Enhancement of Gut Immune Functions by Short-Chain Fructooligosaccharides and Reduction of Colon Cancer Risk

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Short-chain fructooligosaccharides occur in a number of edible plants, such as chicory, onions, asparagus, wheat. . . They are produced industrially from sucrose. They are a group of linear fructose oligomers with a degree of polymerisation ranging from 1 up to 5 (oligosaccharides). Short-chain fructooligosaccharides to a large extent escape digestion in the human upper intestine and reach the colon where they are totally fermented mostly to lactate, short chain fatty acids (acetate, propionate and butyrate), and gas. Butyrate is the most interesting of the short chain fatty acids (SCFA) since, it regulates cell growth and differentiation of colonocyte. In addition to this trophic effect, butyrate stimulates the immunogenicity of the cancerous cells. Short-chain fructooligosaccharides also stimulate bifidobacterial growth. The colonic microflora has a considerable influence on the immune system of the host. The intestinal mucosa, play an important role in the immune system too, it is the largest immunological organ of the body containing. The gut-associated lymphoid tissue (GALT) plays a key role according to its singular interface situation in the body and constitutes an important line of defence which is confronted with a large range of antigenic or immunomodulating substances. Recent founding in animal models clearly demonstrate that pre and probiotic may exert beneficial effects on gut health by enhancing GALT responses directly or indirectly by the mediation of butyrate and lactic bacteria. GALT may play a pivotal role in the rejection of nascent colon tumours. Intestinal microflora modulates the GALT responses and recent founding in animal models clearly demonstrate that pre and probiotic may exert beneficial effects on gut health by enhancing GALT responses directly or indirectly by the mediation of butyrate. The demonstration of the potential health benefits of sc-FOS on reduction risk of colon cancer is an active field of research in human nutrition. The sc-FOS, in animal models, reduce colon tumour development by enhancing both colon butyrate concentrations and local immune system effectors. The objective of this review is to discuss the critical role of GALT and its effectors, associated to butyrate, on colorectal cancer prevention. Both target functions have shown to be enhanced by sc-FOS.

Key words: short-chain fructooligosaccharides; immune functions; bifidobacteria; prebiotic; colon cancer

INTRODUCTION

Short-chain fructooligosaccharides (sc-FOS) are a group of linear Glucosyl $\alpha(1 \rightarrow 2)$(fructosyl)$_n\beta(2 \rightarrow 1)$ fructose polymers with a degree of polymerisation (DP) ranging from 1 up to 5 (oligosaccharides). They have aroused interest in the past decade, mostly because of their nutritional properties. Sc-FOS to a large extent escape digestion in the human upper intestine and reach the colon where they are totally fermented and stimulate bifidobacterial growth. The prebiotic effect of sc-FOS is dose-dependent. It is associated with a decrease of faecal pH and an increase of production of organic acids (lactic acid, short chain fatty acids). Butyrate is the most interesting of the short chain fatty acids (SCFA) since, it regulates cell growth and differentia-

tion of colonocyte. In addition to this trophic effect, butyrate stimulates the immunogenicity (sensitivity to the immune response) of the cancerous cells. The colonic microflora has a considerable influence on the immune system of the host.

The intestinal mucosa also, play an important role in the immune system, it is the largest immunological organ of the body containing. The gut-associated lymphoid tissue (GALT) constitutes an important line of defence. Recent studies suggest that it may play a pivotal role in the rejection of nascent tumours. The demonstration of the potential health benefits of sc-FOS on reduction risk of colon cancer is an active field of research in human nutrition. The sc-FOS, in animal models, reduce colon tumour development by enhancing both colon butyrate concentrations and local immune system effectors. The objective of this review is to discuss the critical role of GALT and its effectors, associated to butyrate, on colorectal cancer prevention. Both target functions have shown to be enhanced by sc-FOS.

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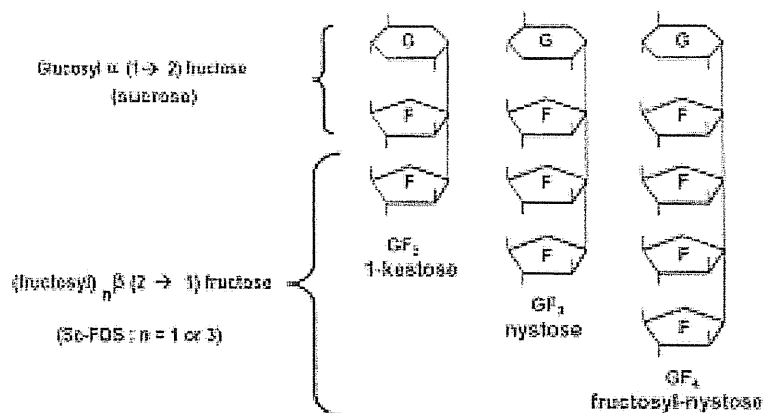
Fig. 1. Short-chain fructooligosaccharides structure of sc-FOS.

