has been well documented. Major functions of the gut microflora include metabolic activities that result in salvage of energy and absorbable nutrients, important trophic effects on intestinal epithelia and on immune structure and function, and protection of the colonised host against invasion by alien microbes. Gut flora might also be an essential factor in certain pathological disorders, including multisystem organ failure, colon cancer, and inflammatory bowel diseases. Nevertheless, bacteria are also useful in promotion of human health. Probiotics and prebiotics are known to have a role in prevention or treatment of some diseases.**

Many species of bacteria have evolved and adapted to live and grow in the human intestine. The intestinal habitat of an individual contains 300–500 different species of bacteria,<sup>1,2</sup> and the number of microbial cells within the gut lumen is about 10 times larger than the number of eukaryotic cells in the human body.<sup>3</sup> The stomach and small intestine contain only a few species of bacteria adhering to the epithelia and some other bacteria in transit. The scarcity of bacteria in the upper tract seems to be because of the composition of the luminal medium (acid, bile, pancreatic secretion), which kills most ingested microorganisms, and because of the phasic propulsive motor activity towards the ileal end, which impedes stable colonisation of bacteria in the lumen. By contrast, the large intestine contains a complex and dynamic microbial ecosystem with high densities of living bacteria, which achieve concentrations of up to  $10^{11}$  or  $10^{12}$  cells/g of luminal contents.<sup>1</sup> These concentrations are similar to those found in colonies growing under optimum conditions over the surface of a laboratory plate.<sup>4</sup> A large proportion of the faecal mass consists of bacteria (around 60% of faecal solids).<sup>5</sup>

Several hundred grams of bacteria living within the colonic lumen affect host homeostasis. Some of these bacteria are potential pathogens and can be a source of infection and sepsis under some circumstances—for instance when the integrity of the bowel barrier is physically or functionally breached. However, the constant interaction between the host and its microbial guests can infer important health benefits to the human host.<sup>6</sup> Recognition of these benefits is drawing particular attention to the functional implications of microflora in host physiology.

### Composition of the flora

Colonisation of the gastrointestinal tract of newborn infants starts immediately after birth and occurs within a few days. Initially, the type of delivery (passage through

the birth canal versus caesarean section) and the type of diet (breast versus formula feeding) might affect the colonisation pattern.<sup>7–10</sup> Other environmental factors also have a major role since differences exist between infants born in developed countries and those born in developing countries, and between infants from different hospital wards.<sup>11–13</sup> Pioneer bacteria can modulate expression of genes in host epithelial cells,<sup>14</sup> thus creating a favourable habitat for themselves, and can prevent growth of other bacteria introduced later in the ecosystem. The initial colonisation is therefore very relevant to the final composition of the permanent flora in adults.<sup>15</sup>

Conventional bacteriological analysis of faecal flora requires meticulous techniques for cultivation of bacteria on various growth media and an array of methods for taxonomic identification of the isolates. Results of such studies<sup>1</sup> have shown that anaerobic bacteria outnumber aerobic bacteria by a factor of 100–1000. The genera *Bacteroides*, *Bifidobacterium*, *Eubacterium*, *Clostridium*, *Peptococcus*, *Peptostreptococcus*, and *Ruminococcus* are predominant in human beings,<sup>1,6</sup> whereas aerobes (facultative anaerobes) such as *Escherichia*, *Enterobacter*, *Enterococcus*, *Klebsiella*, *Lactobacillus*, *Proteus*, etc are among the subdominant genera. Every individual has several hundreds of species belonging to these genera, with a particular combination of predominant species that is distinct from that found in other individuals.<sup>1,16</sup> The species vary greatly between individuals.<sup>16</sup> The composition of the individual's flora can fluctuate under some circumstances, for instance acute diarrhoeal illnesses, antibiotic treatment, or to lesser extent induced by dietary interventions, but individuals' flora composition pattern usually remain constant.<sup>1,16</sup>

Several bacteria that can be seen by direct microscopic examination of diluted faecal specimens cannot be grown in culture media. Unicellular organisms need biodiversity for growth. Thus, 40–80% of the total microscopic counts

### Search strategy and selection criteria

In writing this review, we relied on original articles and reviews that were published in scientific journals and are searchable in database libraries (OVID, PubMed, Medline Plus Databases), and on our current readings on the topic. Due to space limitations, the number of studies quoted has been restricted. We chose articles for citation on the basis of the relevance of its contents without any bias toward author or journal.

