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Review

Dietary fibres as "prebiotics": Implications for colorectal cancer

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A "prebiotic" is a nondigestible food ingredient whose beneficial effects on the host result from the selective stimulation of growth and/or activity of members of the bacterial community that inhabits the human bowel (the gut microbiota). Although much of the prebiotic literature focuses on non-digestible oligosaccharides, such as oligofructose, most dietary fibres that are fermentable carbohydrates could be considered as prebiotics. Early studies suggested that colonic bacteria were risk factors for colon cancer. However, altering the composition or metabolic activity of the bowel microbiota through the use of dietary fibre might be important in reducing the prevalence of colorectal cancer. Mechanisms for beneficial effects of prebiotics might include changing the activity of exogenous carcinogens through modulating metabolic activation and/or detoxification, or stimulating the production of the short-chain fatty acid, butyrate. However, modern analytical techniques suggest that an important consequence of a modified bacterial community could be a change in the expression not only of a range of different bacterial genes in bowel contents, but also in the bowel mucosa of the host. Analogous with observations with probiotics, the stimulation of cytokines and modification of immune responses could be important in producing beneficial effects. Compared with transitory effects of probiotics, the prebiotic action of fermentable carbohydrates potentially provide the opportunity for sustainable modulation of activity of the gut microbiota. However, their mechanisms of action in humans are speculative, and research aimed at providing an integrated view of the gut microbiota and dietary fibre nutrition of humans needs to be developed.

Keywords: Dietary fibre / Immune response / Prebiotics / Probiotics / Review / Xenobiotic metabolising enzymes

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Abbreviations: DF, dietary fibre; **FOS**, fructooligosaccharide; **LAB**, lactic acid-producing bacteria; **RS**, resistant starch; **SCFA**, short-chain fatty acid; **XME**, xenobiotic metabolising enzyme

1 Introduction

Gibson and Roberfroid [1] coined the term "prebiotics", defining it as "a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth

and/or activity of one or a limited number of bacteria in the colon, and thus improves host health". Additionally, it has been stated that only certain dietary fractions that satisfy the following criteria can be considered as prebiotics. They must escape digestion by gastric acid and enzymes in the proximal regions of the gastrointestinal tract and reach the large bowel intact, microbial growth stimulation must be selective for putatively beneficial gut residents without also enhancing the growth of pathogenic species, and finally these alterations in microbiota composition must produce improvements in overall host health [1 – 4].

In contrast to "probiotics", which are "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance" [5], the principle of prebiotics involves ingestion of a substance that selectively induces microbial growth and metabolic activity as opposed to direct administration of bacterial cells as dietary supplements. Thus, prebiotics encompasses a wider range of products than probiotics. In addition, probiotics produce a temporary alteration in the composition of the gut microbiota; changes are not maintained upon discontinuation of supplementation [6 – 10].

Synbiotics refers to the administration of a combination of prebiotics and probiotics [11], combining the objectives of both concepts so as to increase the number of putatively beneficial bacteria in the gut. In theory, this approach could circumvent difficulties in preserving probiotic viability, as the prebiotic substrate would be available to provide a source of nutrient for the probiotic bacteria delivered simultaneously to the gut.

The "dietary fibre hypothesis" for protection against colorectal cancer was advanced by Burkitt [12], based on epidemiological evidence supporting a relationship between diet and colon health. These findings showed lower rates of colorectal cancer in Africa compared to industrialised Western countries, where traditional diets consisted of high amounts of unrefined fibre and high intakes of refined carbohydrates, respectively. It was proposed that alterations in diet could result in colonic disease, such as appendicitis, diverticular disease, adenomatous polyps, and colon and rectal cancer. It was suggested that diet influenced colonic health by inducing changes in stool bulk and content, microbiota composition, total transit time, and intra-lumen pressure. In this review, we consider the possibility of how changes in the composition and activity of the gut microbiota induced by prebiotic effects of dietary fibre (DF) could be important in protection against colorectal cancer.