SHORT-CHAIN FRUCTOOLIGOSACCHARIDES: ORIGIN AND NUTRITIONAL FACTS

By definition, oligosaccharides have a DP lower than 9. Short-chain fructooligosaccharides (sc-FOS) are a group of linear glucosyl $\alpha(1 \rightarrow 2)$(fructosyl)$_n\beta(2 \rightarrow 1)$ fructose polymers with a degree of polymerisation (DP) ranging from 1 up to 5 (Fig. 1).

Short-chain fructooligosaccharides occur in a number of plants such as onions, Jerusalem artichokes, asparagus, wheat, rye, and garlic (8). Pure sc-FOS are produced on a commercial scale from sucrose using a food grade fungal fructosyltransferase, (ACTILIGHT® (Béghin Meiji, France) or MEIOLIGO® (Meiji Seika Kaisha, Japan) or NUTRAFLORA (GTC, USA).

Sc-FOS to a large extent, escape digestion in the human upper intestine (23, 24) and reach the colon where they are totally fermented, mostly to lactate, short chain fatty acids (acetate, propionate and butyrate). Compared with other fermentable products, like cellulose, pectin or lactulose, the fermentation of FOS produces higher percentages of propionic and butyric acid (2, 18). The most important property of sc-FOS is their ability to stimulate SCFA production and bifidobacterial growth.

Unlike other undigestible sugars, such as lactose or lactulose which are hydrolysed by a wide variety of gut bacteria, sc-FOS are only fermented in vitro by a limited range of micro-organisms that include most species of bifidobacteria (except Bifidobacterium bifidum) (11, 20, 22). Indeed, bifidobacteria have relatively high amounts of β-fructosidase, which is selective for the β-(2,1) glycosidic bonds present in sc-FOS. After sc-FOS hydrolysis, fructose serves as an efficient growth substrate for the bifidus pathway of hexose fermentation, which is almost exclusively carried out by bifidobacteria (33).

Numerous studies in humans showed that sc-FOS ingestion led to an increase of faecal bifidobacteria (3, 6, 11, 12, 20–22, 30, 32, 39, 40, 43) have been conducted in healthy subjects using a "control" group and a double or single blind design. The main characteristics and results of the studies conducted in humans are summarised in Table 1. Lastly, Bouhnik et al. (4) observed a significant correlation between the dose of sc-FOS ingested and the faecal bifidobacteria counts at the end of the 7 day period.

BIFIDOBACTERIA AND IMMUNE RESPONSES

It has been reported that bifidobacteria exert various effects on immune system related function, such as, mitogenic activity (14), adjuvant activity (15, 37), promotion of macrophages (14, 35), stimulation of antibody production (41, 44, 45) and antitumour effects (15, 36). Lee et al. (17) tested the immunopontentiating activity (i.e. to stimulate the proliferative response of murine immune cells) of twenty seven micro-organisms in vitro. They showed that bifidobacteria strains have a higher immunopotentiating activity than do Lactobacillus casei or L. acidophilus. Bifidobacterium adolescentis M100-4, originally derived from human intestinal microflora, had the strongest mitogenic activity on splenocytes and Peyer's patches cells. This activity was shown to be dose dependent and was increased after disruption of the cells by sonication, indicating the existence of an intra soluble immunopotentiator.

The intestinal mucosa plays, also, a important role in immunologic response of the body. The gut-associated lymphoid tissue (GALT) constitutes an impor-

Table 1.   Main characteristics and results of clinical studies conducted in healthy
subjects on the prebiotic effects of short-chain FOS ACTILIGHT®.