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are not recoverable by culture,<sup>17,18</sup> although estimates vary between individuals and between studies. Molecular biological procedures can now also be used to investigate the microbial ecology in the colon without use of cultures.<sup>19</sup> Results of an analysis<sup>18</sup> of bacterial genes in human faeces showed that many DNA sequences correspond to previously undescribed microorganisms, and some data<sup>20</sup> suggest that every individual has unique strains of bacteria. Quantitative analysis<sup>21</sup> of faecal bacteria shows important differences between individuals and over time within the same individual that are not always detectable by conventional culture techniques.<sup>22</sup> Molecular procedures have shown that aerobes, including *Escherichia coli*, enterococci, and lactobacilli, achieve very high densities and metabolic activity in the human caecum, since 50% of total bacteria ribosomal RNA in caecal contents correspond to these species.<sup>23</sup> By contrast, these species account for only 7% of bacteria ribosomal RNA in faecal samples.<sup>23</sup> Such species could have an important role in caecal fermentations.

### Main functions of microflora

Use of animals bred under germ-free conditions has provided important information about the effect of the microbial community of the gut on host physiology and pathology.<sup>24</sup> Evidence obtained through such studies<sup>25</sup> suggests that microflora have important and specific metabolic, trophic, and protective functions (panel).

#### Main functions of gut flora

##### Metabolic

Fermentation of non-digestible dietary residue and endogenous mucus: salvage of energy as short-chain fatty acids, production of vitamin K, absorption of ions

##### Trophic

Control of epithelial cell proliferation and differentiation; development and homeostasis of the immune system

##### Protective

Protection against pathogens (the barrier effect)

#### Metabolic functions

A major metabolic function of colonic microflora is the fermentation of non-digestible dietary residue and endogenous mucus produced by the epithelia.<sup>25</sup> Gene diversity in the microbial community provides various enzymes and biochemical pathways that are distinct from the host's own constitutive resources. Overall outcomes of this complex metabolic activity are recovery of metabolic energy and absorbable substrates for the host, and supply of energy and nutritive products for bacterial growth and proliferation. Fermentation of carbohydrates is a major source of energy in the colon. Non-digestible carbohydrates include large polysaccharides (resistant starches, cellulose, hemicellulose, pectins, and gums), some oligosaccharides that escape digestion, and unabsorbed sugars and alcohols.<sup>26,27</sup> The metabolic endpoint is generation of short-chain fatty acids.

Anaerobic metabolism of peptides and proteins (putrefaction) by the microflora also produces short-chain fatty acids but, at the same time, it generates a series of potentially toxic substances including ammonia, amines, phenols, thiols, and indols.<sup>28,29</sup> Available proteins include elastin and collagen from dietary sources, pancreatic enzymes, sloughed epithelial cells and lysed bacteria.<sup>6</sup> Substrate availability in the human adult colon is about 20–60 g carbohydrates and 5–20 g protein per day.<sup>30,31</sup> In the caecum and right colon, fermentation is very intense