2 Prebiotics or dietary fibres: a rose by any other name?

The prebiotic concept was conceived through observations suggesting that certain oligosaccharides were able to selectively stimulate bifidobacterial populations resident in the human gut. As such, oligosaccharides that have remained undigested upon reaching the colon, termed nondigestible oligosaccharides (NDOs), have been the primary focus of much of the research on prebiotics [1, 13]. These NDOs include lactose, lactulose, raffinose, stachyose, fructooligosaccharides (FOSs), glucooligosaccharides (GOSs), xylooligosaccharides, galactooligosaccharides and isomaltooligosaccharides. Evidence suggests that these substrates are fermented and consequently stimulate the growth of particular members of the gut microbiota. Of the FOSs, inulin and oligofructose have had widespread appeal as prebiotics, having been shown to selectively stimulate the growth of bifidobacteria.

A more flexible definition of prebiotics as "potential substrates for bacterial inhabitants of the intestine" [14] would encompass current definitions of DF. The definition currently accepted by the Australia/New Zealand food authorities (FSANZ) is "DF is that fraction of the edible part of plants or their extracts, or analogous carbohydrates, that are resistant to digestion and absorption in the human small intestine, usually with complete or partial fermentation in the large intestine. The term includes polysaccharides, lignins, and oligosaccharides. DF promotes one or more of these beneficial physiological effects: laxation, reduction in blood cholesterol, and/or modulation of blood glucose". Thus, most DFs that are carbohydrates and are fermented in the large intestine, could be potentially considered as prebiotics, including resistant starch (RS), [15] non-starch polysaccharides (NSPs) and NDOs. The important questions are which components of the microbiota will be stimulated by which DFs and how will changes impact on the health of the human host?

3 The bowel microbiota

The human large bowel harbours a complex bacterial community commonly referred to as the "gut microbiota". Modern technologies provide average total bacterial counts in faeces of 1×10^{11} per gram wet weight [6]. These bacteria are predominantly located in the large bowel, although an ileal microbiota can also be detected. The gut microbiota of adult humans contains a wide variety of genera and species [16, 17], but is dominated by obligately anaerobic bacteria that are members of three phylogenetic groups: *Bacteroides-Prevotella* group, *Clostridium coccoides* group, and *Clostridium leptum* group. Prevalent species are variable between individuals, which likely reflect genetically endowed physiological differences between hosts [18]. Bacterial growth is supported by the anaerobic fermentation of dietary components that have escaped digestion by enzymes of the upper gastrointestinal tract, comprised principally of polysaccharides (RS, cellulose, hemicellulose,

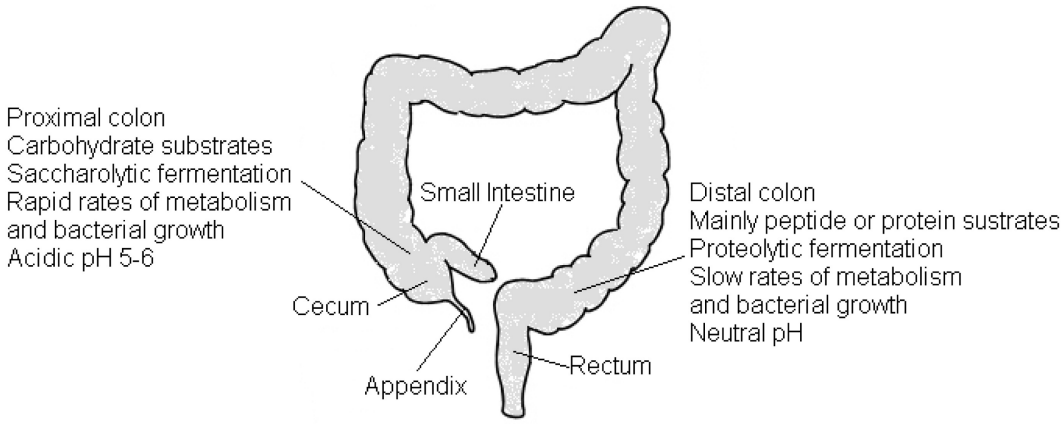

Figure 1. Fermentation in the colon. Adapted from Guarner *et al.* 2003 [120].