Healthy Subjects (n)	Age (yr)	Sc-FOS daily ingestion (g)	Duration (Day)	Bifidobacteria count in stools log CFU/g (mean ± SEM)		Statistical significance (p)	Authors
				Before	After		
6	—	6	30	9.6	9.8	NS	Mitsuoka, et al. *(21)*
23	73 ± 9	8	14	8.8 ± 1.1	9.7 ± 0.5	< 0.005	Mitsuoka, et al. *(22)*
27 (9 × 3)	36.8 ± 9 25.2 ± 3.3	1 3 5	14 14 14	9.8 ± 0.6 9.9 ± 0.6 9.7 ± 0.6	10.2 ± 0.4 10.4 ± 0.4 10.3 ± 0.4	< 0.05 < 0.05 < 0.01	Tokunaga, et al. *(40)*
38	—	8	14	5.2 ± 0.9	6.2 ± 0.6	< 0.01	Rochat, et al. *(30)*
10	20–40	4	14	8.3 ± 1.8	9.4 ± 2.3	< 0.05	William, et al. *(43)*
10	22–39	12.5	12	7.9 ± 0.5	9.1 ± 0.3	< 0.01	Bouhnik, et al. *(3)*
32 (8 × 4)	29.6	2.5 5 10 20	8 8 8 8	8.0 ± 1.1 8.1 ± 0.8 8.0 ± 1.3 8.2 ± 0.9	8.2 ± 1.1 9.1 ± 0.4 9.5 ± 0.3 9.5 ± 0.6	NS < 0.05 < 0.02 < 0.002	Bouhnik, et al. *(4)*

NS: not significant.

tant line of defence.

THE GUT-ASSOCIATED LYMPHOID TISSUE (GALT)

The intestinal mucosa is the largest immunological organ of the body containing over 10^6 lymphocytes/g tissue. Its represent the major part of the body's contact. About 60% of the total immunoglobulin produced daily is secreted into the gastrointestinal tract. The structure of the GALT, the intestinal immune cell distribution among the Peyer's patches, epithelium and lamina propia have been reviewed by Brandtzaeg et al. *(5)*. The GALT-T lymphocytes are not homogenous. There are classified as CD4+ helper/inducer cells and CD8+ suppressor/cytotoxic cells generating different cytokines profiles which distinct yet unproven functions. The majority of intra-epithelial T-cells are CD8+, contrasting with the lamina propia where CD4+ are predominant. The lamina propia is also endowed with lymphocytes belonging to the B-cell lineage. These are mainly memory cells and plasmocytes which 70–90% of them are Ig-A producing cells.

An immune response initiated in the GALT can affect immune responses at other mucosal surfaces. The lymphocytes activated within the Peyer's patches disseminate via mesenteric lymph nodes, thoracic duct and the bloodstream back to the lamina propia, and traffic between other secretory tissues including the respiratory tract and the lacrymal, salivary and mammary glands. The digestive flora is the major antigenic stimu-lus responsible for the migratory pathway and maturation of precursor lymphoid cell present in the Peyers's patches. Recent studies suggest that it may play a pivotal role in the rejection of nascent tumours, and consequently in the reduction of risk of colon cancer.

BUTYRATE—GALT AND COLON CANCER

Butyrate is also known to have preventive effects on colon cancer and adenoma development *(38)*. In the colon, it comes from bacterial fermentation, and constitutes a good substrate for colonocytes. Butyrate oxydation has been shown to make up for more than 70% of the oxygen consumption by the human colonic tissue *(31)*, indicating that butyrate is the prime energy substrate of the colonocyte. It is not only an energy source for colonocytes. Sodium butyrate (NaB) exerts an antiproliferative activity on many cell types. It is an inducer of differentiation of colon carcinoma cell lines. It also has been observed to induce gene expression, to influence the rate of gene expression through its effects on post translational modifications, to induce apoptosis and to reverse the resistance of colonic cancer cells to programmed cell death *(38, 41)*.