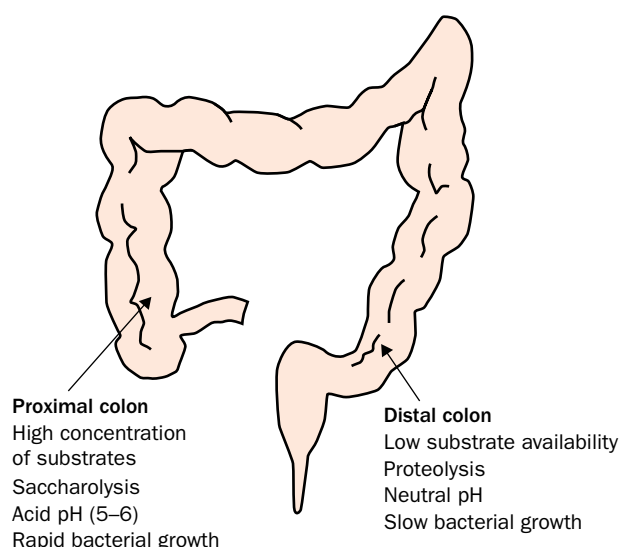


Figure 1: Fermentation in the colon

with high production of short-chain fatty acids, an acidic pH (5–6), and rapid bacterial growth.<sup>26,32,33</sup> By contrast, the substrate in the left or distal colon is less available, the pH is close to neutral, putrefactive processes become quantitatively more important, and bacterial populations are close to static (figure 1).

Colonic microorganisms also play a part in vitamin synthesis<sup>34,35</sup> and in absorption of calcium, magnesium, and iron.<sup>25,36,37</sup> Absorption of ions in the caecum is improved by carbohydrate fermentation and production of short-chain fatty acids, especially acetate, propionate, and butyrate. All of these fatty acids have important functions in host physiology. Butyrate is almost completely consumed by the colonic epithelium, and it is a major source of energy for colonocytes.<sup>26</sup> Acetate and propionate are found in portal blood and are eventually metabolised by the liver (propionate) or peripheral tissues, particularly muscle (acetate).<sup>26,30</sup> Acetate and propionate might also have a role as modulators of glucose metabolism: absorption of these short-chain fatty acids would result in lower glycaemic responses to oral glucose or standard meal—a response consistent with an ameliorated sensitivity to insulin.<sup>38,39</sup> In fact, foods with high proportion of non-digestible carbohydrates all have a low glycaemic index.<sup>40,41</sup> However, results of one study<sup>42</sup> showed no effect of colonic fermentation of carbohydrates on insulin resistance.

#### Trophic functions

**Epithelial cell growth and differentiation**—Possibly, the most important role of short-chain fatty acids on colonic physiology is their trophic effect on the intestinal epithelium. The rate of production of crypt cells is reduced in the colon of rats bred in germ-free environments, and their crypts contain fewer cells than do those of rats colonised by conventional flora, suggesting that intraluminal bacteria affect cell proliferation in the colon.<sup>43</sup> Differentiation of epithelial cells is greatly affected by interaction with resident microorganisms.<sup>14,44</sup> All three major short-chain fatty acids stimulate epithelial cell proliferation and differentiation in the large and small bowel in vivo.<sup>45</sup> However, butyrate inhibits cell proliferation and stimulates cell differentiation in epithelial cell lines of neoplastic origin in vitro.<sup>46</sup> Moreover, butyrate promotes reversion of cells from neoplastic to non-neoplastic phenotypes.<sup>47</sup> A role for

short-chain fatty acids in prevention of some human pathological states such as chronic ulcerative colitis and colonic carcinogenesis has been long suspected although, admittedly, conclusive evidence is still lacking.

**Interactions between gut bacteria and host immunity—**The intestinal mucosa is the main interface between the immune system and the external environment. Thus, that gut-associated lymphoid tissues contain the largest pool of immunocompetent cells in the human body is not surprising.<sup>48</sup> The dialogue between host and bacteria at the mucosal interface seems to play a part in development of a competent immune system. Animals bred in a germ-free environment have low densities of lymphoid cells in the gut mucosa, specialised follicle structures are small, and circulating concentrations of immunoglobulins in the blood are low.<sup>19,24,49</sup> Microbial colonisation of the gastrointestinal tract affects the composition of gut-associated lymphoid tissue. Immediately after exposure to luminal microbes, the number of intraepithelial lymphocytes expands greatly,<sup>50,51</sup> germinal centres with immunoglobulin producing cells arise rapidly in follicles and in the lamina propria,<sup>52</sup> and concentrations of immunoglobulin increase substantially in serum.<sup>49</sup> In mice and rats, a non-pathogenic and non-culturable segmented filamentous bacterium that preferentially attaches to Peyer's patch epithelium stimulates development of mucosal immune architecture and function.<sup>53–55</sup>