pectins, and gums), some oligosaccharides, unabsorbed sugars and sugar alcohols, along with mucus and other secretions of the host [19]. While the human enzyme complement does not normally contain the necessary enzymes to metabolize these materials, certain saccharolytic bacterial species are able to convert them into short-chain fatty acids (SCFAs), mainly acetate, propionate, and butyrate, with lesser amounts of formate, valerate, and caproate. The gases hydrogen, carbon dioxide, methane, and hydrogen sulphide are also produced in conjunction with SCFAs, as well as fermentable intermediates, such as ethanol, lactate, succinate, and pyruvate.

Most bacterial metabolic activity and saccharolytic fermentation occurs in the proximal large bowel, and it is due to this elevated production of SCFAs that the luminal environment is within an acidic pH range, at about pH 5 – 6. Conversely, with depleted carbohydrate substrates reaching the distal colon, rates of bacterial metabolism are significantly lower and are predominantly of a putrefactive nature [20, 21]. Consequently, bacterial populations are presumably adapted metabolically to conditions prevailing in each segment of the colon. These localized differences in fermentation and distribution of SCFAs may be of significance when considering that most lesions and cancer incidences are located in the distal colon (Fig. 1).

4 Altering the composition of bowel microbiota: can it be done?

Much of the current literature on probiotics has centred on lactobacilli and bifidobacteria, probably largely for historical reasons [22]. It is important to realise, however, that neither bacterial group is a major component of the microbiota of adult humans. Indeed, Tannock and colleagues [22 – 24] argue that, with the exception of *Lactobacillus ruminis* and *Lactobacillus salivarius*, lactobacilli detected in human faeces originate from food and oral cavity sources, rather than because they are established colonic inhabitants. Even long-term probiotic feeding does not necessarily change this situation. Tannock and co-workers [6] monitored the detection of lactobacilli in the faeces throughout and after a 6 month feeding study of *Lactobacillus rhamnosus* DR20 in milk to human subjects. For the majority of subjects, the probiotic strain could be detected in faeces only during the 6 months of probiotic administration, experience with the administration of bifidobacteria and other *Lactobacillus* species is no different [7 – 10]. Under these conditions, it is doubtful that probiotics have any impact on the large bowel ecosystem, but perhaps might be effective in the small bowel. Consumption of a probiotic product delivers about 10^9 allochthonous (nonindigenous; formed in another place [33]) bacterial cells to this site with every dose. A realistic probiotic target may not be the modification of the colonic microbiota, but the stimulation of the mucosal immune system of the small intestine.

Prebiotics provide a more attractive approach than probiotics for changing the composition or activity of the human microbiota. For example, Tannock and co-workers [14] used a double-blind cross-over study design, in which they supplemented the diet of human volunteers with three oligosaccharide-containing biscuits, or comparable biscuits containing no oligosaccharides, daily for 3 weeks, with a 2 week washout period between. Although neither the sizes of bacterial populations nor the species composition was affected during that time interval, there was evidence for increased metabolic activity of bifidobacterial and *Colinsella* populations. Other authors, however, have claimed bifidobacterial counts to be increased by the prebiotics inulin and FOSs in human subjects [13, 25 – 31]. The difference between studies may be a function of the type of prebiotic, the dose of prebiotic, and/or the time for which it was administered, as well as differences in analytical methodologies.

The studies to date suggest that the microbiota of a given individual, as happens with ecosystems more generally, has a high degree of compositional stability. After changes are introduced, either through feeding probiotics or prebiotics or through other conditions, such as antibiotic administration, homeostatic mechanisms act to restore the *status quo* [32]. The maintenance of a self-regulating community structure explains the stability of gut microbiota composition. The regulating mechanisms are not known in detail but, as in nature in general, probably involve competition, amensalism (antagonism; 'not sharing the table'), parasitism, and predation [33].