Perrin et al. (personnal communication) have characterize the modification of the phenotype of PROb rat colon adenocarcinoma cells when NaB treated. They focused their study on surface oligosaccharides that could play a role in their tumorigenicity. Blood-group H antigens, formed by the addition of fucose on type
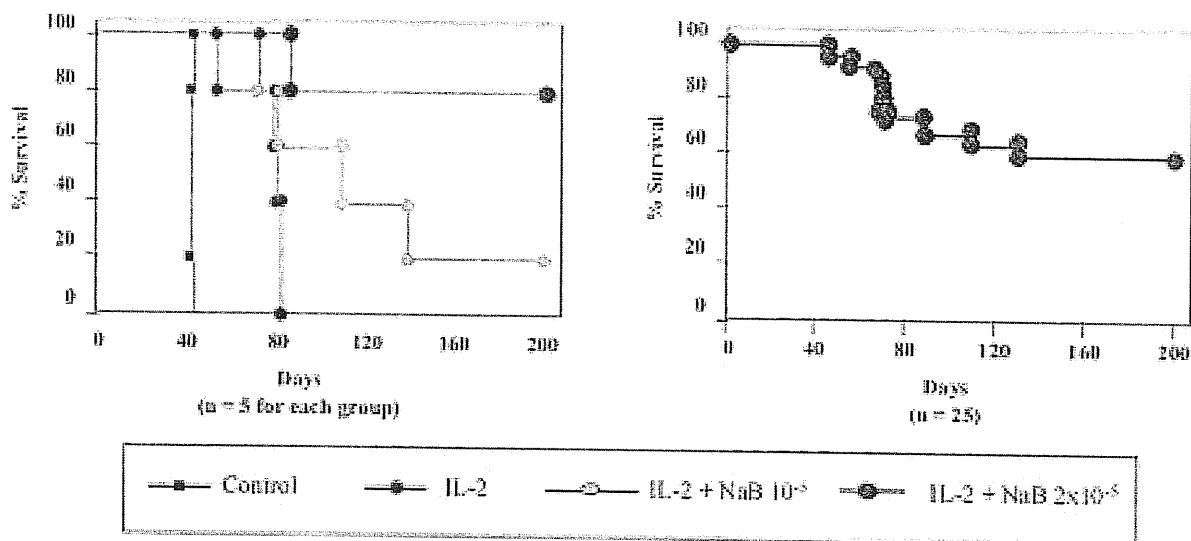
Fig. 2. Effects of butyric acid on experimental carcinogenesis: percentage of survival of rat traited with interleukin 2 (IL-2) and sodium butyrate (NaB), alone or in combination (25).
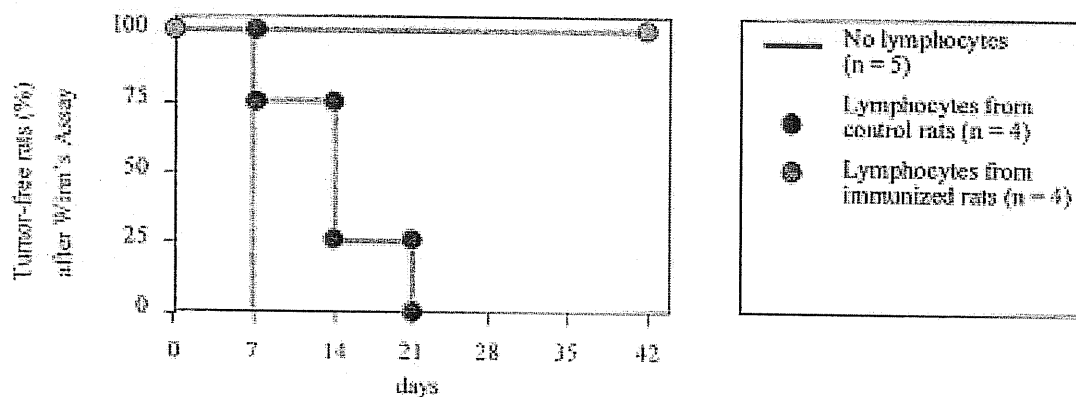

Fig. 3. Effects of butyric acid on experimental carcinogenesis: effects of subcutaneous injection of 10^6 PROb cells alone or mixed with lymphocytes extracted from naive rats or from immunized rats (25).

1,3 precursors, were less expressed on butyrate-treated PROb cells, while $\alpha(2 \rightarrow 3)$ linked sialic acids were enhanced. This phenotype was maintained after sodium butyrate withdrawal, whereas cell growth inhibition was lost. The decrease of H1,3 antigens would be related to the lower activity of $\alpha(2 \rightarrow 3)$ fucosyltransferase(s) and competition between fucoses and sialic acids precursors, borne by CD44v. When subcutaneously grafted, NaB-treated PROb cells induced significantly small tumours. That could result from a more efficient host response, attributable to the phenotype the cancer cells acquired with transient in vitro NaB treatment, since the lower level of H 1,3 antigens was maintained in growing tumours.

