Cellular and Physiological Effects of Probiotics and Prebiotics

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Abstract: We review the present knowledge on the biological mechanisms of action of probiotics and prebiotics. They include direct effects in the intestinal lumen or on intestinal or immune cells, and indirect mechanisms through modulation of the endogenous microflora (composition or functions such as butyrate production) or of the immune system.

Keywords: Probiotics, prebiotics, mechanisms of action, intestinal flora, intestinal immune system, cytokines, butyrate production.

INTRODUCTION AND DEFINITIONS

There is a growing interest in the field of both probiotics and prebiotics as randomised controlled trials (RCTs) have shown that some of them have undoubtful clinical benefits in various physiological or pathological situations [1]. Probiotics have been defined as "non-pathogenic micro organisms (bacteria such as lactobacilli, bifidobacteria, … or yeast) which, when ingested (as living cells) exert a positive influence on host health or physiology" [1]. The term prebiotics has more recently be introduced for "non-digestible food ingredients, which beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, that have the potential to improve host health" [1]. They essentially consist in non-digestible oligosaccharides (NDOs), which stimulate the growth of bifidobacteria (bifidogenic factors) and/or lactobacilli. We summarise here the present knowledge, ideas and questions on the biological mechanisms of action of probiotics and prebiotics; many of these effects are indirect through modulation of the endogenous microflora or of the immune system Fig. (**1**). The great variability of the mechanisms of action is largely explained by the huge variability in their nature, composition and pharmacology.

PROBIOTICS

Pharmacology

Probiotic properties have been documented for micro-organisms, which differ greatly in their genome, enzymatic activities and cell wall composition. Their active constituents include enzymes, immunomodulatory components, and components possessing antagonistic activities against other microorganisms. Probiotics act as vectors, which deliver these active constituents at various places of the gastrointestinal tract (target sites) and noticeably protect them partly from acid in the stomach. The majority of the effects occur only (or mainly) when the probiotics are ingested alive and this suggests that the survival of the probiotic until its target site, could be a desirable property. Pharmacokinetic studies have shown that the survival of probiotics in the gastrointestinal tract varies greatly not only between genera but between species and even strains [2]. The ability to adhere to the intestinal mucosa and/or to intestinal mucus is also an important characteristic, which varies between strains [2]. Many experts consider that this property could favour competitive exclusion of pathogens and immunomodulation. Even the best surviving and adhering probiotic strains usually do not colonise the intestinal mucosa for long periods, and are eliminated after a few days when the host stops ingesting them [2]; however, a few healthy subjects have been shown to be colonised for long periods by some strains [2]. Whether this is good, bad or does not matter is not established at the present time. Probiotics may reach the inductive mucosal immune system through several routes including the specialised M cells of the Peyers patches and dendritic cells [3]. A study performed in mice showed that probiotic lactic acid bacteria could be detected in the dome area of Peyers patches 6-12 hours after their ingestion [4].

Effects on the Other Micro-Organisms Present Within the Intestinal Ecosystem

The first mechanism of action, which was imagined, for probiotics (and which led to the first concept of probiotics) was a modification of the endogenous flora. This mechanism is difficult to assess as the inter-individual variability in the composition of the intestinal flora limits the statistical power of studies. Classical bacteriologic techniques have not been adequate for demonstrating and describing the effect of probiotics on the equilibrium of the fecal flora *in vivo* except for the passage of the probiotic strains in feces [2]. Several authors reported that some ingested bifidobacteria or lactobacilli (but not all of them) decreased the fecal concentrations of *Bacteroides*, clostridia, and *Escherichia coli* and sometimes also increased the endogenous levels of bifidobacteria and lactobacilli. The effects of probiotics on the metabolic activities of the flora have been more convincingly demonstrated and several strains have been shown to reproducibly decrease fecal azoreductase, nitroreductase and ß-glucuronidase activities, which could be