The greatest of the regulatory effectors in the gut ecosystem is doubtless competition, specifically the 'competitive exclusion principle', perhaps better termed the 'niche exclusion principle'. Put simply, two types of organism (species or genotype) with a similar ecology (fill the same ecological niche) cannot live together in the same place. In other words, organisms that are in competition for the identical habitat cannot coexist [34, 35]. The large bowel ecosystem is inhabited by a great diversity of bacterial species. The biodiversity of the community is due to the heterogeneous environments that exist in relation to bacterial nutrients and spatial arrangements. Hundreds if not thousands of niches must exist and different types of bacteria are favoured in each of them. The large bowel community is therefore characterized by a richness of coexisting bacterial species whose populations remain proportionally constant.

5 Evidence that the gut microbiota may enhance the risk of colorectal cancer

Studies in the 1970s indicated differences in the faecal microbiota between populations at different risk of colon cancer, *e.g.* [36–38]. Whereas Finegold *et al.* [38] showed few differences between the faecal microbiota of 13 vegetarian Seventh Day Adventists and 14 nonvegetarian Adventists, more striking differences were found when comparisons were made between different ethnic groups. For example, the faecal microbiota of high-risk colon cancer groups, such as Japanese-Americans, on a Western diet were different with regard to the population levels of a limited number of facultatively anaerobic and obligately anaerobic bacterial species as compared with low-risk groups (Japanese-Americans on a Japanese diet) [37]. It was speculated that the differences in microbiota composition would affect the metabolic transformation of potential carcinogens into reactive chemical species. Moore *et al.* [37] pointed to a wide variation in xenobiotic metabolising activity of different species in each genus, which would imply that a shift in proportions of species could significantly affect the bowel metabolism of an individual. Finegold and Sutter [39] found that changes in diet or dietary supplementation had a lesser effect than potent antimicrobial agents to effect compositional changes in fecal microbiota. However, they suggested that even minor changes could lead to changes in metabolic activity that impact on the production of potential carcinogens or cocarcinogens. Among the harmful effects likely to associate with increased cancer risk is the conversion of relatively benign compounds, such as bile acids, nitrate, and plant glycosides, into reactive metabolites, which are implicated in colorectal carcinogenesis [40–42].

6 Evidence that modifying the gut microbiota might protect against colorectal cancer

Protective effects against cancer, and indeed many of the claims concerning both probiotics and prebiotics, have been attributed to certain Gram-positive, lactic acid-producing bacteria (LABs), such as bifidobacteria and lactobacilli [22, 43]. Much of the proof of probiotic efficacy towards cancer prevention derives from studies on aberrant crypt foci (ACF). Reddy and colleagues [44–46] demonstrated an inhibitory effect of *Bifidobacterium longum* consumption on ACF as a short-term cancer biomarker, experimentally induced through the administration of 2-amino-3 methylimidazo[4,5-*f*]quinoline (IQ) and azoxymethane (AOM). They observed an approximately 50% reduction in AOM-induced ACF occurrence in *B. longum*-treated rats, coupled with a reduction in faecal β-glucuronidase. Rowland and colleagues [47] also correlated the reduction in AOM-induced ACF with caecal β-glucuronidase activity and ammonia concentration in rats treated with *B. longum* and inulin in combination or not. β-Glucosidase activity was also increased with *B. longum* and inulin consumption. Tsuda and co-workers [48] and Challa and co-workers [49] have combined *B. longum* consumption with lactoferrin and lactulose, respectively, and have reported reduction in AOM-induced ACF in rats. The second group, however, was able to demonstrate the effectiveness of *B. longum* without the influence of lactulose [49]. Other species of bifidobacteria, such as *Bifidobacterium* sp. *Bio*, produce similar effects, lowering ACF incidence by about 50% [50]. However, the study by Arimochi and colleagues showed otherwise, where bifidobacteria had no influence on AOM-induced ACF formation. In these studies, significant declines in ACF development were found with *L. acidophilus* and *C. perfringens*, although these effects were not correlated with a reduction in β-glucuronidase activity [51]. The variability of effects seen in the different studies may relate to differences in animal models, differences in mode of administration, and possibly also differences in the baseline animal diet used. More important, perhaps, is the question as to whether all types of ACF are predictive of colorectal cancer [52]. Thus, although statistically significant differences in ACF have been reported, these cannot be considered as proof that colorectal cancer in humans, who have

not been treated with high doses of carcinogens, would be affected by probiotics.