Butyrate stimulates the immunogenicity of the cancer cells (25). The phenotype of the weakly immuno-genic rat colon cancer PROb cells was modified with sodium butyrate. After a 4-days in vitro sodium butyrate treatment, the lymphokine-activated killer cell sensitivity, the expression of Major Histocompatibility Complex class I, and the intercellular adhesion molecule 1 of PROb cells, were increased in a dose-dependent manner.

Perrin et al. (25) tested the efficiency of interleukin 2 (IL-2) and sodium butyrate (NaB), alone or in combination, against experimental widespread carcinomatosis induced in rats by intraperitoneal injection of 2 × 10^6 PROb colon carcinoma cells. IL-2/butyrate combination resulted in cases of complete cure of carcinomatosis with specific protection against PROb cells (Fig. 2 and 3). IL-2 secretion probably leads to activation of non-specific effectors such as natural killer and/or lym-

phokine-activated killer cells resulting in a rapid clearance of otherwise immunogenic sodium butyrate-treated cells. The complete regression of tumour masses may be attributed, to butyrate-induced decrease of tumorigenicity and increase of immunogenicity of the cancer cells.

So, it may be advantageous to provide indigestible carbohydrates as an indirect source of butyrate to the large bowel.

SHORT CHAIN FRUCTOOLIGOSACHARIDES AND COLON CANCER

Classically defined as non-starch polysaccharides, fibre now include other sources of fermentable substrate for microflora, such oligosaccharides, resistant starch (10). Dietary fibres have been proposed as protective agents against colon cancer but results of both epidemiological and experimental studied are debatable and prevention programme have been limited to general lifestyle guidelines (42). These conflicting results may relate to the heterogeneity of the fibre and basal diet, feeding protocol, chosen biomarker, and/or stage of colon carcinogenesis. Among fibres, carbohydrates producing large amounts of butyrate appear to be greatest interest as butyrate is an energy yielding substrate for colonocytes, affects cellular function, is an antineoplastic agent in vitro, and has been implicated in the protective effect of fibre in rodents (19, 34). It has been hypothesised that protection against colon cancer may be restricted to butyrate producing fibres.

To investigate the effects of different type of fibres, rats (26) and Min mice (28) have been used: chemically induced and spontaneous cancer models. The relevance of animal models, as compared to human colon tumour studies depends on the criteria considered (29). Azoxymethane (AOM) induced tumours are similar to human tumours in many histological, biochemical, immunological and cellular aspects; but many of the tumours do not follow the adenoma to carcinoma progression, frequently arising de novo from flat mucosa. This model permits the investigation of early stages of carcinogenesis, the end-point being a consensual precancerous marker, the aberrant crypt foci. The mouse model (Min mice) is a model for both familial adenomatous polyposis and sporadic colon cancer. The Min mice are heterozygous for a non-sense mutation of the Apc gene, the murine homologue of APC. The Min mouse model adenomas are pertinent by their genetic origin, but they are more frequent in the small bowel than in the colon, as opposed to the human situation. These studies provided data on later stages of

colon cancerogenesis, and the end-point was the number of detectable tumours.

A two part randomised blinded study in rats, mimicking a prospective study in humans, was performed using a low fibre control diet (CD) and three high fibre diets: starch free wheat bran (WB) type III resistant starch (RS) and short-chain fructooligosaccharides (sc-FOS) (9). Using a randomised block design, 96 inbred rats were fed for 16, 30 or 44 days to determine the period of adaptation to the diet, fermentation profiles, and effects on the colon, including mucosal proliferation on day 44. Subsequently, 36 rats fed the same diets for 44 days were injected with azoxymethane and checked for aberrant crypt foci 30 days after. After fermentation had stabilised (44 days), only RS and sc-FOS produced large amounts of butyrate, with a trophic effect in the large intestine. No difference in the mucosal proliferation between the diets was noted at this time. In the subsequent experiment one month later, fewer aberrant crypt foci were present in rats fed high butyrate producing diets (RS, $p = 0.022$. sc-FOS $= 0.043$) (Fig. 4). Similar effects on a reduction of the number of aberrant crypt foci in CF1 mice treated with AOM and fed diets containing sc-FOS and exogenous bifidobacteria, were published by Koo and Rao (16). Campbell et al. (7) evaluated in rats the effects of selected indigestible oligosaccharides on caecal and faecal SCFA concentration, pH, total large bowel wet weight and wall weight, and concentrations of intestinal microbiota. The duration of the study was 14 days. The sc-FOS containing diet resulted in higher caecal butyrate concentrations compared with the control, or with the cellulose or xylooligosaccharide containing diets.