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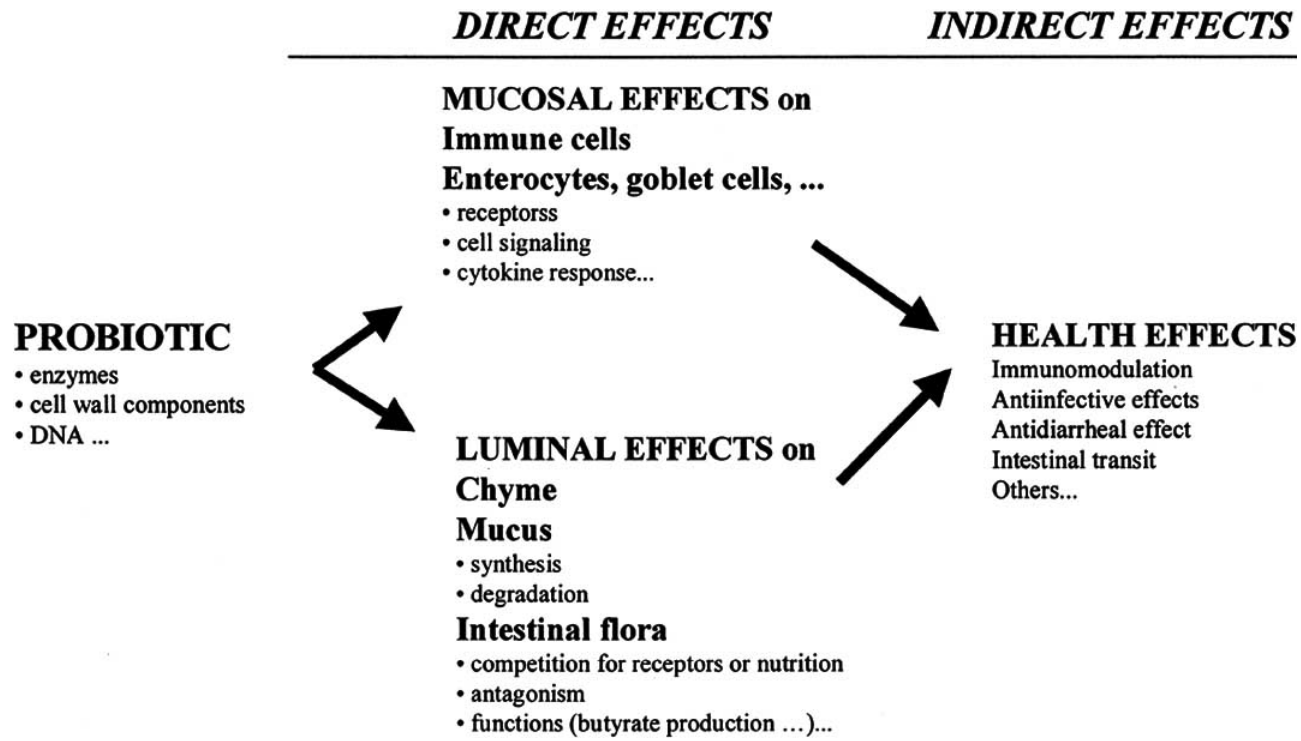

Fig. (1). Mechanisms of action of probiotics.

involved in colonic carcinogenesis [5]. Instability of the flora is associated with the risk of intestinal infections or diseases and probiotics may conceptually help stabilise the ecosystem. However, this hypothesis is not yet proven.

Microbe-microbe interactions are easier to study in *vitro* in simplified models but the extrapolation of the results obtained in such models is hazardous. Protective effects of probiotics against intestinal infections have been observed in animal models [4]. The evidence in humans is fair (positive double-blind RCTs) for *Clostridium difficile* and *Helicobacter pylori* [1]. The interactions, which may occur, between a probiotic and members of the endogenous flora or pathogens include competition for essential nutrients, production of antimicrobial factors, modifications of ecological conditions (pH for example…) and competition for adhesion sites in the intestine. In addition, probiotics may decrease the capacity of some pathogens to secrete toxins, or destroy toxins or inhibit toxin adhesion and effects (see below).

Production of Antimicrobial Factors

Many probiotic strains have antagonistic properties *in vitro* against pathogens. For example, the cell-free supernatant of *L rhamnosus* Lcr35 inhibited the growth of enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), *Klebsiella pneumoniae, Shigella flexneri, Salmonella typhimurium, Enterobacter cloacae, Pseudomonas aeruginosa, Enterococcus faecalis* and *C. difficile* [4]. The mechanisms involved are not always the same as probiotics may produce a range of antimicrobial factors. For example, lactobacilli produce lactic acid, which is deleterious to many micro-organisms and some strains also produce antibacterial proteins. Among those, bacteriocins display a wide antibacterial spectrum against gram-positive (but not gram-negative) bacteria [4].

Competition for Adhesion

Adhesion of pathogens to the host mucosa is the first step during many intestinal infections. Some probiotics inhibit adherence of various pathogens to the mucus and/or to the epithelial cells and may thus avoid invasion [6]. For example, Bernet *et al.* [6] reported a dose-dependent inhibition of adherence of ETEC, EPEC and *S. typhimurium* to Caco-2 cells by strains of bifidobacteria or lactobacillus. In some cases, the supernatant from probiotic cultures has the same effect, indicating that in these cases, secretion products are probably involved [6]. For example, the culture supernatant of *L. johnsonii* La1 interferes with the growth, urease activity, and adhesion to cultured human epithelial cells of *H. pylori* [6]. Heat killed micro-organisms exhibited antiadhesion properties in a few reports [6], however in the majority of the studies the inhibition of pathogen adhesion or invasion was observed only or mainly with living probiotic cultures. The majority of the *in vitro* studies have used intestinal cell lines. However, Reid and co-workers [6] demonstrated that lactobacillus strains which originated from the human urovaginal tract, had adhesive properties and inhibited the colonisation of uroepithelial cells by pathogens. Depending on the probiotic, the antiadhesion property may result from non-specific steric hindrance of pathogen adhesion receptors or from specific blockade by a proteinaceous or non proteinaceous constituent or metabolic product of the probiotic. For example, the inhibition of the adherence of *E coli* to a reconstituted basement membrane by *L. crispatus* JCM 5810 involves its S-layer protein [7]. In some cases, probiotics such as *Saccharomyces boulardii* have the ability to bind pathogens [8].