Many and diverse interactions between microbes, epithelium and gut-associated lymphoid tissue are involved in modelling the memory mechanisms of systemic immunity. For instance, flora have been implicated in oral tolerance. The systemic response to a specific antigen can be abrogated after ingesting the same antigen. This effect persists for several months in mice with conventional flora, whereas in germfree mice systemic unresponsiveness persists for only a few days.<sup>56</sup> After oral administration of ovalbumin, germ-free mice maintain a Th2 immune response and produce IgE antibodies against ovalbumin. Interestingly, the abnormality can be corrected by reconstitution of conventional flora, but this procedure is only effective in neonates and not in older mice.<sup>57</sup> The interaction between gut-associated lymphoid tissue and flora early in life seems to be crucial for appropriate development of complex mucosal and systemic immunoregulatory circuits.

In adults, immunity may be constantly reshaped by persistent interactions between the host and its bacteria that take place in the gut. Commensal organisms try to circumvent the immune response. For instance, *Bacteroides fragilis*, a predominant species in the human colon, can change its surface antigenicity by producing distinct capsular polysaccharides.<sup>38</sup> Surface diversity seems to allow the organism to escape immunosurveillance and maintain an ecological niche of predominance in the intestinal tract. However, host defences adapt and keep an active control of bacterial growth.

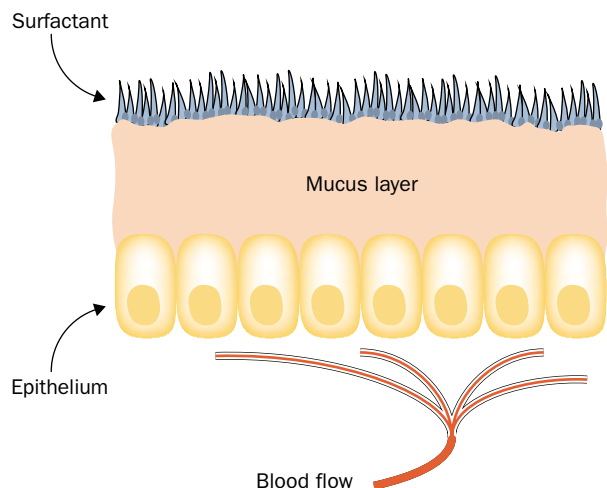
The immune response to microbes relies on innate and adaptive components, such as immunoglobulin secretion. Most bacteria in human faeces are coated with specific IgA units.<sup>59</sup> Innate responses are mediated not only by white blood cells such as neutrophils and macrophages that can phagocytose and kill pathogens, but also by intestinal epithelial cells, which coordinate host responses by synthesising a wide range of inflammatory mediators and transmitting signals to underlying cells in the mucosa.<sup>60</sup> The innate immune system has to discriminate between potential pathogens from commensal bacteria,

with use of a restricted number of preformed receptors. Mammalian cells express a series of toll-like receptors, which recognise conserved motifs on bacteria that are not found in higher eukaryotes.<sup>61</sup> The system allows immediate recognition of bacteria to rapidly respond to an eventual challenge. For example, incubation of non-pathogenic bacteria with inflamed human intestinal mucosa elicits different types of immediate cytokine responses, which are transduced to the underlying tissue and promote changes in the phenotype of lamina propria lymphocytes.<sup>62</sup>

#### *Protective functions: the barrier effect*

Resident bacteria are a crucial line of resistance to colonisation by exogenous microbes and, therefore, are highly relevant in prevention of invasion of tissues by pathogens (figure 2). Germ-free animals are very susceptible to infection.<sup>63,64</sup> Colonisation resistance also applies to opportunistic bacteria that are present in the gut but have restricted growth. The equilibrium between species of resident bacteria provides stability in the microbial population within the same individual under normal conditions. However, use of antibiotics can disrupt the ecological balance and allow overgrowth of species with potential pathogenicity such as toxigenic *Clostridium difficile*, associated with pseudomembranous colitis.<sup>65</sup>