The results of some studies suggest that prebiotics are protective against colorectal cancer. For example, feeding the NDO lactulose has also led to a reduction in dimethylhydrazine-induced DNA damage in rat colons inoculated with human microbiota [53]. Min mice, a model for human colon cancer, have also been used to demonstrate this effect with short-chain FOSs [54]. Parameters such as faecal mutagen excretion were also improved by NDOs in human studies [46, 55, 56]. It should be noted, however, that many of the reported studies considered indirect parameters, such as bacterial enzyme activities and changes in caecal weights, and did not measure carcinogenesis *per se.* Perhaps more importantly, colonic or faecal bifidobacterial counts were not done to confirm a direct relationship between the observed effects and prebiotic consumption. Therefore, much of the available data merely imply the relationship between changes in bacterial composition and ACF reduction or carcinogenesis, and do not provide concrete evidence for the involvement of the growth stimulation of bifidobacteria or other resident microbes [57].

7 Mechanisms by which prebiotics might protect against cancer

We have previously considered the more general question of how dietary fibres, including prebiotics, could protect against cancer [58]. Here we consider only those mechanisms likely to be mediated through the microbiota.

7.1 Faecal bulking

Faecal weight is increased by both poorly and extensively degraded DF, although for nonfermentable DFs this is probably a direct effect of the DF itself [59]. Faecal bulking, as well as reduced transit times has been associated with protection against colorectal cancer. Indeed, the DF source wheat bran has effectively demonstrated both a reduction in transit time, enhanced faecal bulking, and an observed decrease in colorectal cancer risk [60]. Mechanisms for this protection have been postulated to be the decreased probability of colonic carcinogen exposure due to reduced time duration in the colon and through the reduction in carcinogen concentrations, respectively [58].

7.2 Changes in colonic pH

A prebiotic-induced decrease in luminal colonic pH may function to improve mineral solubility and uptake, namely calcium, magnesium, and iron. In particular, enhanced bacterial fermentation has also been shown to have this effect on calcium ions, through the fermentation of such substances, as phytate (myoinositol hexaphosphate), which binds to divalent cations, such as calcium. Further, a calcium-SCFA exchange system is suggested to be in place in the colon. Improved calcium absorption would provide adequate calcium for various physiological processes, thus limiting the depletion of calcium content from the bone [61, 62]. Additionally, calcium is suggested to be beneficial towards colorectal cancer, with increasing evidence that it inhibits proliferation and enhances differentiation and apoptosis of mucosal cells [63]. Further, an acidic luminal environment may reduce procarcinogenic enzyme activity, such as those of 7α-hydroxylase and nitroreductase [64].

7.3 Modulating the activity of exogenous carcinogens

7.3.1 Carcinogen binding to bacteria

Binding of carcinogens to bacterial cell walls has been suggested to protect against colorectal cancer. El-Nezami and colleagues [65–68] demonstrated such binding with aflatoxin B_1 (AFB_1), a fungal dietary contaminant causing mutagenic and carcinogenic effects in both animals and humans. Binding of AFB_1 was strain-specific, with *Lactobacillus rhamnosus* strain GG (LBGG) and *Lactobacillus rhamnosus* strain LC-705 (LC-705) being most effective. *In vivo*, health benefits would work through preventing intestinal contact and absorption, hepatic metabolism, and enhancing excretion.

In considering the case of AFB_1 as an example, the physical sequestration of the carcinogen has been implicated as the main mechanism for the reduced contact and absorption into the intestinal mucosa and metabolic transformation by the liver into mutagenic and carcinogenic metabolites. It was clearly shown not to be due to detoxification of the carcinogen, in that nonviable heat and acid-treated LBGG and LC-705 still demonstrated carcinogen-binding properties [65]. It is believed that this binding involves bacterial cell surface carbohydrates. Further, new noncovalent or hydrophobic interactions were also found to be significant in the treated cells, as was demonstrated with the binding of the dietary mutagenic pyrolyzate, 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1) to a *Lactococcus* strain. Of minor significance is the electrostatic interactions produced by the presence of metal cations, especially with divalent cations which are chelated by AFB_1 and bound by bacterial cell walls to lessen bacterial AFB_1 binding [69].