Antitoxin Effect

S. boulardii has a beneficial effect against *C. difficile* infections, which has been demonstrated in various models.

Two blinded randomised placebo controlled trials demonstrated that it significantly reduces the risk of recurrence of frequently relapsing infections [1]. The effect of *S. boulardii* on *C. difficile* toxins was suspected when some authors observed a therapeutic effect of the probiotic yeast in animals despite the absence of effect on *C. difficile* concentrations in feces [8]. Further trials showed that the fecal concentration of the toxins of the pathogen were reduced by *S. boulardii* or by its culture supernatant and that the active compound was a 54 kD serine protease [8].

Effects on the Immune System or Cells

Recent trials have underscored the importance of a continuous cross talk between intestinal microbes (including probiotics) and the immune system of the host. This system comprises « non specific and innate » lines of defense such as macrophages and polymorphonuclear leukocytes (PMNL), which perform phagocytosis, and specific defenses carried by lymphocytes. The gut immune system consists of organised lymphoid aggregates (Peyer's patches, appendix, and mesenteric lymph nodes), which are often referred to as "gut associated lymphoid tissue (GALT)" and are mainly "inductive sites" for the immune response. In non-organised lymphoid elements in the epithelium and lamina propria these cells are effector sites of the response. The GALT is functionally connected with the other mucosal tissues throughout the body (bronchial tree, salivary and lacrymal glands, mammary gland, genital mucosae) and antigen exposure in the GALT can generate an immune response not only in the gastrointestinal tract but also in other mucosae such as in the lung and in the vagina. This results from the trafficking of mucosal lymphocytes through the body, which is regulated by a recognition between their adhesion molecules and those expressed in the mucosa especially on endothelial cells.

Cellular Recognition of Probiotics and Signal Transduction

Knowledge is rapidly increasing on the mechanisms involved in the subtle recognition of micro organisms in the gastrointestinal tract and on the triggering of adapted responses. Many microbial signals are specifically recognised including bacterial lipopolysaccharide (LPS), peptidoglycan cell wall constituents, formylated peptides and nucleotides. The host innate immune response distinguishes signals from pathogens and commensals by pattern recognition receptors or Toll-like receptors (TLRs) which are expressed differentially among immune cells and by intestinal epithelial cells (IEC). The latter discriminate between commensal and pathogenic bacteria by the intracellular Card4/Nod2 protein [4]; if the signal corresponds to a pathogen, IEC may react by expressing inflammatory and chemoattractive cytokines, especially TNF-α and IL-8. The immune cells express multiple TLRs to recognise the microbial environment and trigger an adapted response. TLR2 recognises lipoproteins and peptidoglycans and triggers the host response to Gram-positive bacteria and yeast, TLR1 and TLR6 participate in activation of macrophages by Gram-positive bacteria, TLR4 mediates responses to LPS primarily from Gram-negative bacteria, and TLR5 recognise flagellin. TLR9 recognises special features of microbial DNA and this seems to be one of the modes of action of some probiotics, which exhibit immunomodulating properties [9]. The bacterial DNA differs from the eukaryote DNA as it contains a high proportion of oligonucleotides containing unmethylated CpG motives (ODN CpG). Interestingly, some of these ODN CpG stimulate lymphocytes whereas eukaryotic DNA and methylated oligonucleotides do not [7]. Stimulation of dendritic cells by ODN CpG is associated with production of T_H1 cytokines such as interleukin-12 (IL-12). Rachmilewitz *et al.* showed that the probiotic mixture VSL#3 had a beneficial effect on chemically induced colitis in mice and that this effect was derived from the DNA as VSL#3 unmethylated DNA was effective while VSL#3 -methylated DNA and control eukaryote DNA were ineffective [10]. This discovery should probably lead to important progress in the near future, especially in the selection of new immunomodulating strains.

The transduction of the microbial signal is also an important physiological step in the host cell and the role of nuclear factor-˙κ (NF-κB) is essential. Madsen *et al.* recently showed that the VSL#3-DNA down-regulated proinflammatory cytokine secretion by attenuation of the NF-κB pathway in intestinal epithelial cells [11]. Non-pathogenic micro organisms may also attenuate potential proinflammatory responses by blocking degradation of the counterregulatory factor IκB [112]. Dahan *et al.* showed that *S. boulardii* exerted a preventive effect on EHEC infection in T84 cells by interfering with a transduction pathway implicated in the control of tight-junction structure and decreased IL-8 secretion via inhibition of the NF-κB and MAP Kinase signalling pathways [13].

Cytokine Responses

Cell activation results in the secretion of various cytokines which coordinate the whole inflammatory reaction in the mucosa. Release of interleukin-12 (IL-12) regulates T cell and NK cell responses, induces the production of interferon-γ (IFN-γ), and favours the differentiation of TH1 lymphocytes. On the opposite, IL-4, IL-5 and IL-10 induce the preferential differentiation of TH2 lymphocytes and TGF-β, IL-10 drive B cell switch to IgA isotype and promote oral tolerance (TH3 type of response).