Several mechanisms have been implicated in the barrier effect. In vitro, bacteria compete for attachment sites in the brush border of intestinal epithelial cells.<sup>66</sup> Adherent non-pathogenic bacteria can prevent attachment and subsequent entry of pathogen enteroinvasive bacteria into the epithelial cells.<sup>66</sup> Furthermore, bacteria compete for nutrient availability in ecological niches and maintain their collective habitat by administering and consuming all resources—eg, in the gnotobiotic mouse mono-colonised with *Bacteroides thetaiotaomicron*.<sup>67</sup> The host actively provides a nutrient that the bacterium needs, and the bacterium actively indicates how much it needs to the host. This symbiotic relationship prevents unwanted overproduction of the nutrient, which would favour



**Figure 2: The mucosal barrier**

The mucosal barrier separates the internal milieu from the luminal environment. The function of the barrier depends on the integrity of the mucosa—from the endothelium through to the epithelial cell lining—and the reactivity of dynamic defensive factors such as mucosal blood flow, epithelial secretions, and immunocompetent cells. The mucus layer is formed by the interaction of various mucosal secretions, including mucin glycoproteins, trefoil peptides, and surfactant phospholipids. However, resident bacteria are the crucial line of resistance by exogenous microbes.

intrusion of microbial competitors with potential pathogenicity for the host. Finally, bacteria can inhibit the growth of their competitors by producing antimicrobial substances called bacteriocins.<sup>68,69</sup> The ability to synthesise bacteriocins is widely distributed among microbial collectivities of the gastrointestinal tract. The host can control production of such substances since most of them are protein compounds degradable by digestive proteases. Thus, the role of bacteriocins is mainly restricted to localised niches.

### Translocation of bacteria

The passage of viable bacteria from the gastrointestinal tract through the epithelial mucosa is called bacterial translocation.<sup>70</sup> Translocation of endotoxins from viable or dead bacteria in very small amounts probably constitutes a physiologically important boost to the reticuloendothelial system, especially to the Kupffer cells in the liver. However, dysfunction of the gut mucosal barrier can result in translocation of many viable microorganisms, usually belonging to gram-negative aerobic genera (escherichia, proteus, klebsiella). After crossing the epithelial barrier, bacteria can travel via the lymph to extraintestinal sites, such as the mesenteric lymph nodes, liver, and spleen. Subsequently, enteric bacteria can disseminate throughout the body producing sepsis, shock, multisystem organ failure, or death of the host. Much work has been done on bacterial translocation in animals, and translocation occurs notably in haemorrhagic shock, burn injury, trauma, intestinal ischaemia, intestinal obstruction, severe pancreatitis, acute liver failure, and cirrhosis. The three primary mechanisms in promotion of bacterial translocation in animals are overgrowth of bacteria in the small intestine; increased permeability of the intestinal mucosal barrier; and deficiencies in host immune defences.<sup>71</sup>

Bacterial translocation can occur in human beings during various disease processes.<sup>72</sup> Indigenous gastrointestinal bacteria have been cultured directly from the mesenteric lymph nodes of patients undergoing laparotomy. Data suggest that the baseline rate of positive mesenteric lymph node culture could approach 5% in otherwise healthy people. However, in disorders such as multisystem organ failure, acute severe pancreatitis, advanced liver cirrhosis, intestinal obstruction, and inflammatory bowel diseases, rates of positive culture are much higher (16–40%).<sup>72</sup> Bacterial translocation is associated with a significant increase in development of postoperative sepsis in patients undergoing surgery.<sup>73</sup> Intestinal bacteria are probably involved in development of multisystem organ failure in human beings, even though massive release of proinflammatory mediators because of intestinal hypoperfusion is perceived as the key event.<sup>72</sup> In patients with cirrhosis, bacterial translocation can cause spontaneous bacterial peritonitis, an important complication of advanced liver disease.<sup>74</sup> In this setting, overgrowth of bacteria within the small bowel has a bigger role than do colonic sources.<sup>75</sup>