The same fibre diets have been tested in Min mice aged 6 or 7 weeks ($n = 40$) (28). Each group was fed ad libidum for 42 days either the control low-fibre diet (CD) or one of the three high-fibre diet (WB, RS, sc-FOS). Gut tumours and small intestine lymphoid nodules were counted (Fig. 5). Neither WB nor RS modified the number of tumours. However, sc-FOS dramatically reduced the incidence of colon tumours and concomitantly developed GALT. Interestingly, the sc-FOS effect was limited to the colon; they were no significant differences between diets in the number of tumours found in the small intestine suggesting strongly that events specific to the colon were involved. A bifidogenic effect probably occurred in the experiment because Howard et al. (13) demonstrated that dietary supplementation with the same sc-FOS enhanced the population of bifidobacteria in mouse colon as soon as 14 days.

Fig. 4. Effects of short chain fructooligosaccharides on experimental carcinogenesis: mean number of aberrant crypt foci per rat fed with control low-fibre diet (CD), wheat bran (WB), resistant starch (RS) or short chain fructooligosaccharides (sc-FOS) (26).

Fig. 5. Effects of short-chain fructooligosaccharides on experimental carcinogenesis: gut tumours and small intestine lymphoid nodules in Min mice (n = 40) fed ad libidum for 42 days with control low-fibre diet (CD), wheat bran (WB), resistant starch (RS) or short-chain fructooligosaccharides (sc-FOS) (28).

To obtain an insight into the GALT response to changes in the colonic ecosystem, the authors looked at the lymphoid tissue of the small intestine. The examination of the colon for that purpose was impossible because treatment of this tissue, to study colon tumours, made impossible to accurately evaluate colon lymphoid follicles. A significantly higher number ($p < 0.05$) of macroscopically detectable lymphoid nodules were noted in the small intestine with the sc-FOS diet. This suggests that immune system may play a role in inhibiting tumour formation by eliminating cells that express antigens if they are immunogenic enough to allow the expansion of immune cell specific for these antigens.

To investigate whether T cell status may influence colon tumour formation in Min mice fed a sc-FOS diet, Pierre et al. (27) have chosen to immunodeplete mice with antibodies against target T cells (CD4+ and CD8+), rather than NK cells, which do not affect the incidence

of intestinal neoplasia in *Min* mice *(9)*. *Min* mice depleted of CD4+ and CD8+ lymphocytes developed twice as many tumours as immunocompetent mice.

To investigate the response of the tissue to sc-FOS at the effector molecule level, Bassonga et al. *(1)* assessed the expression of cytokines present in the colon. They chosen to study the mRNAs since certain cytokines (e.g. IL-15) are frequently not translated or secreted by resting cells. They used a multiprobe ribonuclease protection assay to study the expression of selected cytokines in the colon of C57BL/6 and *Min* mice fed low fibre diet (CD) or a sc-FOS enriched diet (sc-FOS).

Five cytokines were consistently detected regardless of the animals or diet (IL-4, IL-5, IL-13, IL-15 and IFN γ). IL-4, IL-5 and IL-13 were expressed at low but comparable levels and were not sensitive to the diet. IL-10, IL-9, IL-6 and IL-2, were not detected. The IL-15 mRNA was frequently highly expressed in both *Min* groups but a level significantly higher ($p = 0.01$) in the *Min* group fed sc-FOS as compared to the *Min* group fed CD. IFN γ mRNA, when detected, showed the same pattern of expression as IL-15. The fact that IFN γ appears to be modulated in the same way as IL-15 support the hypothesis that IL-15 could be secreted in an active form, since IFN γ, a cytokine produced by activated T-cells and which stimulates cytotoxic activity, is a target of active IL-15.

CONCLUSION

Short-chain fructooligosaccharides have aroused interest in the past decade, mostly because of their nutritional properties. To a large extent, sc-FOS escape digestion in the human upper intestine and reach the colon where they are totally fermented, mostly to lactate and short chain fatty acids (acetate, propionate and butyrate). The most important property of sc-FOS is their ability to specifically stimulate bifidobacterial growth and to induce butyrate production. Recent studies have shown that these both effects with the stimulation of the activity of some compound of the GALT play an important role in the colon cancer prevention.

The demonstration of the health benefits of sc-FOS and the stimulation of immune rejection of nascent tumours offers new perspective for the prevention of colon cancer and opportunities for better understanding of GALT to colon cancer.

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