In vitro experiments using either isolated CaCO-2 cells or the same cells co-cultured with immunocompetent cells showed that micro-organisms do not all elicit the same cytokine secretion pattern [14]. For example, challenge of CaCO-2 cells with *L. sakei* induced expression of IL-8, MCP-1, IL-1β, and tumour-necrosis factor-α (TNF-α) mRNA in the presence of underlying leucocytes. Leucocyte sensitised CaCO-2 cells produced TNF-α and IL-1β whereas IL-10 was exclusively secreted by human peripheral blood mononuclear cells (PBMC) [14]. CaCO-2 cells alone remained hyporesponsive to the bacterial challenge. *L. johnsonii* showed reduced potential to induce proinflammatory cytokines but increased transforming growth factor beta mRNA in leucocyte sensitised CaCO-2 cells. TNF-α was identified as one of the early mediators involved in cellular cross talk. Chirstensen *et al.* have shown that the cytokine response of dendritic cells also varied after challenge with different lactobacilli [15]. Clearly, all probiotics do not share the same immunomodulating properties but can even have opposite effects on some

parameters. Moreover, the dose of probiotics also strongly influenced the nature of the immune response in this model.

In vivo studies have confirmed that probiotics may influence cytokine production at mucosal surfaces and by blood leukocytes. For example, Maassen *et al.* [4] reported increased expression of TNF-α, IL-2 and/or IL-1β in the gut villi of mice given *L. reuteri* and *L. brevi*. A large variation in the ability of different lactobacilli strains to induce pro- and anti-inflammatory cytokines was also observed.

Intake of probiotics has also been shown to enhance cytokine production *in vivo* in human subjects, and by PBMC *ex vivo* following appropriate stimulation. For example, several studies have shown that ingestion of yoghurt bacteria in large doses (10^{11} to 3 10^{12}/d), led to stimulation of the capacity of PBMC to produce IFN-γ [4].

Effects on Innate Immunity

Human *in vitro* co-cultures produced by the co-cultivation of IEC lines and PBMC using transwell culture technique have allowed important progress in our understanding on the microbial-mucosal interactions. The group at the Nestlé research centre in Lausanne has shown using such models that there are two major cytokine/chemokine responses of IEC exposed to bacteria including probiotics. The first type is a NF-κB mediated inflammatory response resulting in the production of IL-8, MCP-1, IL-1β, and TNF-α (which was only transient with comensal strains). A second class of lactobacilli including *L. johnsonii* and *L. gasseri* induced the immunoregulatory cytokine TGFβ in the absence of any pro-inflammatory event [14].

In vivo studies have confirmed that some probiotic strains could significantly stimulate the innate immune function in healthy subjects. Several studies have shown an enhancement of the phagocytosis of PMNL in healthy humans consuming various probiotics (for example *L. johnsonii* La1, *B. lactis* Bb12, *B. lactis* HN019 or *L. rhamnosus* HN001) (but not all) in fermented milks [4]. Pelto *et al.* reported that *L. rhmanosus* GG modified the expression of leucocyte receptors involved in phagocytosis. Indeed, while a milk challenge increased significantly the expression of the phagocytosis receptors CR1, FcgammaRI and FcalphaR in neutrophils and CR1, CR3 and FcalphaR in monocytes of milk-hypersensitive subjects, this effect was prevented by the probiotic [16]. Other studies have shown an enhancement of NK cell activity and an increase in the proportion of circulating NK cells in volunteers who consumed yoghurt or probiotics [4].

Effects on Humoral Immunity

Cellular effects on dendritic cells and on the native immune system may lead to effects on humoral immunity and antibody secretion. In a randomised, placebo-controlled study performed in children suffering from acute rotavirus gastroenteritis, Kaila *et al.* [1] observed that children receiving the probiotic *L. rhmanosus GG* had significantly higher numbers of circulating IgG, IgA- and IgM-secreting cells compared with children receiving a placebo. At convalescence, a significantly higher proportion of children in the *probiotic* group exhibited rotavirus-specific IgA antibody-secreting cell response (90% vs. 46% in the placebo group). This may be one of the mechanisms to explain how some probiotics (and especially *L. rhmanosus GG*) significantly shorten or prevent acute gastroenteritis, especially rotavirus gastroenteritis in infants [1].

Link-Amster *et al.* [17] reported that the consumption of fermented milk containing *B. bifidum* and *L. johnsonii* La1 enhanced the specific serum IgA antibody response following vaccination with *S. typhi* Ty21 in healthy volunteers. This was also observed with other probiotics and other oral vaccines [4], and several studies showed a significant increase in total IgA in blood of healthy subjects receiving probiotics. Some authors hypothesised that immunomodulation may be due to an increased transport of antigens across the mucosal barrier (via increased intestinal permeability) or up-regulation of antigen presenting molecules and co-stimulatory molecules on immune cells, which induced or increased the number of B cells [4]. Several teams have indeed shown that some probiotics may modulate intestinal permeability (see below), however, this is not a constant finding. One of our studies confirmed that the serum concentrations of IgA slightly increased when healthy volunteers received the probiotic *L. johnsonii* La1. However, we showed that their intestinal permeability to proteins of various sizes was not significantly altered.