Perhaps more germane to the current discussion is whether carcinogen binding demonstrated *in vitro* can be extrapolated to an *in vivo* situation. Bolognani and co-workers [70] showed that while certain LAB are indeed able to effectively bind a range of dietary carcinogens *in vitro*, with differing species and carcinogen specificities, no reduction in *in vivo*

mutagenicity was detected in animal studies. Thus, they concluded that binding of carcinogens to the faecal microbiota does not exert a significant influence on intestinal absorption, metabolic transformation, and distribution. They have offered explanations pertaining to the rise in pH between the stomach and the small intestine or changes in other relevant conditions that could have reversed binding *in vivo.* In addition, varying nutritional states prior to treatment may have contributed to disagreement among studies [70].

7.3.2 Modulation of xenobiotic metabolising enzymes

A xenobiotic is "a chemical which is not a natural component of the organism exposed to it", and many, if not most, human carcinogens are xenobiotics. A range of enzymes (xenobiotic metabolising enzymes or XME) are classed as either phase 1 or phase 2, which function to convert these exogenous compounds into reactive metabolites or carry out conjugation reactions in order to detoxify reactive compounds for excretion, respectively [71]. Phase 1 enzymes include the cytochrome P450s (CYP) and phase 2 enzymes include glutathione *S*-transferase (GST) and NAD(P): quinone reductase (quinone reductase), UDP-glucuronosyltransferase (UGT), sulphotransferases, and *N*-acetyl transferase (NATs) [72–74]. While the liver is predominantly responsible for biotransformation of ingested compounds, as it contains the majority of the XME, the colon and other tissues also show activity [75].

Helsby *et al.* [75] showed that wheat bran fed at 10 or 20% dietary levels to Wistar rats led to changes in the levels of activity and expression of several XMEs, both in hepatic and colonic tissues. Other authors have shown differential effects of wheat bran, carrot fibre, and oat bran, to suggest that the nature or source of the DF influences which, if any, enzyme activities are modified [76]. However, the extent to which bacterial modification is associated with these changes in expression of XMEs is not always clear.

There are at least two possible mechanisms by which prebiotics may affect hepatic or colonic XMEs through actions on the microbiota [77, 78]. Digestion and fermentation of DF carbohydrates leads to the production of SCFAs, of which butyrate in particular has been shown to induce phase II enzymes. Other authors [75, 78] have also pointed out that the action of colonic esterases may lead to the release of hydroxycinnamic acids from certain DFs in the human colon, and these acids also have modulatory effects on XMEs in mammalian cells.

Chemical transformations also occur directly due to metabolic activities of the microbiota. Depending on the microbial species and state of metabolism, enzymes produced by the microflora may include nitroreductases, azoreductases, 7α-hydroxysteroid-dehydrogenase/7α-dehydroxylase, hydrolases, and β-glucuronidase, all of which function to convert compounds to reactive metabolites. For example, β-glucuronidase exhibits its harmful effects by hydrolysing glucuronic acid-heterocyclic amine (HCA) conjugates into reactive metabolites to damage both the colon and the liver through the enterohepatic circulation [79]. β-Glucosidase and sulphatase may also be present. Certain bacteria, such as *Bacteroides* species, appear to enhance mutagenicity *via* potent carcinogens, such as HCA, through these mechanisms. Additionally, exchanges between the liver and the intestine takes place through the enterohepatic circulation [80].