### Colon cancer

The molecular genetic mechanisms of colorectal cancer are well established, but environmental factors such as diet might also have a major role in development of sporadic colon cancer. Dietary fat and high consumption of red meat, especially processed meat, are associated with high risk of colon cancer.<sup>76</sup> By contrast, a high intake of fruits and vegetables, whole grain cereals, fish, and calcium has been associated with reduced risk.<sup>76,77</sup> Dietary factors and genetic factors interact in part via events taking place in the

lumen of the large bowel.<sup>77</sup> The effect of diet on the carcinogenic process could be mediated by changes in metabolic activity and composition of the colonic microflora.

Intestinal bacteria could play a part in initiation of colon cancer through production of carcinogens, cocarcinogens, or procarcinogens. In healthy people, diets rich in fat and meat but poor in vegetables increase the faecal excretion of N-nitroso compounds,<sup>78</sup> a group of genotoxic substances that are known initiators and promoters of colon cancer. In fact, such diets also increase the genotoxic potential of human faecal water.<sup>79</sup> Another group of carcinogens of dietary origin are the heterocyclic aromatic amines that are formed in meat when it is cooked. Some intestinal microorganisms strongly increase damage to DNA in colon cells induced by heterocyclic amines, whereas other intestinal bacteria can uptake and detoxify such compounds.<sup>80</sup>

Bacteria of the bacteroides and clostridium genera increase the incidence and growth rate of colonic tumours induced in animals, whereas other genera such as lactobacillus and bifidobacteria prevent tumorigenesis.<sup>81–85</sup> A descriptive human study<sup>16</sup> compared the composition of the faecal flora of people with different risks of colon cancer. High risk of colon cancer was associated with presence of *Bacteroides vulgatus* and *Bacteroides stercoris*. Low risk was associated with presence of *Lactobacillus acidophilus*, *Lactobacillus S06* and *Eubacterium aerofaciens*. Although the evidence is not conclusive, colonic flora seem to be a major environmental factor that modulates risk of colonic cancer in human beings.

### Inflammatory bowel diseases

Resident bacterial flora have been suggested to be an essential factor in driving the inflammatory process in human inflammatory bowel diseases.<sup>86</sup> In patients with Crohn's disease, intestinal T lymphocytes are hyper-reactive against bacterial antigens, and Pirzer and colleagues<sup>87</sup> suggested that local tolerance mechanisms are abrogated in such patients. In addition, patients with Crohn's disease or ulcerative colitis have increased intestinal mucosal secretion of IgG type antibodies against a broad spectrum of commensal bacteria.<sup>88</sup> Immuno-inflammatory responses mediated by IgG can damage the intestinal mucosa since, unlike normal IgA responses, they activate the complement and the cascade of inflammatory mediators.<sup>48</sup> Moreover, patients with inflammatory bowel diseases have higher amounts of bacteria attached to their epithelial surfaces than do healthy people.<sup>89</sup> These bacteria are from diverse genera and some of them, especially bacteroides, were identified within the epithelial layer, in some instances, intracellularly.<sup>89</sup> Thus, unrestrained activation of the intestinal immune system by elements of the flora could be a key event in the pathophysiology of inflammatory bowel disease. Some patients with Crohn's disease (17–25%) have mutations in the *NOD2/CARD15* gene, which regulates host responses to bacteria.<sup>90</sup>

The idea that resident bacteria of the normal flora are involved in intestinal mucosal inflammation is lent support by data from animal studies. Treatment with wide-spectrum antibiotics has been shown to mitigate mucosal inflammation in rats and mice with inflammatory bowel disease.<sup>91,92</sup> Resident bacteria are necessary for development of spontaneous colitis in HLA-B27 transgenic rats,<sup>93</sup> and in mice deficient in interleukin 10.<sup>94</sup> However, in some instances bowel colonisation could have a protective role—as seen in germ-free mice with dextran sodium sulphate-induced colitis.<sup>95</sup> These observations can be explained by the immature immunity seen in germ-free animals. In