Protecting Effect Againts Atopic Eczema (allergy)

Erica Isolauri and co-workers demonstrated in convincing randomised controlled trials that *B. lactis* Bb12 and *L. rhamnosus* GG had significant preventive effects upon the development of atopic eczema in infants [18]. The probiotics were given prenatally to mothers who had at least one first degree relative with atopic eczema, allergic rhinitis or asthma and to the infants for 6 months. The mechanisms for this effect are still debatable. Rautava *et al.* reported that *L. rhamnosus* GG consumption increased the concentration of TGFβ in the mother's milk [19]. An influence on the composition of the intestinal flora could also be involved. Interestingly, *L. rhamnosus* GG administration did not prevent birch pollen allergy or apple allergy in a series of allergic teenagers [18], and one may therefore imagine that probiotics may have a greater chance for prevention of allergy when given very early in life. This theory is in keeping with the results from population-based studies, which suggest that increased exposure to bacteria in early life are protective against allergy.

Effects on Enterocytes, Mucus Production and Intestinal Permeability

Endogenous bacteria and/or probiotics influence not only immune cells but also enterocytes and mucus producing cells. These actions may result in many cellular and tissular effects, especially modulation of intestinal permeability, mucus production and cell turnover in the intestinal villi.

Trophic Action

Several studies showed that the ingestion of *S. boulardii* produces trophic intestinal effects including increases in the specific and total activities of brush-border membrane enzymes in the jejunal mucosa of growing rats but also healthy adults [8]. Buts *et al.* suggested that this effect might be due to the endoluminal release of spermine and spermidine contained in the yeast cells as these polyamines could reproduce the trophic effect on the mucosa in rats [20].

In addition, after oral treatment of rats with *S. boulardii*, there is a marked stimulation of sodium-dependent D-glucose uptake into brush border membrane vesicles with a corresponding accumulation of the sodium D-glucose cotransporter-1 (ref in 21). *L. reuteri* R2LC and *L. plantarum* DSM 9843 significantly increased bowel mucosal mass in rats with methotrexate induced enterocolitis [22]. Ichikawa H *et al.* reported that the oral administration of *L.casei* or *Clostridium butyricum* increased the crypt cell production rate of the jejunum, ileum, cecum and distal colon in rats fed an elemental diet [23].

Effect on Intestinal Permeability and its Cellular Mechanisms

Many researchers are interested in the effect of probiotics on intestinal permeability as an increase in permeability is involved in the pathogenesis of many mucosal diseases (e.g. inflammatory bowel diseases, celiac disease, intestinal infections, allergy). Paracellular tight-junctions are major determinants of intestinal permeability. Several trials have shown that probiotics may protect tight-junctions from pathological leakages that are observed in various infectious or inflammatory conditions. For example, *L. brevis* and *L. plantarum* 299v reduced the increase in permeability induced by *E. coli* in rat intestine [4]. IL-10 knock out mice develop a colitis presenting similarities with Crohn's disease and associated with an increased permeability. Colitis and permeability disorders were prevented in IL-10 knock out mice treated for 4 weeks with the probiotic cocktail VSL#3 [24]. Moreover, VSL#3 directly applied to T84 monolayers placed in Ussing chambers increased transepithelial resistance and decreased mannitol flux (i.e. enhanced intestinal permeability) [10]. Experiments performed in T84 cells showed that *S. boulardii* maintains the tight-junction structure (the distribution of the zonula occludens ZO-1 tight-junction associated protein was analysed by confocal microscopy) of these cells during EPEC infection [25]. This yeast also abolished the phosphorylation of myosin light chain in EHEC infected cells, which is one of the transduction pathways implicated in the control of tight-junction structure [13].

Electrolyte Transport

Many pathogenic micro-organisms induce diarrhoea by increasing chloride secretion by enterocytes. Resta-Lenert and Barrett recently reported that *S thermophilus* ATCC19258, and *L acidophilus* ATCC4356 had no effect on chloride secretion in the human intestinal epithelial cell lines HT29/cl.19A and CaCO-2 but blocked the effect of EIEC on chloride secretion. A normal transport function was also restored by the spent medium of the two probiotics [26]. The antisecretory effect of *S. boulardii* has been extensively studied in various models [8]. Several studies have shown that this yeast and its supernatant exerted a protective effect against *E. coli* heat labile toxin and cholera toxin (which both increase chloride secretion via cAMP activation in enterocytes) [8]. As pertussis toxin inhibited this protective action, it was concluded that the effect might be related to the ability of a protein from the yeast to bind a receptor that negatively regulates adenylate cyclase activity. Interestingly, *S. boulardii* also inhibited the chloride secretion induced by the calcium-dependent pathway.