7.4 The butyrate hypothesis

Bacterial digestion and fermentation of DFs leads to the production of SCFAs. It has been suggested that butyrate, in particular, has functions at the level of gene expression, preventing malignant transformation by reducing cell proliferation, and inducing differentiation and apoptosis [81–85]. *In vivo* studies provide evidence for bacterial modulation of colonocyte proliferation, differentiation, and apoptosis, that have correlated with the production of SCFAs in a limited number of animal studies [86]. Such effects are of importance as proliferation, differentiation, and apoptosis are often used as intermediate markers to determine the progression of colorectal cancer. However, discrepancies of outcomes of butyrate action have been observed between colorectal cancer cell lines, where proliferation is inhibited and differentiation stimulated [87], as well as cancerous cells being restored to normal cell types [88], while proliferation is stimulated *in vivo* [86]. Indeed, for such a widely cited hypothesis, it is disappointing that most of the "evidence" derives from *in vitro* experiments that may not relate to human exposure.

7.5 Modulation of gene expression in the faeces and caecum

Until relatively recently, knowledge of both the composition and function of the gut microbiota has been limited to culturable bacterial strains, and through undertaking numerous phenotypic assays. However, newer technologies enable identification of species that are currently nonculturable, and greatly expand our understanding of their activity [16, 23, 89–93]. These new technologies have been applied to studying the existence and nature of inter-microbial and host-bacterial relationships. Hooper and colleagues [94, 95] have considered one avenue by which bacterial residents of the gut communicate with the host to provide a growth advantage towards itself. This involves the fucosylated glycoconjugates, Fucα1,2Gal-glycans, and distinctions between production in conventionally raised and germ-free mice. The presence of filamentous segmented bacteria in the mouse intestinal tract leads to the upregulation of the

murine gene (α[1 – 2] asialo GM1 fucosyl-transferase) involved in the fucosylation of the asialo GM1 glycolipid associated with enterocytes [92]. This phenomenon has also been reported in relation to colonization of formerly germfree mice with an anaerobic, Gram-negative gut resident, *Bacteroides thetaiotaomicron* [93]. This bacterial species is able to utilize L-fucose, salvaged from intestinal glycoconjugates, as an energy source. The induction of fucosylation of glycoconjugates in the bowel of mice was shown to be dependent on a critical concentration of *Bacteroides* cells and on the ability of the bacteria to utilize L-fucose. A mutant strain, unable to utilize L-fucose, was less efficient at inducing fucosylation. Linkage between L-fucose utilization and the system that is concerned with signalling enterocytes to induce fucosylation of intestinal glycoconjugates is mediated by the bacterial protein FucR [94]. It is proposed that the *Bacteroides*, by influencing host biochemistry, ensure that L-fucose is constantly available as an energy source in a highly competitive environment. These kinds of studies open doors to an understanding of the complexity of microbiota-host relationships and how modification of microbiota composition, if it is possible, might alter enterocyte gene expression.

It is also apparent that at least some members of the gut microbiota are able to influence physiological development of gut tissues. For example, Hooper and co-workers [94, 95] extended their investigations on *B. thetaiotaomicon*-host signalling by correlating the presence of these bacteria in the gut with changes to murine gene expression. DNA microarrays and real-time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) were used to assess differences in gene expression between germ-free mice and those colonised with *B. thetaiotaomicron.* Genes involved in lipid absorption, intestinal barrier function, xenobiotic metabolism, gut motility, postnatal intestinal maturation, and angiogenesis were found to have been affected by *B. thetaiotaomicron.*

7.6 Modulation of immune response

The response of the immune system towards the gut microbiota is, in general, one of tolerance [96]. Nevertheless, the gut-associated immune cells doubtless monitor the antigenic composition of the intestinal milieu, and respond accordingly when microbial content is altered. Different bacterial strains seem to elicit different immune responses, which may either be systemic or localized, innate or acquired, and cellular or humoral. Immunity can be stimulated by enhancing inflammatory and regulatory cytokines, macrophage T lymphocyte ($CD4^+$ and $CD8^+$) and natural killer (NK) cell activity [97].

In experimental animal studies, *Bifidobacterium longum*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus helveticus* and *Lactobacillus rhamnosus* have all been shown to enhance phagocytosis. Interferon-α (IFN-α), IFN-β, and IFN-γ have been induced by *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* [98, 99]. Further, certain lactobacillus strains, such as *L. casei*, *L. acidophilus* and *L. delbrueckii* subsp. *bulgaricus* have been shown to stimulate immunoglobulin A (IgA) antibody levels, although the significance of this to the host is unknown [100].