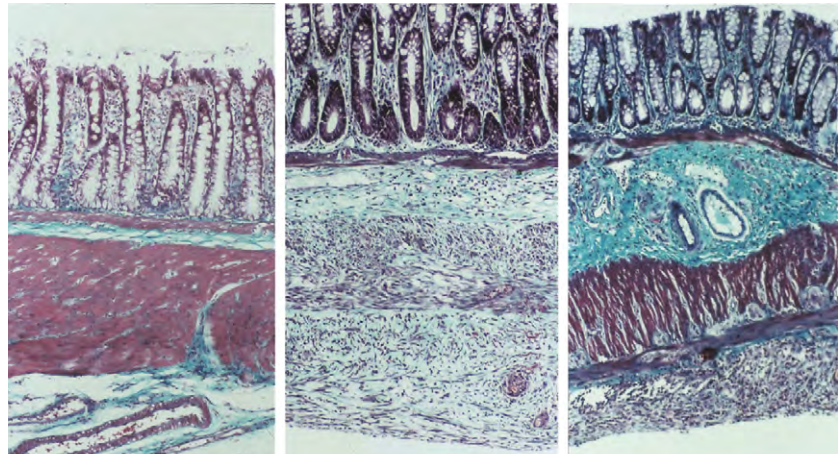
addition different species of bacteria can induce different effects on immuno-inflammatory mechanisms.<sup>96</sup> Several species of the commensal microflora, including some anaerobes, invade the mucosa after induction of colitis,<sup>96</sup> and various species of bacteroides are especially prone to induce transmural inflammatory lesions. Enteric bacteria differ in their fibrogenic capability and these differences seem to be linked to the type of the inflammatory response they produce.<sup>97</sup> Some aerobic bacteria provoke a severe acute inflammatory reaction that is circumscribed to focal areas of abscesses, but local deposition of collagen is negligible. Conversely, some anaerobes (*Bacteroides fragilis*, *Bacteroides uniformis*, and *Clostridium ramosum*) induce a mild granulocyte response but a widely diffuse infiltration of mononuclear cells, associated with accumulation of collagen in the tissue (figure 3). Non-viable bacteria inocula do not induce the full effect. Thus, some anaerobes have the potential to induce diffuse fibrogenic responses when invading the intestinal wall.

In inflammatory bowel diseases in human beings, direct interaction of commensal microflora with the intestinal mucosa stimulates inflammatory activity in the gut lesions.<sup>98,99</sup> Faecal stream diversion has been shown to prevent recurrence of Crohn's disease, whereas infusion of intestinal contents to the excluded ileal segments reactivated mucosal lesions.<sup>98</sup> In ulcerative colitis, short-term treatment with an enteric-coated preparation of broad-spectrum antibiotics rapidly reduced mucosal release of cytokines and eicosanoids and was more effective in reduction of inflammatory activity than were intravenous steroids.<sup>99</sup> However, antibiotics have limited effectiveness in clinical management of inflammatory bowel disease, since induction of antibiotic resistant strains substantially impairs sustained effects. At present, investigators are assessing use of probiotics, rather than antibiotics, to antagonise bacteria for therapeutic purposes, and clinical trials offer a promising perspective.<sup>100,101</sup>

### Probiotics and prebiotics

Bacteria can be used to improve human health. A bacterium that provides specific health benefits when consumed as a food component or supplement would be called a probiotic. A consensus definition of the term was issued a few years ago and states that oral probiotics are living microorganisms that upon ingestion in specific numbers, exert health benefits beyond those of inherent basic nutrition.<sup>102</sup> According to this definition, probiotics do not necessarily colonise the human intestine. The crucial point is to show a distinct health benefit achieved by consumption of a specific strain. The effect of a bacterium is strain specific and cannot be extrapolated even to other strains of the same species. For demonstration of probiotic activity, well-designed clinical trials are needed, which should be controlled, randomised, and double-blinded.<sup>102</sup> The same criteria should apply to prebiotics, which are non-digestible food ingredients that beneficially affect the host by selectively stimulating growth, or activity, or both, of one or a restricted number of bacteria in the colon.<sup>103</sup>