Interactions with Mucus

The intestinal mucosa is covered by a mucous gel, which is produced by the goblet cells and acts as a barrier protecting the mucosa against harmful components present within the luminal environment. Some microorganisms may degrade mucus, or increase its synthesis or adhere to mucus. Several authors reported that a series of probiotics including *L. rhamnosus* GG did not degrade mucus [27]. A fascinating study by Mack *et al.* showed that *L. plantarum* 299v increased the expression levels of mucins MUC-2 and MUC-3 mRNA in HT-29 cells [28] and *L. rhamnosus* G G mediated the up-regulation of the MUC-2 mRNA and protein in CaCO-2 cells. Furthermore, the two probiotics inhibited the adherence of EPEC to the HT-29 intestinal epithelial cells, but not to control non-epithelial cells suggesting a protective role of mucin. In a recent study, the same authors confirmed that preincubation of some probiotic strains of *Lactobacillus* with EPEC inhibited the adherence of the enteropathogen to intestinal epithelial cells. Interestingly, not all *Lactobacillus* strains have this capacity and the mutant strain of Lp adh- and LaDDS, which did not adhere to intestinal epithelial cells were ineffective [29].

Direct effects in the Intestinal Lumen and on Gastrointestinal Motility

Some of the effects of ingested probiotics are due to their enzymatic properties in the host. The best example is the digestion of lactose by yoghurt lactase. Lactose maldigestion is frequent in adults and in general due to the physiological decline of intestinal lactase activity after weaning. A series of studies showed that the digestion of the lactose contained in yoghurt was better than that contained in milk [1] and two mechanisms (which do not exclude each other) were found to be involved. The first is the slower gastro-intestinal transit time of yoghurt which gives more time for lactose digestion. The other is a probiotic effect, which is suppressed when the bacteria present in yoghurt are experimentally destroyed (by heat). We showed using an intestinal perfusion technique that the lactase contained in the yogurt bacteria was delivered and active in the small bowel of human volunteers [1]. Corthier *et al.* recently proposed an additional mechanism as they observed (using luciferase as a reporter gene system) that orally administered *S. thermophilus* produced ß-galactosidase in the gastrointestinal tract of mice [30]. Another study by the same team also showed that the probiotic *L. casei* DN-114 001 could initiate protein synthesis during its transit in mice [31]. Interestingly, yoghurt bacteria, which are very sensitive to bile, are more potent upon lactose digestion than bile salt resistant bacteria with similar lactase contents and this led to the idea of using bile salt sensitive bacteria (such as *Lactococcus lactis*) to deliver enzymes in the small intestine.

Among other examples of direct enzymatic effects of ingested probiotics, an enhanced digestion of a sucrose load has been shown in infants with sucrase deficiency when they consumed *S. cerevisiae* (i.e. a yeast, which contains the enzyme sucrase) [1]. Buts *et al.* showed that the endoluminal release of an aminopeptidase by *S. boulardii* upgraded endoluminal *N*-terminal hydrolysis of oligopeptides in suckling rats [21]. Sidhu *et al.* [32] showed that gavage of rats with *Oxalobacter formigenes* (a bacterium

which degrades oxalate) reduced urinary oxalate excretion and suggested evaluation of this strain in the prevention of oxalate kidney stone disease. Several authors propose genetic manipulation of probiotic vectors to improve their therapeutic activities in the intestine and this technique may have wide applications in the future. For example, Drouault *et al.* showed that genetically modified *Lactococcus lactis* containing lipase (from *Staphylococcus hyicus*) helped lipid digestion in pigs with pancreatic ligation [32]. Steidler *et al.* showed that genetically modified *Lactococcus lactis* producing IL-10 significantly protected mice against experimental colitis [32].

The endogenous flora influences intestinal transit but the mechanisms for this effect are poorly understood. Two randomised controlled studies indicated that the probiotic *B. animalis* DN-173010 shortened the transit time in the sigmoid colon in healthy women [1]. In one of the two studies, we showed that the probiotic treatment did not affect faecal weight, pH, bacterial mass or faecal bile acids. Thus, the mechanism for the effect remains unknown [1].

PREBIOTICS

The concept of prebiotics has been only recently introduced, and studies proving clinical efficacy or searching for mechanisms are limited [1]. We also discarded from our analysis the trials, which were performed with synbiotics (i.e. mixtures of probiotics and prebiotics) since they usually do not allow one to establish which component is effective and the mechanisms for the effects.

Pharmacology

Prebiotics are short chain or long chain carbohydrate molecules, which are not absorbed in the small bowel, and are fermented in the colon into short chain fatty acids (SCFA) and gas. They influence the endogenous ecosystem (by definition) usually increasing the population of bifidobacteria and often decrease the colonic pH [1]. As long as they are not fermented, prebiotics (especially the short chain ones) exert an osmotic effect in the intestinal lumen, which is negatively related to their molecular weight. This increases the water flow rate, and may induce borborygmi, abdominal pain, and eventually diarrhoea if the capacity of the colon to absorb water and electrolytes is exceeded. This effect largely contributes to the action of high doses of prebiotics in the treatment of constipation [1].