Human studies have also demonstrated various immune stimulatory effects, such as phagocytic activity, NK cell activity, and total and bacteria-specific IgA levels associated with probiotic administration of certain bifidobacterial and *Lactobacillus* strains, such as *Bifidobacterium lactis*, *L. rhamnosus* GG, *L. acidophilus*, and *L. plantarum* [101 – 108]. Cytokine production, in particular IFN-γ, was enhanced by *L.delbrueckii ssp bulgaricus* and *S. thermophilus* [109]. Other cytokines reported to be increased by LAB supplementation were IL-1β, IL-6, IL-10, IFN-γ, and tumor necrosis factor-α (TNF-α) [99, 110 – 112]. However, *L. casei* Shirota and *L. salivarus* have not shown such immune effects [8, 10]. These studies have encompassed a wide range of patient profiles, from children to adults, and from healthy volunteers to Crohn's disease and human immunodeficiency virus (HIV) patients. Without measurement of clinical outcomes in health or disease situations, however, the results of these studies remain somewhat inconclusive.

Discrepancies exist between experimental animal and human studies, perhaps due to experimental conditions and/or differences in bacterial strains, or innate differences in the immune systems of different animal species. For instance, *L. casei* Shirota was not found to have immune effects in human studies whereas an enhancement of NK and type 1 helper cell activity, IFN-γ, IL-12, and TNF-α production, coupled with the inhibition of IgE production was found in animal studies [113, 114]. Evidence for the enhancement of the cytokines IL-12, TNF-α, IFN-γ has also been found in other animal studies and NK cell activity in human trials [115 – 118]. Inflammatory processes, including the release of pro-inflammatory cytokines, are an essential part of the human body's defence against invading pathogens. However, excessive production of pro-inflammatory cytokines, or their production in the wrong biological context may lead to chronic inflammation, which is associated with greatly enhanced cancer risk [119].

8 Conclusions and future directions of research

The DF hypothesis in relation to colorectal cancer was proposed as the result of the observation that consumption of unprocessed fibres of the African diet was associated with decreased risk of gastrointestinal disorders, as opposed to

highly processed fibres of the Western diet. The concepts of probiotics and prebiotics were formulated on the basis of historical views that certain bacterial inhabitants of the gut established a mutually beneficial relationship with the human host. Despite evidence that modulation of the composition of the gut microbiota through administration of fermentable DFs will likely be impossible to achieve, attempts to alter the quality and quantity of bacterial metabolites in the gut remains an interesting prospect, especially in relation to reducing the prevalence of colorectal cancer. Enhanced elimination or detoxification of exogenous carcinogens through modulation of XMEs may be particularly important.

Fermentable DF carbohydrates, through their effects on the gut microbiota, may also produce beneficial effects in human physiology through novel mechanisms that have so far been neglected. Aside from indirect effects of the activities of the gut microbiota, direct communication (cell-to-cell signalling) between the microbiota and host tissues might be important because of inducible changes in the expression of host genes. Genes involved in intestinal barrier function, xenobiotic metabolism, gut motility, and angiogenesis will all have likely relevance for cancer. Finally, changes in the metabolic activity of the microbiota, through the upregulation of genes encoding the synthesis of particular polysaccharides, peptides, or proteins, might modulate the functioning of the immune system. These are essential to counteract the long-term implications of stimulated inflammatory processes in cancer.

Perhaps the most important lesson to be learned from a review of the literature concerning DFs, prebiotics, and colorectal cancer is that a much more integrated approach to scientific research in this area is required. Studies of the impact of dietary supplementations on the aetiopathogenesis of colorectal cancer should, in future, be conducted as team efforts in which nutritionists, pathologists, and microbial ecologists can together be involved. Otherwise, unrealistic expectations will continue concerning the modulation of the composition and metabolic activities of the gut microbiota and its impact on host-microbiota relationships.

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