Specific interactions of bacteria with the host might result in measurable benefits for the host. The mechanisms of action have been studied extensively, but further research is needed.<sup>104–106</sup> Some probiotics are useful in prevention and treatment of acute diarrhoeal conditions. Co-administration of probiotics to patients on antibiotics significantly reduced antibiotic-associated diarrhoea in children<sup>107,108</sup> and adults.<sup>109,110</sup> Probiotics can be used to prevent such antibiotic-associated diarrhoea.<sup>111</sup>



**Figure 3: Histological sections from rat colon stained with Masson's trichrome** (Left) the layered structure of a healthy colonic wall has a prominent muscularis propria (red). Collagen fibres can be seen mainly within the submucosa and serosa (green). (Middle) the distorted structure of the colonic wall 1 week after intramural inoculation with a suspension of *Bacteroides fragilis*. The muscularis propria has been substituted by fibroblasts secreting collagen (green). Transmural involvement of the colonic wall from the submucosa to the serosa can be seen, together with thickening and fibrosis of the serosa. (Right) in rats pretreated with a neutralising antibody against transforming growth factor  $\beta 1$ , the fibrotic response is clearly reduced. The smooth muscle cells (red) did not transform into fibroblasts.

Supplementation of an infant formula with probiotics also prevents diarrhoeal disease in chronically hospitalised children.<sup>112</sup> *Lactobacillus rhamnosus* strain GG has also been useful as a prophylaxis of diarrhoea in undernourished children, especially in those who are not breastfed.<sup>113</sup> Children with acute gastroenteritis who received a probiotic supplement (either *L. rhamnosus*, *Lactobacillus reuteri*, or *Lactobacillus casei*) also had significantly decreased duration of diarrhoea.<sup>114–119</sup> Probiotics are most effective in acute diarrhoea caused by rotavirus infection.<sup>119</sup> Use of probiotics has also reduced faecal excretion of rotavirus.<sup>112,116</sup>

Bacteria used as a starter culture in yoghurt improve digestion of lactose and eliminate symptoms of intolerance in people who do not efficiently absorb lactose. This beneficial effect is due to presence of microbial  $\beta$  galactosidase (lactase) in the fermented milk product. Live bacteria are essential for the effect, since heated or pasteurised yoghurts did not prevent lactose malabsorption and symptoms of intolerance.<sup>120,121</sup> Prevalence of lactose malabsorption in adult populations is about 5–15% in northern European and American countries and 50–100% in African, Asian, and South American countries.<sup>122</sup> People who are intolerant of lactose tend to eliminate dairy products from their diets and thus compromise their intake of calcium. These properties of yoghurt bacteria are thought to be a very reliable way to achieve adequate calcium intakes through dairy products in adults.

Orally administered probiotics can enhance specific IgA responses to rotavirus in infected children<sup>123</sup> or to *Salmonella typhi* in adults undergoing vaccination with an attenuated strain.<sup>124</sup> In healthy people, two different

probiotics administered in a fermented milk product transiently colonised their gut and enhanced phagocytic activity of circulating leucocytes for a few weeks while colonisation persisted,<sup>125</sup> lending support to the idea that enteric bacteria elicit immune responses at local and systemic levels. In a clinical trial,<sup>126</sup> *L rhamnosus* strain GG was given prenatally to mothers with family history of atopy and postnatally to their infants for 6 months. Compared with placebo, the probiotic significantly reduced incidence of atopic eczema during the 2-year follow-up period.

Probiotics and prebiotics have been shown to prevent colon cancer in several animals, but their role in reduction of risk of colon cancer in human beings is not established.<sup>127</sup> However, probiotics have been shown to reduce the faecal activity of enzymes known to produce genotoxic compounds that act as tumour initiators in human beings.<sup>128–132</sup>

## Recommendations

A better understanding of our relations with the microbial world should help in prevention of diseases such as atopy, colon cancer, and inflammatory bowel diseases.

### Conflict of interest statement

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