An important characteristic of the pharmacology of many prebiotics is the adaptation to regular consumption. Indeed, regular consumption of some NDOs such as lactulose results in changes in the metabolic activity of the colonic flora (bacterial adaptation), which increases its ability to ferment the NDO and includes a fall in hydrogen net excretion [33]. A lower risk of diarrhoea has been reported in some but not all studies when prebiotics (especially lactulose) were consumed regularly (clinical adaptation).

Effects on the Composition of the Intestinal Microflora

A significant increase in faecal bifidobacteria counts was repeatedly obtained in humans ingesting short chain FOS at a dose >=10g/d. Moreover, the increase in bifidobacteria counts was correlated with the dose of ingested material [34]. This effect was also reported with other NDOs such as FOS, with longer chains obtained by partial hydrolysis of inulin, galacto-oligosaccharides, xylo-oligosaccharides, and lactulose [35, 36]. The same NDOs also often increase faecal concentrations of lactobacilli. The mechanism is not fully understood but it is known that bifidobacteria possess the enzymes necessary for the fermentation of these sugars. However, the mechanism may not only be due to the feeding of bifidobacteria but also modification of the ecological conditions (pH decrease) favouring the growth of bifidobacteria while disfavouring that of Bacteroides and/or clostridia [35]. Some of the effects may change with time because of adaptation of the endogenous flora. Indeed, Le Blay *et al.* [37] reported that the FOS-induced increase in intestinal lactic acid-producing bacteria was lost during chronic FOS consumption in rats, but the butyrogenic properties of FOS were maintained in the same animals. Modifications of the flora, which occur during prebiotic consumption, are usually beneficial and may provide some protection against infections [38]. However, a recent paper suggested that they might sometimes be deleterious. Indeed, Ten Bruggencate *et al.* showed that FOS dose-dependently impaired the resistance to salmonella infection in rats. In these experiments, the number of salmonella and their translocation was increased in the animals receiving the prebiotics [39].

Effects of Prebiotics on Butyrate, Other SCFA and Colonic pH

Prebiotics are fermented into SCFA and decrease colonic pH. This effect is sometimes not observed in feces for the prebiotics, which are rapidly fermented in the proximal colon as SCFA are absorbed downstream [34, 40]. Fermentation is a metabolic process performed by microorganisms under anaerobic conditions (in humans this happens in the colon), which transform substrates (mainly carbohydrates) into SCFAs and H2 and CO2 gas. The main SCFAs are acetate, propionate and butyrate and their relative proportions may be influenced by prebiotics. They acidify the colonic environment and play a major role in colon as well as systemic physiology. Indeed, all of them allow energy salvage and propionate may decrease blood cholesterol by inhibiting its hepatic synthesis [41]. Butyrate is the major fuel for colonocytes and is preferentially used in place of glucose [41]. Its physiological effects are numerous Fig. (**2**) and noticeably it may regulate some gene expression (through histone acetylation - DNA methylation), especially genes implicated in the control of epithelial proliferation-differentiation-apopotosis processes. Colonic diseases such as diversion colitis but also inflammatory bowel disease and colon cancer seem to be associated with a decrease in faecal butyrate contents and many studies have shown that butyrate administration may be useful for treating or preventing experimental colitidies [41]. Noticeably also, these diseases are more frequent in the distal colon in humans (colon cancer, ulcerative colitis) than in the proximal colon where the SCFA concentrations are higher. Although some bacterial species produce more butyrate than others (some fusobacteria or clostridia for example), the production of this SCFA is carried out by numerous members of the endogenous flora.

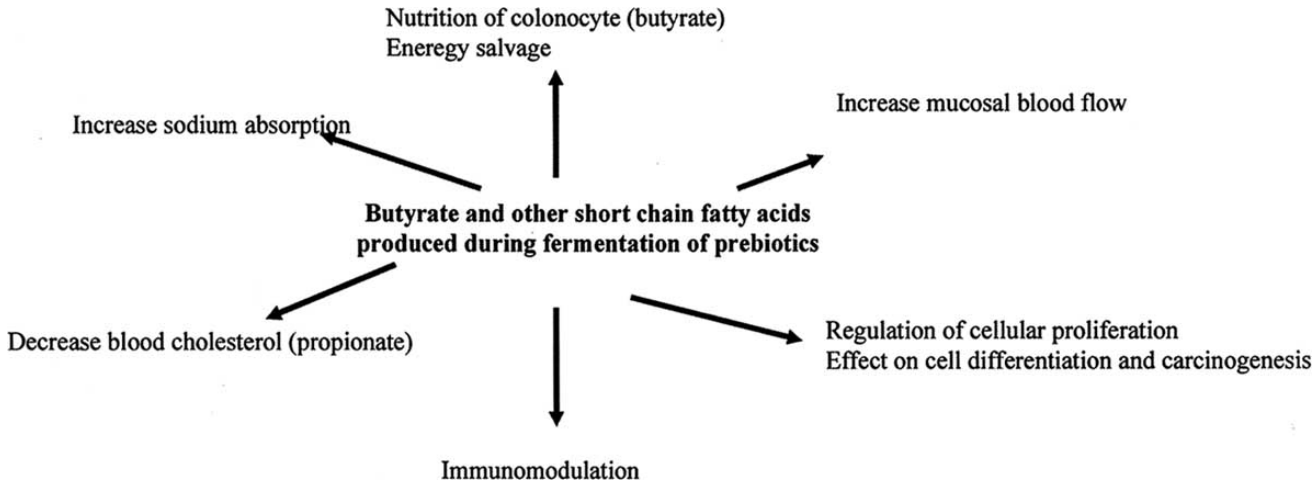

Fig. (2). Physiological effects of butyrate (produced by fermentation in the colon).

Some prebiotics, especially fructo-oligosaccharides (FOS) and some resistant starches increase the proportion of butyrate production in the colon [42]. They have therefore received a special interest and their effects have been studied in animal models and humans. Several studies have shown that FOS administration decreased intestinal tumour formation (and/or relevant surrogate markers such as the aberrant crypt foci) in rodents. For example, Pierre *et al.* showed that short chain FOS reduced colonic tumour occurrence in Min mice (a model simulating the late steps of colonic carcinogenesis which occur in humans) [43]. The same FOS and resistant starch (so only fibres promoting a stable butyrate-producing ecosystem) also proved to decrease the rate of aberrant crypt foci in rats [44]. Interestingly, some of these effects may be linked to immunomodulating properties of butyrate. For example, FOS administration increased the intestinal lymphoid follicles in Min mice [43].

As SCFA may influence colon motility, SCFA modifications may be one of the mechanisms for use of prebiotics in the treatment of constipation. However, this hypothesis remains unproven [1, 33, 41].

Colonic acidification decreases some metabolic activities and especially the 7-α-deshydroxylase, which converts the primary bile salts into carcinogenic secondary bile salts. The bacterial 7-α-dehydroxylase is inhibited when the pH is below 6.5 and colonic acidification is thus a way to decrease secondary bile salt production and colon cancer risk. Several studies have shown that lactulose administration to healthy volunteers lowered the faecal concentrations of secondary bile salts [45]. A study by Roncucci *et al.* reported in 1993 that lactulose decreased the recurrence rate of colon adenomas [46]. We recently showed that FOS chronic ingestion increased SCFA production and faecal primary bile salts excretion in humans with colonic polyps suggesting potential for this prebiotic for colon cancer prevention (Boutron-Ruault *et al.* submitted).

Other Effects and Clinical Consequences

As mentioned above, several RCTs have demonstrated that lactulose is an effective treatment of constipation [1]. The mechanism involved in the laxative effect seems multifactorial. At high doses, lactulose (and probably all NDO) can induce osmotic diarrhoea, however, at low doses (at which lactulose has a significant effect in patients), the osmotic effect is limited by fermentation. An increase in faecal hydration, in the faecal bacterial mass, and a stimulation of colon motility by end products may participate to the clinical efficacy. RCTs have shown that lactulose and lactitol have a beneficial therapeutic effect in humans with hepatic encephalopathy. This neurologic disorder, which occurs in subjects or animals with severe liver disease, is due to substances derived from the metabolism of the gut flora including ammonia and which are not metabolised by the sick liver [1]. Several mechanisms of action for the beneficial effects have been shown including stimulation of colonic bacterial growth, incorporation of ammonia into bacterial proteins, colon acidification (which reduces ammonia absorption), laxative effect, and possibly the shift of the colonic production of medium chain fatty acids to short chain fatty acids [47]. Lipid-lowering effects of FOS inulin have repeatedly been observed in rodents but human studies have provided conflicting results. Doses used, species specificities or experimental conditions may strongly influence the results but as stressed in recent reviews a general conclusion cannot be drawn yet [48]. Numerous investigations performed essentially in animal models have shown repeatedly that some NDOs, such as inulin, FOS or transgalacto-oligosaccharides (TOS), stimulate calcium absorption and may have beneficial effects for prevention of bone demineralisation. Several mechanisms have been hypothesised for this potential effect: an increase in calcium absorption by lowering of the intestinal pH or stimulation of calcium absorption by SCFA [49]. Scholz-Ahrens *et al.* reported that dietary FOS and calcium had some effect on bone structure in ovariectomised rats and that this was probably not mediated by polyamines produced by the stimulated endogenous flora [50].

CONCLUSION

Substantial evidence suggests that probiotics and prebiotics can significantly influence health but that they are not a panacea. Molecular tools, especially DNA microarray

techniques should allow us to progress in our understanding on how commensal or exogenous micro organisms modulate expression of genes involved in several important intestinal functions including immunity. To understand better the mechanisms is of paramount importance to progress in the development of new products despite two main difficulties, which are that many effects are indirect and that probiotics are not simple molecules.

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